Bone Research Society

ANNUAL MEETING 2022

6-8 JULY 2022 • MANCHESTER, UK

Invited Speaker Abstracts,

Oral Communications Abstracts &

Poster Abstracts

Speaker Abstracts

RBD1.1

Trials in FD pain: What have we learnt?

Professor Roland Chapurlat

Lyon, France

Abstract

Pain is one of the possible symptoms in fibrous dysplasia of bone/McCune-Albright syndrome (FD/MAS). Some patients never experience bone pain, but roughly two thirds do have bone pain at some point during their lifetime. This pain has to be distinguished from joint pain that also may arise in the patients with bone deformity. The pain may be of variable intensity, of various characteristics (nociceptive, neuropathic-like) and spontaneously vary over time.

Several studies have addressed the treatment of pain in FD/MAS. The first trials were observational studies using intra-venous bisphosphonates (BP). A reduction greater than 50% in the intensity of bone pain could be obtained with pamidronate and later with zoledronate. Two placebo-controlled randomized trials using oral BPs (risedronate, alendronate) failed to demonstrate any benefit on bone pain. Several small case series using denosumab at various dosages have shown mixed results on bone pain, at the expense of a severe overshoot of bone turnover in a few patients at treatment discontinuation. The inhibition of the interleukin-6 pathway has been evaluated in a placebo-controlled randomized trial of tocilizumab. No significant change has been observed in this secondary endpoint of the trial, although a small subset of patients might have benefited.

Designing clinical trials in FD/MAS is challenging because a substantial improvement of bone pain is expected in the placebo group, due to the placebo effect, regression to the mean and the natural evolution. The research agenda on the mechanisms of bone pain in FD/MA remains open, because we do not know what is causing pain in this disease and whether this is related to bone turnover, that was targeted in previous clinical trials. Therapeutic improvement will probably emerge when the pathophysiological pathways of bone pain in FD/MAS are identified.

RBD1.2

Osteoporosis in Duchenne Muscular Dystrophy: Current standards of care and the path forward

Professor Jarod Wong

Glasgow, United Kingdom

Abstract

Duchenne muscular dystrophy (DMD) is a rare X-linked form of muscular dystrophy affecting about 1 in 4000 male live births, that occurs due to loss of dystrophin protein with resultant muscle fragility. Oral glucocorticoid therapy has been adopted world-wide as standard of care in DMD over the last 20 years. Glucocorticoid is usually commenced between 4-7 years of age and continued indefinitely with proven long-term benefits on skeletal muscle by prolonging ambulation, maintaining upper limb function and positive benefits on cardio-respiratory outcomes. Osteoporosis and resulting vertebral fractures are very common in these boys due to the multiple and escalating insult on the growing skeleton. Vertebral fractures are observed in as high as 75% of boys with DMD on long term oral daily glucocorticoid especially when routine spine imaging is performed. Current international standards of care recommend annual lateral spine imaging to identify vertebral fracture in boys on glucocorticoid therapy. Initiation of treatment with intravenous bisphosphonates is recommended following first long bone fracture and upon identification of moderate or severe vertebral fracture even in the absence of back pain. Addressing delayed puberty and hypogonadism with exogenous testosterone is also critical to improve bone mass accrual. I will discuss the extent of bone morbidity in boys with DMD, the rationale of the current international standards of care of management of osteoporosis in this population together with the available evidence to support such recommendations. I will also highlight the work of implementing standards of care in the UK via DMD Care UK, a project funded by patients (Duchenne UK) in collaboration with clinicians nation-wide. Finally, I will discuss areas for future development which include the consideration of the use of bisphosphonates prior to first fracture, the need for consideration of osteo-anabolic bone protective therapies, and a pathway for monitoring and management of osteoporosis in the growing adult population of men with DMD.

RBD1.4

Impact of FGF23 suppression on FGF23 mediated disorders in adults

Professor Judith Bubbear

Abstract

Fibroblast growth factor-23 (FGF23) is a bone derived hormone that acts in the kidney to suppress phosphate reabsorption and vitamin D synthesis. Excess levels of FGF23 therefore cause renal phosphate wasting leading to inadequate mineralization of bone, a spectrum of skeletal abnormalities, physical impairment, weakness and pain. Elevated levels of FGF23 are pathological in conditions including X-linked hypophosphataemia (XLH) and Oncogenic Osteomalacia.

Burosumab is an anti-FGF23 antibody that is licenced for the treatment of X-linked hypophosphataemia in children and adults. It is currently only available in England as part of a drug company funded early access scheme and is in the process of being evaluated by NICE.

This session will discuss real life use of Burosumab in adults as part of this early access programme in adults with XLH, drawing on the experience at the Royal National Orthopaedic Hospital. It will cover efficacy, side-effects, and practical considerations of treatment. Use in Oncogenic Osteomalacia will also be discussed.

S1.2

Epidemiology in the digital era

Professor Rachel Cooper

Manchester, United Kingdom

Abstract

In recent decades the face of epidemiology has changed dramatically with important implications. This transformation has been driven in part by major technological advances that have made possible more detailed measurement of exposures and outcomes at a greater scale than ever before. Also contributing is a digital revolution within health care that has created new opportunities for research using electronic health record data. To handle the complexity and scale of the data now available novel analytical methods have had to be adopted at pace. This presents many new opportunities for research on musculoskeletal health and function but there are also pitfalls to be avoided if we are to realise the full potential of these transformations for public and patient benefit.

S2.1

Emerging therapies for osteogenesis imperfecta

Professor Frank Rauch

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Abstract

Osteogenesis imperfecta (OI) is a heritable disorder that is mainly characterized by frequent fractures. In the large majority of individuals with OI, the condition is caused by dominant variants in one of the collagen type I encoding genes, COL1A1 or COL1A2. As the underlying genetic defect can presently not be corrected, medical treatments aim at symptomatic improvements through bone-strengthening drugs.

The traditional treatment approach of OI is based on intravenous infusions with bisphosphonates. Bisphosphonates decrease bone resorption and reliably increase bone mineral density in growing children with OI. However, intravenous bisphosphonates have not been evaluated in adequately powered randomized-controlled trials. Newer drugs are typically using antibodies to achieve therapeutic effects. Antibodies against RANKL decrease osteoclast activity and thereby lead to increased bone mineral density, but there are concerns about 'rebound' hypercalcemia when such antibodies are administered to children. Anti-sclerostin antibodies are used to stimulate bone formation. There is preliminary evidence that the sclerostin antibodies can increase bone mineral density in adults with OI, but the effect in children is not known at present. Studies in mice have found that OI is associated with increased activity of transforming growth factor (TGF) beta. Therefore, TGF-beta inhibiting antibodies are a potential approach to treat OI. Phase I studies suggest that TGF-beta antibody treatment is feasible in adults with OI, but pediatric data are not yet available.

Thus, several new antibody-based treatments are being developed for OI. Given that antibody treatments can be expected to have only a short-lived effect on bone, it seems likely that they will need to be combined with long-acting agents such as bisphosphonates to maintain any treatment gains that are achieved with antibody drugs.

S2.2

New Molecular Targets in Osteoarthritis

Professor Tonia Vincent

Oxford, United Kingdom

Abstract

Osteoarthritis (OA) is the most common form of joint disease, affecting most people over 60, and younger individuals following joint trauma. Once considered an inevitable consequence of repeated mechanical insult over many decades, it is now being recognised as a disease driven by mechanosensitive signalling that drives both detrimental mechano-inflammatory pathways as well as those that promote tissue repair. Careful agnostic molecular analyses, genetic modification in murine models of disease and human clinical trials have helped to shape the new landscape. Classical inflammatory cytokines such as IL1 and TNF have not proved to be effective targets in randomized controlled studies. Indeed, treatments repurposed from rheumatoid arthritis have all failed. Rather, a very different picture is emerging that supports the promotion of intrinsic repair of cartilage as a more effective strategy. This aligns well with basic cartilage biology, recent large scale genome wide association studies and phase II clinical trials. Taken together these studies not only change how we view OA target discovery, but provide the tantalizing possibility that cartilage might be recoverable in the OA joint.

S3.1

Osteomorphs: a new cell entity regulating bone resorption

Dr Michelle McDonald

Darlinghurst, Australia

Abstract

Osteoclasts are long lived highly specialised bone resorbing cells which form through the fusion of mononuclear pre-cursor cells and are believed to follow a linear fate and undergo apoptosis at the end of their life cycle. A number of anti-resorptive therapeutics target these cells, either preventing their resorptive function, Bisphosphonates, or inhibiting their formation, Denosumab (Anti-RANKL). These agents have achieved great success in preventing bone loss and fractures in patients with osteoporosis, amongst other diseases, however complications from their long term use has led to treatment cessation. In the case of Denosumab, treatment cessation has led to rebound bone loss and increased fractures, providing new challenges for its use in the clinic. We visualised the dynamics of osteoclasts in real time within live bone tissue leading to the discovery of a new fate for these complex cells. Further, this novel cell biology provides an improved understanding of patient response to anti-resorptive therapy.

We developed a novel intravital imaging methodology to visualize osteoclast dynamics on the intact endocortical surface of tibia in live mice. Employing a double reporter mixed bone marrow chimera model and using sRANKL to stimulate osteoclasts and osteoprotegerin-Fc (OPG:Fc) to mimic Dmab we examined osteoclast dynamics and function. We showed that in addition to apoptosis, osteoclasts undergo fission to form osteomorphs, a novel intermediate cell of the osteoclast lineage. These osteomorphs were then shown to re-fuse, confirming that the process of osteoclast recycling as an alternative cell fate to apoptosis. Using RNAseq we defined the osteomorph as a novel cell population, distinct from osteoclasts and osteoclast pre-cursors. Interestingly, osteomorph specific genes were associated with bone phenotypes in mice. We also showed accumulation of osteomorphs and their rapid re-fusion following withdrawal of OPG:Fc, providing a mechanism for the rapid bone loss and fractures suffered by patients following Denosumab withdrawal.

S3.2

Periosteal cells for skeletal repair and disease modelling

Dr Scott Roberts

London, United Kingdom

Abstract

The periosteum is a fibrous membrane that envelopes bones at all exterior mineralised surfaces. It is composed of an outer fibrous layer and an inner cambium layer, the latter containing cells primed to differentiate into bone during physiological skeletogenesis, and cartilage for endochondral ossification during fracture repair. The periosteum's osteogenic function has been known since the 18th century, however, it is only recently that the field has begun to understand the identity, potency and activation of the cells contained within this tissue. This research is critical to allow the use of periosteal cells for skeletal repair strategies and disease modelling. Indeed, the periosteum is widely known to be involved in structural progression associated with the inflammatory disease ankylosing spondylitis, whilst loss of the periosteum increases the risk of fracture non-union. Our research has focussed on identifying specific signalling pathways that are involved with periosteal cell activation and tissue formation. For example, several of our studies reveal the importance of the IL-6/JAK/STAT3 axis in the expansion and differentiation of periosteal cells. Fundamentally, this links periosteal biology to inflammatory cues, which may be indicative of why periosteal new bone formation is observed following traumatic injury (fracture) or in inflammatory diseases. These insights have been taken forward to model ankylosing spondylitis through investigation of IL-17 cytokines, critical disease mediators, in conjunction with an in vitro biomimetic periosteal bone formation assay. This study revealed IL-17F to be approximately equipotent to IL-17A in its ability to promote human periosteal cell osteogenic differentiation, which is divergent from the lesser role that it appears to have in other inflammatory processes. Fundamentally, these data suggest that neutralisation of both IL-17A and IL-17F may be required to optimally suppress pathological periosteal bone formation observed in ankylosing spondylitis. In addition to inflammatory cascades, bone morphogenetic protein (BMP) signalling (amongst other pathways), is a critical modulator of tissue formation from periosteal cells. Antagonists of this pathway, such as GREM1 and Noggin, have a negative effect on periosteal cell proliferation and function. Indeed, GREM1 expression is suppressed during periosteal osteogenic differentiation. Interestingly, both of these antagonists are enriched in fracture non-union tissue. We have discovered that the early activation of BMP signalling is critical for in vivo tissue formation from isolated human periosteal cells. Subsequently, this notion was tested where periosteal cell priming with BMPs appear crucial for cell function, with BMP2 and BMP6 appearing to be the strongest effectors of chondrogenic differentiation. Interestingly, self-assembling bilayer tissues derived from human periosteal cells in BMP-driven differentiation can repair the joint surface. Despite the progress that we and other groups have made towards understanding periosteal cell biology, the identity of the (stem) cell at the apex of the differentiation hierarchy remains undefined. Single cell RNA sequencing is shedding light on the identity of this elusive cell, and at the same time casting doubt on some of the previously suggested markers. Nevertheless, with each publication identifying a new aspect of this fascinating cell, we as a field move closer towards realising its true potential.

S4.1

Bone Mechanoadaptation at the whole-organ level

Dr Andrew A Pitsillides

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Abstract

Bone mechanoadaptation is thought to adhere to principles that predict straight shape, yet most bones have obvious curvature. This paradox may endure as most in vivo loading studies examine only small segments of a bone rather than integrating these response at a 'whole-organ' level. Bone shape is governed by growth-related endochondral ossification and (re)modelling drift after birth that is deemed to be mechano-adaptively guided. Many questions regarding how loading influences whole-organ level bone shape are, however, unanswered. Does loading evoke changes that possess spatial hierarchy? How persistent are load-induced shape changes or are they – as mechanoadaptive principles predict – completely reversible? Do mechanoadaptive changes in osteoblasts and osteoclast remodelling activity unerringly correlate with local tissue-level strains? Here, historical and new studies using a range of approaches will be re-explored to examine this paradox and to address some of these questions. They will reflect briefly upon bones derived from distinct embryonic origins or are formed either by intramembranous or endochondral ossification, the role of early embryo loading, and the possibility that skeletal tissues exhibit 'modular' rather than uniform relationships with mechanical load and also with osteotrophic factors (e.g. PTH). The focus will then shift briefly to the cellular bases of these responses, will consider how strain patterns differ with sex, and then how load history modify the deficient load-related response linked to ageing. Finally, data will be presented from experiments using CT-based, 4-dimensional imaging and computational analysis to monitor acute and chronic whole bone shape adaptation and remodelling in vivo in our well-established non-invasive mouse tibia loading model. Results confirm the expected reversibility of acute load-induced structural changes in some cortical regions, which adhere to strain magnitude-related regulation of (re)modelling and occur where load-induced anti-resorptive activity is initially focused. They also show, however, that loading drives significant and extensive changes in tibia shape and increases in mass that are lasting. These lasting modifications were found to be independent of local strain magnitude and occurred where initial load responses were principally osteogenic. This indicates that loading stimulates nonlinear remodelling responses to strain that culminate in greater curvature, suggesting that whole organ level mechanoadaptation does not adhere with target strain-related principles, and that vast regions of the whole bone organ retain a lasting structural strain magnitude-independent load 'memory' where greater curvature is optimised for load predictability without sacrificing strength.

S5.1

Sclerostin in breast cancer bone metastasis

Professor Hanna Taipaleenmäki

Hospital of the Ludwig-Maximilians-University (LMU), Munich, Germany

Abstract

Bone is the most common site for breast cancer metastases and approximately 70% of breast cancer patients with an advanced disease suffer from bone metastases. Active breast cancer cells severely disturb physiological bone remodeling by inhibiting osteoblast-mediated bone formation and stimulating osteoclast-mediated bone resorption, leading to an osteolytic disease. Osteolytic metastases cause skeletal-related events (SREs), including pathological fractures and pain that require palliative interventions. In breast cancer patients, SREs are often associated with muscle weakness, a debilitating condition that decreases mobility, well-being, and life quality of the patients. Due to the osteolytic nature of the disease, the current bone-targeted treatments are based on anti-resorptive agents that prevent bone destruction but cannot heal the existing lesions or cure the disease. Therefore, novel and more efficient therapies are needed.

Sclerostin is a soluble Wnt antagonist secreted by the osteocytes. Sclerostin prevents canonical Wnt ligands from binding to their receptor Lrp5/6 thereby activating Wnt signaling and osteoblast-mediated bone formation. Due to its strong bone anabolic effect, anti-sclerostin antibody (Scl-Ab) has been approved for the treatment of postmenopausal osteoporosis.

Interestingly, aggressive metastatic breast cancer cells also secrete sclerostin. Cancer-derived sclerostin binds to Lrp5 on osteoblasts, leading to an inhibition of Wnt signaling and osteoblast differentiation. Through this mechanism, sclerostin likely contributes to cancer-induced reduction in osteoblast activity while favoring bone destruction and further tumor growth. Consistently, treatment of bone metastases–bearing mice with Scl-Ab alleviates tumor growth in bone and bone destruction without increasing metastases at other sites. In bone, Scl-Ab has a strong dual effect restoring cancer-induced impairment of bone formation and inhibiting pathological bone resorption. Furthermore, Scl-Ab treatment protects from cancer-induced loss of muscle function in tumor-bearing mice through its anti-resorptive action. Importantly, treatment with Scl-Ab improves survival of bone metastases-bearing animals. Together, these findings reveal that therapeutic targeting of sclerostin reduces breast cancer-induced bone metastases and muscle weakness in mice, indicating its potential as a novel therapeutic option for breast cancer patients.

S6.1

Reframing the gut-bone axis

Dr Carolina Medina Gomez

Rotterdam, Netherlands

Abstract

For more than a decade, researchers have assembled evidence of the impact of the microbial communities residing in the human gut and the musculoskeletal system. The effect of the gut microbiome (i.e., the collection of microorganisms residing in the gut, their genomes, and their interactions) on bone and muscle tissue involves complex mechanisms including immunomodulation, modifications in hormone levels, nutrient absorption, microbial synthesis and luminal availability of vitamins, and translocation of other microbial signaling molecules -as short-chain fatty acids- across the endothelium. Nevertheless, this research field is still in its infancy, and as such, laboratory procedures and analytical pipelines are continuously being improved. On top of this constant evolution, host lifestyle and diet can greatly impact the gut microbiome, which in turn influence host metabolism, contributing further to discrepancies observed between some microbiome studies. Despite these important caveats, the basis of our current knowledge is solid, with evidence arising from diverse sources, from fundamental (in vitro and in vivo animal) studies to interventional trials. In this seminar, I will summarize the most important investigations supporting the role of the gut microbiome in bone and muscle metabolism, including perturbation experiments, primarily performed in mouse models, and how the gathered knowledge from them is being translated to clinical practice by the design and execution of, for instance, probiotics (i.e., live microorganisms) clinical trials. At the same time, I will critically assess these findings through the lens of our current knowledge in the microbiome field and discuss a selection of challenging topics to further improve microbiome research. I will highlight the research reports on this topic during the last year and the work we have performed in our own laboratory. Finally, I will hypothesize where the field is moving to become a crucial driver of the personalized healthcare revolution.

S6.2

Preventing falls & fractures in older populations: an 'appetite' for change

Dr Marc Sim

Perth, Australia

Abstract

One in three individuals over 65 years will experience a fall annually. In Australia, falls are the leading cause of hospitalised injuries and injury deaths among older individuals. Most alarmingly in this age group, 50% of falls result in fracture, incurring an average in-patient hospital stay of 9 days. In addition to the trauma and injury associated with the fall and/or fracture itself, patients often have prolonged impaired mobility remaining functionally dependent upon others.

Falls have many contributing factors, making prevention complicated. Besides muscle function, which comprises strength and physical function, other well-established falls risk factors include visual impairment, use of multiple medications, chronic disease, and the physical environment (e.g. tripping hazards). Exercise has emerged as one key factor in lowering fall and fracture risk, likely through its ability to improve muscle function and prevent chronic disease. But exercise is only one piece of the puzzle: nutrition is also significant. From a dietary perspective, attention has primarily been on the benefits of supplementing protein , Vitamin D and calcium. However, the importance of other aspects of diet for musculoskeletal health to prevent falls and fracture remain largely unknown.

An emerging research area is role of vegetables and their components to improve musculoskeletal health, especially in the prevention of diseases such as sarcopenia and osteoporosis. When considering clinical outcomes, superior muscle function observed with higher consumption of vegetables (≥ 3 serves/day) may explain lower long-term injurious fall and hip-fracture risk. However, due to differences in nutrient profile, specific vegetable types appear to be more advantageous for musculoskeletal health. Notably, cruciferous vegetables (e.g. broccoli, cauliflower, cabbage) may provide the greatest benefits. Nitrate is another nutrient found in high concentrations within green leafy vegetables and beetroot. Traditionally, health benefits of dietary nitrate have typically centred around improved vascular function and reduced risk for cardiovascular events; another underappreciated risk factor for poor musculoskeletal outcomes.

Recently however, diets rich in vegetable-derived nitrate have been linked to better muscle strength of the upper and lower-limbs, as well as physical function in adults across the lifespan. Notably, both green leafy and cruciferous vegetables are rich sources of Vitamin K that is essential for the carboxylation of the bone-derived protein osteocalcin, which has been implicated in the material properties of bone. Our recent data also indicates dietary Vitamin K may play an important role for fracture prevention. In summary, this talk will cover the importance of diet for musculoskeletal health, whilst also examining novel strategies to screen for nutritional deficiencies (e.g. muscle biomarkers, DXA whole-body imaging) to identify and manage individuals with high risk of falls and fractures.

Oral Communications Abstracts

MBOC1.1

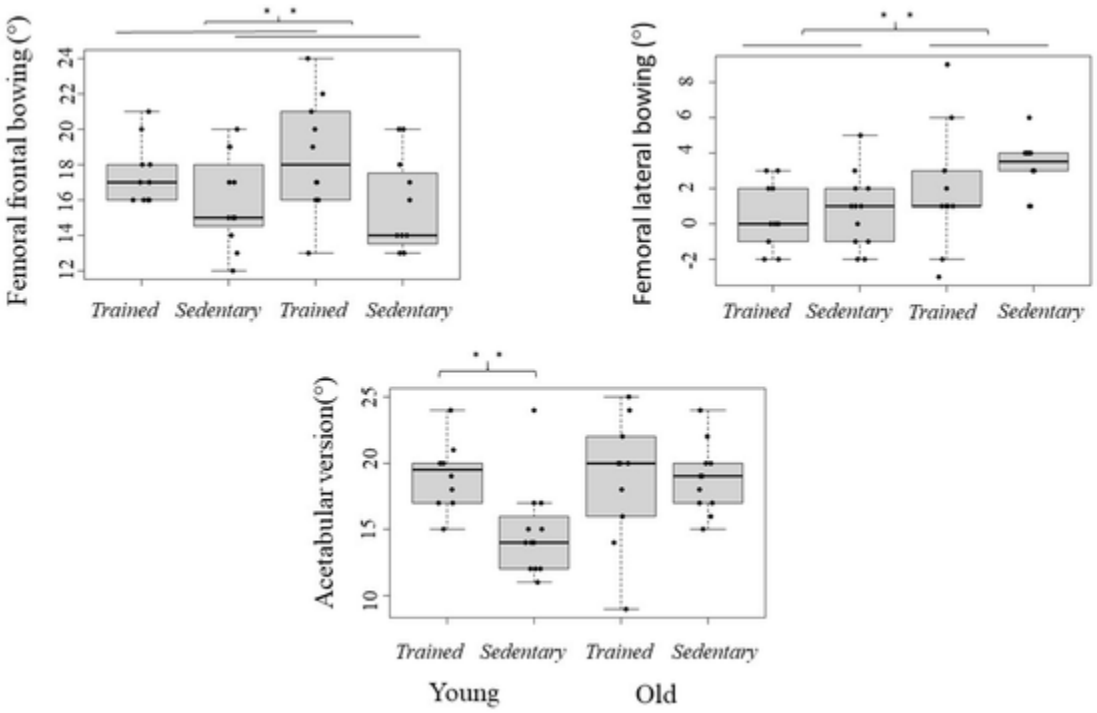
Associations between long-term exercise participation and lower limb bone geometry in young and older adults

Mr Matteo Scorcelletti[1], Dr. Jochen Zange[2], Mr Jonas Böcker[2], Mr. Wolfram Sies[2], Dr. Patrick Lau[2], Dr. Uwe Mittag[2], Prof. Christoph Clemen[2], Prof. Neil Reeves[1], Prof. Jörn Rittweger[2], Dr. Alex Ireland[1]

[1]Manchester Metropolitan University, Manchester, United Kingdom. [2]Institute of Aerospace Medicine DLR, Cologne, Germany

Abstract

Background: Features of lower limb bone geometry including femoral anteversion are associated with clinical outcomes, such as risk of fractures and osteoarthritis. In addition, they are associated with movement kinematics, such as muscle lever arms and hip-contact forces. Therefore it is important to find determinants of skeletal geometry. Lower limb geometry changes dramatically during development, in part due to adaptation to the forces it has to withstand during physical activity. However the effects of physical activity in adulthood on lower limb geometry, and associations between geometry and muscle function are relatively unexplored. Methods: 43 healthy adult males were recruited; 10 young trained (24±2y), 12 young sedentary (29±4y), 10 older trained (65±4y) and 11 older sedentary (67±5y). Trained individuals were recruited among regional class and world-class athletes. Features of hip and lower limb bone geometry including acetabular coverage and version, total and regional femoral torsion, and femoral and tibial lateral and frontal bowing, and frontal plane lower limb alignment were assessed using magnetic resonance imaging (MRI). Muscle function was assessed using mechanography, with peak vertical jump power and peak one-legged hop force recorded. Associations between age, training status and geometry were assessed using multiple linear regression, whilst associations between geometry and muscle function were assessed by linear mixed effects models with adjustment for age and training status. Results: Trained individuals had 2° (95%CI: 0.6° to 3.8°; $p<0.009$) higher femoral frontal bowing and older individuals had 2.2° (95%CI: 0.8 to 3.7°; $p<0.01$) higher lateral bowing. An age-by-training interaction indicated 4° (95%CI: 7.1° to 1.4°);$p=0.005$) greater acetabular version in trained individuals in the young group only . Lower limb geometry measures were not associated with muscle function (all $p > 0.05$). Conclusions: These results suggest that the ability to alter skeletal geometry via exercise in adulthood is limited. This reinforces the importance of exercise during development as a strategy to optimise life-long skeletal health. Whilst skeletal geometry is associated with clinical outcomes and gait, it does not appear to be associated with peak muscle function


Femoral frontal bowing (°)
12 14 16 18 20 22 24
Trained Sedentary Trained Sedentary
Femoral lateral bowing (°)
-2 0 2 4 6 8
Trained Sedentary Trained Sedentary
Acetabular version(°)
10 15 20 25
Trained Sedentary Trained Sedentary
Young
Old

MBOC1.2

Catastrophic vertebral fracture cascade in boys with Duchenne muscular dystrophy on long-term glucocorticoids

Dr Nicola Crabtree, Dr Vrinda Saraff, Dr Suma Uday, Ms Jenny Anscombe, Ms Tahera Elwell, Professor Nicholas Shaw

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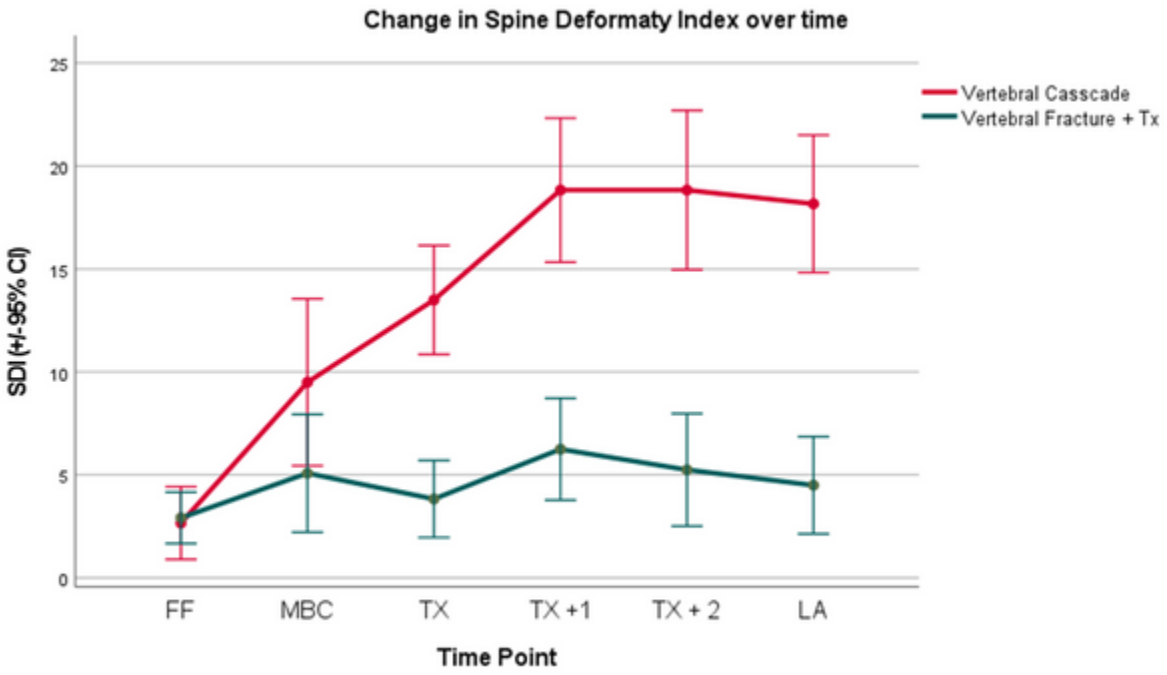
Abstract

Vertebral fractures are a recognised sequelae in boys with DMD managed with oral glucocorticoids(GC). In severe cases, boys can develop vertebral fractures throughout their entire spine, a scenario referred to as “vertebral fracture cascade (VFC)”. Assessing the evolution of VFC may advance our understanding and improve the prediction and prevention of these often-painful vertebral fractures (VF).

The aim of this work was to conduct a retrospective review of VF in boys with DMD undergoing routine bone health assessments. Number and grade of VF was documented using the spine deformity index (SDI) in conjunction with a modified Genant semi-quantitative VF assessment tool. Clinical details including GC regime, mobility, pubertal status, bisphosphonate treatment and bone density were collected in boys with at least 4 years follow-up and at least one VF. Results are presented at the time of; first fracture(FF), referral to the metabolic bone clinic (MBC), treatment initiation (TX), 1 (TX1) & 2 (TX2) years post and last assessment (LA)

Fifty boys (mean age = 9.4 years) met the criteria and were grouped accordingly: vertebral fracture cascade on bisphosphonate treatment (VFC (n=7)), vertebral fracture on bisphosphonate treatment (VFTx (n=22)), & untreated vertebral fractures (UVF (n=21). At FF there were no significant differences in age, height, weight, BMI SDS or SDI. However, VFC & VFTx boys had significantly lower bone density. (BMAD Z-Score = -1.1(0.7), -1.2 (0.9) & -0.8(1.0) for VFC, VFTx & UVF, respectively). Between the groups significant differences were noted for steroid regime (daily steroids: VFC =100%, VFTx =41% & UVF =21%) and pubertal status (pubertal delay: VFC =100%, VFTx =14% & UVF =14%) but not for mobility (immobile: VFC =86%, VFTx =73% & UVF =71%). The average time between FF and MBC and between MBC and TX was 20 & 5 months, respectively. Between FF and TX1 there was a highly significant interaction between time and SDI, such that after one year of bisphosphonate treatment VFC boys had a mean SDI of 18 compared to 6 for VFTx boys. This difference persisted over time (Figure below).



These data suggest that early initiation with bisphosphonate therapy, timely assessment and if appropriate, intervention in pubertal development and optimisation of steroid regime, may reduce the probability of a catastrophic vertebral fracture cascade.

MBOC1.3

ANTI-RANKL THERAPY PREVENTS GLUCOCORTICOID INDUCED BONE LOSS AND IMPROVES MUSCLE FUNCTION IN DUCHENNE MUSCULAR DYSTROPHY

Dr. Soher Jayash[1], Ms Dounia Hamoudi[2], Dr. Louise Stephen[1], Dr. Shuko Joseph[3], Dr. Anteneh Argaw[2], Dr. Sze Choong Wong[4], Prof. Jérôme Frenette[2], Prof. Colin Farquharson[1]

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Abstract

Background: Duchenne muscular dystrophy (DMD) is a rare genetic condition characterised by progressive muscle wasting. Whilst there is still no cure, steroids are known to slow muscle wasting but their use results in bone loss. Bisphosphonates are the standard treatment to prevent bone loss in glucocorticoid-treated DMD. Targeting RANKL may have advantages in DMD by ameliorating dystrophic skeletal muscle function in addition to their anti-resorptive properties. However, the potential effects of anti-RANKL treatment and upon discontinuation in glucocorticoid-induced animal models of DMD is unknown and needed prior to exploration in the clinical research setting.

Methods: Dystrophin-deficient Dmdmdx (mdx) mice (5-week-old) were treated with IgG (control), glucocorticoid [deflazacort; 1.2mg/kg/day], anti-mouse RANKL [4mg/kg/3d] or both deflazacort and anti-RANKL for 8-weeks. (n=5-8 mice/group). Bone and muscle structure and function were determined. In a second study, we determined the impact on the structure and biomechanical properties of bone in deflazacort-treated mdx mice after discontinuation of anti-RANKL, followed by one or two doses of intravenous bisphosphonate [zoledronate; 0.1mg/kg].

Results: Anti-RANKL and deflazacort improved grip force performance of mdx mice (both $p<0.05$) although a synergistic effect of both treatments was not noted. However, anti-RANKL alone improved ex vivo contractile properties of dystrophic muscles ($p<0.05$). This functional improvement was associated with a reduction in muscle damage, fibrosis, serum creatine kinase concentrations, and inflammatory cell number (all $p<0.01$). Micro-CT analysis of lumbar vertebrae revealed higher trabecular BV/TV ($p<0.05$) and lower trabecular separation ($p<0.001$) with anti-RANKL treatment compared to deflazacort only. In the second study, when compared with bones from deflazacort-treated mice, anti-RANKL treatment followed by one or two doses of zoledronate for 8 weeks resulted in higher cortical BMD ($p<0.01$), trabecular BV/TV, thickness and number (all $p<0.01$), failure load ($p<0.05$), work to failure ($p<0.01$) and max and yield load ($p<0.05$) of lumbar vertebrae. Similar results were obtained with the tibiae where cortical BMD ($p<0.001$), trabecular number ($p<0.05$), failure load ($p<0.01$) and stiffness ($p<0.05$) were higher with deflazacort followed by anti-RANKL treatment relative to deflazacort alone. These differences were absent in mice whose anti-RANKL treatment was discontinued for 8-weeks and not followed by zoledronate.

Conclusions: Anti-RANKL treatment improves bone mass in glucocorticoid-treated mdx mice. Both anti-RANKL and glucocorticoid treatments improved muscle function in mdx mice. Bisphosphonate administration after anti-RANKL discontinuation may be required to inhibit a rebound acceleration of

bone turnover and reduce fracture risk, despite the low bone turnover state in glucocorticoid-induced osteoporosis.

RBDOC1.1

Treatment with an AAV vector expressing ENPP1-Fc prevents ectopic tissue calcification and restores bone parameters in ENPP1 deficient mice

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Abstract

Ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) is the major enzyme that generates extracellular pyrophosphate (PPi), an inorganic metabolite with potent anti- calcification activity. Loss-of-function mutations lead to a state of ENPP1 Deficiency and hypopyrophosphatemia, which in turn cause extensive calcification of the heart, arteries, and kidney, impaired growth and early mortality. The acute infantile form of ENPP1 Deficiency (referred to as Generalized Arterial Calcification of Infancy (GACI)) presents with pathological mineralization in multiple tissues, arterial stenoses, growth impairment, and a 50% mortality rate in the first year of life. Currently, there are no approved treatment for this rare disease.

We developed an adeno-associated virus vector expressing a modified human ENPP1-Fc under liver-specific promoter (AAV-ENPP1) as a potential therapy for ENPP1 Deficiency.

A dose-response study was performed to investigate the pharmacological effects of AAV-ENPP1 in Enpp1asj-2J/asj-2J mice, a murine model of ENPP1 deficiency. These mice have a large deletion/insertion mutation in the Enpp1 gene and closely mimic the clinical phenotype of patients. At ~ 2 weeks of age, Enpp1asj-2J/asj-2J mice were intravenously dosed either with vehicle or one dose of AAV-ENPP1 at 1x1010 (low), 1x1012 (medium), 1x1014 (high) vg/kg. Clinically relevant endpoints, including plasma PPi levels, tissue calcium content assessed by a colorimetric assay and micro-CT analysis of bone were measured after 10 weeks. Significant increase in tissue calcium levels were recorded in aorta, kidneys, spleen and vibrissae (muzzle skin) in the vehicle-treated group. At 12 weeks of age, vehicle-treated mutant mice also showed profound defects in femora, including lower trabecular number and thickness in females, thinner cortical bone and lower growth plate volume. One single intravenous injection of the AAV vector resulted in a robust and durable increase in plasma ENPP1 activity for the duration of the study. AAV administration also led to a dose dependent increase in PPi levels. Treatment at high dose prevented pathological calcification in all the tested organs and restored bone parameters in Enpp1asj-2J/asj-2J mice. Those findings demonstrate the potential of this gene therapy to treat ENPP1 Deficiency.

RBDOC1.2

An appraisal of a spine screening programme for vertebral fracture and its management in Duchenne Muscular Dystrophy: Is it time to reconsider the threshold for treatment with bisphosphonate?

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Abstract

Objectives:

To describe the outcome of a spine screening programme for vertebral fracture [VF] in Duchenne muscular dystrophy [DMD] and management with bisphosphonate.

Methods:

Annual lateral spine imaging was introduced in the neuromuscular clinic in 2015. Lateral spine radiographs were reported by a single observer using the semi-quantitative genant method for this study.

Results:

Nineteen boys with DMD on glucocorticoid commenced bisphosphonate [18 Zoledronate, 1 Pamidronate] at a median age of 12.6 years (Range 7.2, 18.5) from 2016-2021. 13/19 (68%) had painful VF, 4/13 (31%) had a previous fragility long bone fracture. 4/19 (21%) had VF with no back-pain of whom one had a previous fragility long bone fracture.

2/19 (11%) had reduction in vertebral height with back-pain but did not meet radiological criteria for VF, one of whom had a previous long bone fracture.

4/19 (21%) had VF on first imaging and commenced on bisphosphonate: two with severe but asymptomatic VFs, one with mild VFs and back-pain; another with moderate VFs and back-pain.

5/19 (26%) had VF on first imaging but commenced treatment with follow-up. All five had no back-pain at first imaging: 3/5 (60%) with mild VF and 2/5 (40%) with moderate VF. 2/5 with mild VF showed progression with follow-up [increasing number/severity of VF] and developed back-pain. 1/5 showed stability of moderate VFs but developed back-pain. Two others showed progression with increasing number/severity of VF but remained asymptomatic.

8/19 (42%) had no VF on first imaging but developed mild VF with follow-up. 7/8 remained asymptomatic upon identification of mild VFs. With further follow-up and at initiation of bisphosphonates, all developed back-pain with increasing number and severity of VFs.

Therefore, in our experience, 10/13 (77%) boys who were not treated with bisphosphonate in line with current international recommendations, with asymptomatic mild VF showed progression of VFs, with 9/10 developing back pain with follow-up.

Conclusion:

Over three-quarter of boys with DMD with asymptomatic mild VF who were not treated with bisphosphonate in accordance with international recommendations showed progression of VFs and developed back pain. We believe that the threshold for initiation of bisphosphonates in DMD should now be reconsidered. Given the challenges of identification of mild VFs, our data calls for the consideration of prophylactic treatment in a sub-group of boys with highest risk for VF. Research into clinical factors that predict development of VF in these boys is now needed.

RBDOC1.3

Longitudinal assessment of physical function in adults with X-linked hypophosphatemia following initiation of burosumab therapy

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Abstract

Background: X-linked hypophosphatemia (XLH) is a rare hereditary disorder of phosphate metabolism mediated by elevated levels of fibroblast growth factor 23 (FGF23). In addition to skeletal effects of this condition, large deficits in multiple components of physical function including lower limb power, functional capacity and mobility have previously been reported in adults with XLH. Burosumab is a monoclonal antibody to FGF23 shown to be effective for skeletal complications when used as a therapy for XLH, but its effects on physical function are unknown.

Purpose: Therefore, we assessed multiple components of physical function in adults with XLH prior to and following six months of burosumab therapy.

Methods: Nine adults (six female, mean age of 40.6±16.5 years) were recruited from specialist centres in London and Bristol. During clinical visits for initial burosumab treatment and at six-month follow-up, physical function assessments were performed. In detail, lower limb function (jump power) was assessed by mechanography, mobility by short physical performance battery (SPPB), functional capacity by six-minute walk test (6MWT) and upper limb function by handgrip strength. Differences between baseline and follow-up were assessed with paired t-tests.

Results: Lower limb power increased by 13% (P=0.049) from baseline to six months. Whilst SPPB score increased by 20% the statistical evidence was weaker (P=0.062), and there was no evidence to support increases in 6MWT (+7.6±21.5%, P=0.291) or handgrip strength (+6.0±10.4%, P=0.131).

Conclusions: Burosumab treatment is associated with improvements in lower limb function and mobility, whereas deficits in 6MWT performance appear little affected. This was a small-scale, observational study hence study power and robustness of study design were limited. Future larger-scale and controlled studies should be performed to further investigate the effects of burosumab therapy on physical function.

OC1.1

Functional validation of the osteoporosis GWAS candidate FUBP3 in knockout mice

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Abstract

Osteoporosis and fragility fractures affect millions of people, resulting in morbidity, mortality and enormous healthcare costs. Reduced bone mineral density (BMD) is a major risk factor for fracture and is a highly heritable trait. We and others have used Genome Wide Association Studies (GWAS) to reproducibly associate non-coding polymorphisms at the 9q34.11 locus with BMD and height. The closest gene is FUBP3, a DNA and RNA binding protein and transcriptional activator of c-myc which is expressed in osteoblasts and osteoclasts, but with no previously confirmed role in bone biology. We hypothesised that FUBP3 was the causative gene underlying the association with BMD and height at 9q34.11 and investigated its functional role in the skeleton in vivo.

FUBP3 deficient mice (n=6-9 per sex, per genotype, per age, local ethical approval) had short stature with correspondingly short caudal vertebrae (3-7% reduction vs. WT, $p<0.01$, ANOVA). Prenatal skeletal development was normal with no defects in intramembranous or endochondral ossification on alcian blue and alizarin red staining of P1 specimens. In keeping with their short stature, FUBP3 deficient mice had a smaller overall width of the tibial growth plate (16.7% reduction vs. WT, $p<0.01$, t-test) with smaller hypertrophic ($p<0.001$) and proliferative ($p<0.001$) zones. However, growth plate zones remained proportionate with no evidence of abnormal chondrocyte differentiation or growth plate disorganisation.

Bone mineral content, measured by x-ray microradiography, was decreased in femurs (vs. WT, $p<0.05$, Kolmogorov-Smirnov test) and caudal vertebrae (vs. WT, $p<0.01$, Kolmogorov-Smirnov test), but markedly decreased in lumbar vertebrae (vs. WT, $p<0.001$, Kolmogorov-Smirnov test). Bone trabecular microarchitecture was altered in adult FUBP3 deficient mice with reduced femoral and lumbar vertebral trabecular thickness (5-10% reduction vs. WT, $p<0.05$, t-test) and trabecular BMD (vs. WT, $p<0.05$, t-test). FUBP3 deficient femurs and lumbar vertebrae were weak, with reduced femoral maximum load on three point bend testing (9-24% reduction vs. WT, $p<0.05$) and reduced yield load (15-23% reduction vs. WT, $p<0.05$, t-test) and maximum load (12-20% reduction vs. WT, $p<0.05$, t-test) on lumbar vertebral compression. Cortical bone parameters, including cortical porosity, were not significantly different between FUBP3 deficient and wild type mice. Neither examination of calcein double labelled lumbar vertebral sections nor TRAP stained femoral sections revealed an alteration in osteoblastic bone formation or osteoclastic bone resorption parameters .

Together, these data provide the first demonstration of a functional role for FUBP3 in the skeleton and confirm FUBP3 as the causative gene at the 9q34.11 GWAS locus.

OC1.2

Characterising the heterogeneity of the adult periosteum by single-cell RNA sequencing: towards the identification of a periosteal stem cell

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Abstract

The periosteum plays a crucial role in bone homeostasis and repair through its resident skeletal stem/progenitor cell (SSPC). The adult periosteum contains cells with mesenchymal stromal cell (MSC) behaviour; expressing MSC markers and demonstrating capabilities of forming cells of osteogenic, chondrogenic and adipogenic lineages both *in vivo* and *in vitro* (Roberts et al., 2014). Despite this, specific surface markers to prospectively isolate stem cells from the periosteum are lacking, with contradictory data surrounding genetic markers of periosteal SSPCs. Indeed, recently both Cathepsin K and CD200 have been postulated to identify periosteal SSPCs. Additionally, the transcription factor Prrx1 has been proposed as a marker of the osteochondrogenic cell population within periosteal tissue. Using a novel *Prrx1eGFP* transgenic mouse in combination with single-cell RNA sequencing (scRNASeq) we aim to define the heterogeneity of the adult periosteum and candidate markers of SSPCs.

Periosteal GFP-positive and negative populations were sorted to ensure representation of rare cells within the analysis. 20,000 cells were processed with the Chromium Single Cell Gene Expression Analysis (10x Genomics). Cell ranger was used to analyse the raw data. Loupe software was then used to visualise the processed results. On average, the read count for each cell within the GFP-positive sample was 19,013, resulting in approximately 2,285 genes per cells. Within the GFP-negative sample, the average read count per cells was 23,873, resulting in approximately 1,305 genes per cells. When the dataset was combined, 10 cell clusters could be identified, with the GFP-positive cells distinct from GFP-negative. These clusters contained separated populations expressing genes suggestive of unipotent osteogenic progenitors, bipotent osteo-chondroprogenitors and SSPCs within the *Prrx1eGFP*-positive subpopulation.

Interestingly, previously suggested markers (including cathepsin K) showed widespread expression throughout the 6 *Prrx1GFP*-positive clusters, indicating that these markers are no more specific than Prrx1. Subsequently, we identified a putative SSPC cluster and uncovered a plausible selection strategy based on surface markers. This study not only highlights the heterogeneity of the adult periosteum, but also sheds further light on the identity of the stem cell component of this remarkable tissue. This selection strategy could aid in the identification of a human periosteal SSPC for applications towards bone regeneration strategies and disease modelling.

OC1.3

Describing the genetic architecture of hip shape: findings from UK Biobank

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Abstract

Background

Hip shape is an important risk factor for hip osteoarthritis (HOA). A previous genome-wide association study (GWAS) identified eight independent single nucleotide polymorphisms (SNPs) associated with hip shape, three of which were associated with hip osteoarthritis (HOA). In this study, we aimed to further characterise the genetic architecture of hip shape, based on a GWAS of hip shape derived from hip dual-energy X-ray absorptiometry (DXA) scans obtained in UK Biobank study (UKB).

Methods

Hip shape was quantified in left hip DXA images from UKB. GWAS was performed on ten orthogonal hip shape modes (HSMs) (which together explained 86.3% of the variability in hip shape) in 38,175 individuals, derived by statistical shape modelling (BoneFinder, University of Manchester). Analyses were adjusted for age, sex, genotyping chip, and 20 ancestry principal components. GCTA-COJO was implemented to identify conditionally independent SNPs at each genome-wide significant HSM-associated loci. LD score regression was used to estimate genetic correlations between HSMs, and between HSMs and other traits based on GWAS summary statistics for alpha angle (AA) (UKB), cam morphology (UKB), HOA (GO consortium), narrowest neck width (NNW) (UKB) and femoral head diameter (fHD) (UKB).

Results

We identified a total of 233 conditionally independent signals associated with the first ten HSMs at genome-wide significance ($p<5x10-8$) which mapped to 201 loci (based on a 1mb sliding window). SNP heritability (h2) ranged between 0.21-0.36 for all modes (except HSM5 h2 = 0.07). Two HSMs showed evidence of genetic correlation with AA at $P<0.05$, five with cam morphology, four with HOA, six with other HSMs, six with NNW, and eight with fHD (Figure 1). All loci identified in our previous GWAS were replicated, with those mapping to endochondral bone formation genes (e.g., *SOX9*, *PTHLH*, *RUNX1*,

FGFR4, and *HHIP*) associated with three or more HSMs. Of the 193 novel loci identified, 12 were related to two or more HSMs, 10 were related to HOA, 13 to fHD, and 21 to NNW.

Conclusions

We report the largest hip shape GWAS to date, identifying 233 conditionally independent signals mapping to 201 loci. All previously reported hip shape SNPs were replicated in our study. Loci mapping to HSMs were also related to conventional hip geometry measures, and to HOA, in keeping with the evidence of a genetic correlation between these traits. Further investigations are in progress to determine the genetic mechanisms linking hip shape, hip geometry and HOA.

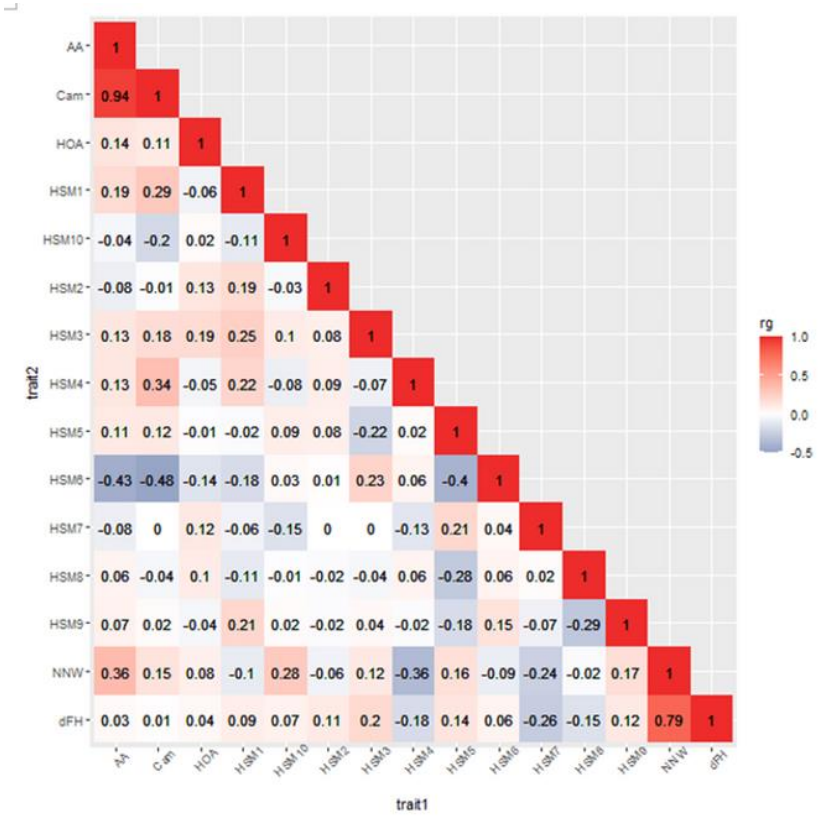

Figure 1. Genetic correlations between hip shape modes and other traits.

Abbreviations: HSM (hip shape mode), HOA (hip osteoarthritis), AA (alpha angle), NNW (narrowest neck width), dFH (diameter of the femoral head).

OC2.1

Towards a sclerosteosis treatment: effect of small molecule Wnt/β-catenin pathway inhibitors on osteoblast function

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Abstract

Sclerosteosis (OMIM accession number 269500) is an ultra-rare, severe autosomal recessive sclerosing skeletal dysplasia found predominantly among the South African Afrikaner population. Generalised skeletal overgrowth in patients, such as myself, results in acute clinical symptoms due to increased bone density and thicker trabeculae and cortices. The condition has no available treatment and is managed through difficult surgical interventions.

Sclerosteosis is caused by SOST gene mutations that result in loss of functional sclerostin. Sclerostin is primarily expressed by osteocytes and is a key regulator of bone formation. Indeed, it functions by antagonising canonical Wnt signalling in osteoblast lineage cells to reduce proliferation, alkaline phosphatase activity and mineralisation, and to increase apoptosis. Loss of sclerostin also leads to enhanced canonical Wnt signalling and decreased apoptosis in osteocytes. Taken together, any sclerosteosis therapeutic would need to recapitulate the key aspects of sclerostin function in osteoblasts and osteocytes, which is otherwise lacking in these patients

In our earlier work, recombinant sclerostin and various fusions thereof were investigated as possible sclerosteosis treatments. These proved effective in osteoprogenitor cells *in vitro* but showed limited efficacy in SOST knockout mice *in vivo* (1), suggesting that sclerostin replacement therapy would potentially lack efficacy as a sclerosteosis treatment. Interestingly, previous studies have shown that small molecule inhibitors of the canonical Wnt pathway modulate bone homeostasis and bone cell function. Herein, as a next step in the pursuit of a treatment for sclerosteosis, we explore the efficacy of such small molecule Wnt pathway inhibitors as an alternative approach to suppressing osteoblast function. Modulation of Wnt signalling via Porcupine O-Acyltransferase; or CDC Like Kinase 2 and Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A inhibition resulted in an abrogation of MC3T3-E1 (subclone 14; mouse pre-osteoblast) mineralisation. Importantly, inhibition of co-activator CREB binding protein/β-catenin-mediated signalling resulted in widespread cell toxicity at higher concentrations, with no effect on mineralisation at lower concentrations. Inhibition of Tankyrase 1/2 and interaction between β-catenin and Transcription Factor 4 did not affect mineralisation status of MC3T3-E1 cells. These data suggest certain small molecule Wnt inhibitors appear to modulate osteoblast function in an analogous fashion to sclerostin and as such may be candidates for a sclerosteosis therapeutic.

1. Dreyer et al., 2021. Recombinant sclerostin inhibits bone formation in vitro and in a mouse model of sclerosteosis. J. Orthop. Transl. 29, 134.

OC2.2

Sclerostin deficiency results in reduced osteoclastogenesis *in vitro*: a further mechanism for high bone mineral density?

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Abstract

Sclerostin, encoded by the SOST gene, is a negative regulator of bone formation and mediator of bone mechanotransduction. Sclerostin is secreted by mature osteocytes and binds to LRP4/5/6 receptors on osteoblasts to inhibit Wnt/β-catenin signalling. Mutations in the SOST gene, or in regulatory elements, cause an increase in osteoblast-mediated bone formation and result in increased bone mass, as seen in Sclerosteosis and Van Buchem disease. This high bone mass phenotype is replicated in SOST knockout (KO) mice. Interestingly, Romosozumab (anti-sclerostin antibody) treatment of osteoporotic patients produces a marked reduction in bone resorption markers along with the predicted robust induction of bone formation markers. This indicates that sclerostin has a dual role on both osteoclasts and osteoblasts.

Herein, we explore the *in vitro* potential of bone marrow progenitors from SOST KO mice in comparison to C57BL/6, with an aim to elucidate whether genetic loss of SOST affects osteoclastogenesis. Bone marrow progenitors were isolated from 6-week-old age/sex matched mice, due to SOST KO mice experiencing the most rapid gains in long bone mass between 1 and 2 months of age. One million cells from each mouse strain were cultured on dentine discs (5mm) for 7 days in the presence of RANKL and MCSF, with 2 days of acidification to promote resorption. TRAP staining enabled the assessment of osteoclast resorption and number. Osteoclasts, preosteoclasts and resorption area were quantified via image analysis.

Marrow from SOST KO mice yielded 65.5% fewer osteoclasts than C57BL/6 ($p<0.0001$). Furthermore, the area of preosteoclasts and total resorption area was reduced by 14.3% ($p<0.05$) and 52.7% ($p<0.0001$), respectively in the SOST KO compared to C57BL/6. Interestingly, *in vitro* treatment with recombinant sclerostin (0-500ng/ml) did not modulate osteoclast formation or function, indicating that the anti-osteoclastogenic role of SOST deletion is likely indirect. Indeed, treatment of ovariectomised rats with a sclerostin neutralising antibody has previously been shown to upregulate osteocyte OPG expression, resulting in a modified RANKL:OPG ratio. Our data indicate a clear reduction of *in vitro* osteoclastogenesis from SOST KO mice despite the absence of any osteocyte/osteoblast paracrine signals, which may indicate 'cell memory' from the *in vivo* environment. Although the precise mechanism for this phenomenon is unknown, it may be due to epigenetic regulation of osteoclast precursors and/or sclerostin modulation of bone marrow. This study sheds further light on the cellular basis of the extreme bone mass phenotype observed due to sclerostin deficiency.

OC3.1

Understanding periosteal osteochondral differentiation through the construction of microRNA-based protein interaction networks

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Abstract

Endochondral ossification involves the replacement of a hyaline cartilage template with bone and is the result of coordinated skeletal cell differentiation towards chondrocyte and osteoblast lineages. We have previously shown that humanized culture of human periosteum-derived progenitor cells (hPDCs) enhances osteochondrogenic cell vigour *in vitro*, and results in increased bone formation *in vivo*. Despite this striking effect the regulatory mechanisms involved in altering hPDC potency are not known. microRNAs (miRNAs) are small non-coding RNAs that contribute to multiple homeostatic and pathological processes, including stem cell fate decisions. This study aimed to construct a microRNA-mRNA regulatory network that underpinned hPDC osteochondrogenic fate.

hPDCs (p5, 6 donors, 18.6±8.9 years old, pooled, n=3) were cultured in humanized conditions (human serum [HS], 47 donors, pooled, n=3) or FBS. Differentially expressed miRNAs (DEMIs) were identified using the nCounter System and the Human miRNA Expression Assay, containing 800 human miRNAs (normalized to the geometric mean of the top 100 microRNAs). Subsequent analysis via TargetScan and PANTHER allowed the identification of putative target genes and regulated pathways, respectively. Target genes were cross-referenced with paired RNAseq data to identify high confidence miRNA targets. Finally, protein-protein interaction (PPI) networks were constructed using STRING to identify hub genes that may control osteochondrogenic fate determination. Functional analysis of selected hub genes was carried out *in vitro* and *in vivo*.

The above analysis identified 16 DEMIs, with miR-145-5p and miR-4454 being the top downregulated and upregulated miRNAs, respectively (2.17- and 3.45- fold in HS vs FBS; $p \leq 0.05$). *In silico* analysis of potential target genes revealed 1503 mRNA targets. Upon analysis and comparison to differentially expressed genes (DEGs) in a paired RNAseq dataset (≥2-fold; $p \leq 0.05$; 1319 genes), 122 DEGs were identified to common (4.5% overlap). Interestingly, FGFR3 was identified as a key hub gene within the PPI network, and was predicted in by pathway analysis. Functional analysis revealed that hPDCs with activated FGFR3 signalling displayed increased FGFR3 ($p \leq 0.01$) and SOX9 ($p \leq 0.0001$) expression when cultured under chondrogenic conditions. Furthermore, these cells formed bone with cartilaginous remnants when implanted subcutaneously in immunocompromised mice on calcium phosphate scaffolds which was not seen with control cells . Fundamentally, this workflow of combining miRNA analysis with RNAseq and *in silico* analysis has revealed a regulatory network controlling osteochondral differentiation from hPDCs. This network contains further potential mediators, which may have relevance to diseases of aberrant osteochondral differentiation such as osteoarthritis and fracture repair.

OC3.2

Sex differences in osteoblast matrix composition divergently impacts vascular endothelial cell behaviour.

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Abstract

Background. Physiological bone formation during development and repair is modulated by osteoblast (OB)-derived vascular endothelial growth factor (VEGF) in a sexually dimorphic manner in males and females [1]. While our previous findings have indicated that this sexual dimorphism is driven in part by the skeletal vasculature, the contributions of the OB-extracellular matrix (ECM) composition are yet to be defined. Herein, this study sought to investigate whether the ECM profiles of male and female OBs are distinct and if this leads to divergence in vascular cell behaviour.

Materials and Methods. OBs were isolated from 4-day old male and female C57BL/6 mice and cultured separately for 7 days before direct-contact co-culture with labelled bone marrow-derived endothelial cells (BMECs). BMEC survival on the OB-ECM was assessed by cell counts. Raman spectroscopy of individual male and female OBs (n=25 cells) was performed to characterise matrix composition and the extent of mineralisation. As a control, the impact of soluble factors on BMEC number was also assessed by treatment with male and female OB-conditioned media (CM).

Results. Following 24 hours of direct-contact co-culture with male OBs, BMEC numbers were 1.39-fold higher than in co-cultures with female OBs (P=0.005). Male and female OB-CM did not divergently affect BMEC number (P=0.53) thus this sexual divergence is due to matrix composition. Raman spectroscopy of OBs revealed divergence in amorphous calcium phosphate and carbonated apatite precursors of hydroxyapatite mineral, with males producing higher levels (3.22 and 1.33-fold, respectively) than female OBs. Collagen-specific proline and hydroxyproline levels were 1.52 and 2.12-fold higher in female versus male OB cultures, respectively. This correlated with sex-specific changes in the stability of the collagen helices being 1.41-fold higher in female versus male cultures, suggesting the male OBs are able to advance into the mineralisation phase while female OBs are primarily synthesising the collagenous matrix.

Conclusions. Defining the mechanisms regulating sex-specific OB-ECM production could offer a new therapeutic route to effectively target pathological skeletal angiogenesis in men and women. Defining sex differences in osteoblast cell function could also address health inequalities around gender that currently exist clinically.

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OC4.1

Cam morphology does not appear to cause osteoarthritis: findings from a Mendelian randomisation study

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Abstract

Background

Cam morphology, a bulging of the anterolateral aspect of the femoral head, has been associated with hip osteoarthritis (HOA) in conventional observational studies. It is postulated to cause HOA through impingement of the femoral head on the acetabulum, and surgical procedures to remove cam lesions are being developed with the aim of preventing HOA. However, the studies which provide the evidence of this association are liable to unmeasured confounding and reverse causation. To overcome this, we aimed to use Mendelian randomisation (MR) to establish whether a causal relationship exists between cam morphology and HOA.

Methods

Hip dual-energy X-ray absorptiometry (DXA) scans in UK Biobank (UKB) had alpha angle (AA), a measure of cam morphology, quantified using an automated approach. Two genome-wide association studies (GWASs) were performed, one of AA in UKB, with replication in the Rotterdam Study, and one of HOA in the Genetics of Osteoarthritis consortium. Genetic instruments of AA and HOA were identified by linkage disequilibrium clumping of GWAS summary statistics (p-value <5x10-8 & r2 <0.001). Bidirectional two-sample MR was conducted using the inverse variance weighted (IVW) method. Sensitivity analyses were done using MR Egger and weighted median (WM) approaches.

Findings

GWAS of AA (mean AA 47.8º, range 32-115º, n=38,173) identified 6 independent loci associated with AA, namely TGFA, TNFAIP8, TIAM2, LMX1B, SOX5 and UQCC1. The TNFAIP8 association signal colocalised with eQTL expression in degraded human cartilage. The GWAS of HOA (n=621,469) revealed 72 independent genetic loci. The 6 and 72 genetic instruments of AA and HOA, respectively were used in bidirectional MR. The results showed no consistent causal effect of AA on HOA but rather the reverse was seen, a genetic predisposition to HOA increases AA (Table 1).

Interpretation

GWAS of AA identified 6 independent loci all with biologically plausible roles in hip shape development. Our analysis suggests TNFAIP8, expressed only in degraded cartilage, contributes to the development of cam morphology following joint degeneration. MR analyses suggested that a genetic predisposition to HOA leads to cam morphology, whereas cam morphology does not appear to influence HOA risk. Therefore, targeting cam morphology as a way to prevent HOA is unlikely to be successful.

Table 1. Bi-directional MR results.

	AA vs HOA		HOA vs AA	
	OR [95% CI]	P	Beta [95% CI]	P
IVW	1.87 [0.94-3.72]	0.07	0.07 [0.04-0.11]	$3.08x10^{-06}$
MR Egger	0.01 [0.00-4.02]	0.21	0.16 [0.06-0.26]	$2.70x10^{-03}$
WM	1.27 [0.93-1.74]	0.14	0.05 [0.02-0.09]	$3.03x10^{-03}$

OC4.2

Physical activity volume and intensity distribution profile in relation to bone, lean and fat mass in children: The Physical Activity and Nutrition in Children Study

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Abstract

Background: Physical activity (PA) volume and the distribution of activity intensity may provide a novel comprehensive profile for bone mineral content (BMC), lean mass (LM), and fat mass (FM). This study aimed to assess the PA volume and intensity distribution profile with total-body-less-head BMC, LM, and FM in pre- and early-pubertal children.

Methods: This study utilised cross-sectional data from the Physical Activity and Nutrition in Children Study, an ongoing longitudinal study in a population sample of Finnish children (290 children (158 females) aged 9 to 11 years). Average-acceleration (a proxy metric of PA volume) and intensity-gradient (a metric of PA intensity distribution) were calculated from accelerometer-assessed PA. Linear regression was used to examine the associations of PA volume and intensity with dual-energy X-ray absorptiometry assessed total-body-less-head BMC, LM, and FM.

Results: In females, PA volume was positively associated with BMC when adjusting for LM and FM (unstandardised regression coefficient (ß) = 0.26, p = 0.035), though not when adjusting for LM only. In females, PA volume was not associated with LM or FM. PA intensity was not associated with any outcome in females (Figure 1). In males, PA volume was positively associated with BMC when adjusting for LM and FM (ß = 0.47, p = 0.002), though not when adjusting for LM only. In males, PA volume was positively associated with LM (ß = 7.33, p = 0.014), and negatively associated with FM (ß = -20.62, p = 0.013). PA intensity was negatively associated with BMC in males (ß = -0.13, p = 0.015), and was not associated with LM or FM (Figure 1). There was no interaction between PA volume and intensity in females or males for any outcomes. The high-volume (> sex-specific mean for PA volume) physical activity profiles included at least 2 minutes of high-intensity activity, with 2 hours of activity equivalent to slow walking, and several hours of light activity.

Conclusions: A higher volume of PA may be associated with improved BMC in females and males, and with improved LM and reduced FM in males. Adjusting for LM and FM altered the relationships between PA with BMC, emphasising the importance of considering LM and FM alongside bone. The high-volume

PA profiles included short periods of high intensity activity, with several hours of light activity, suggesting that increasing PA volume at any intensity may be crucial for the development of bone health in pre- and early-pubertal children.

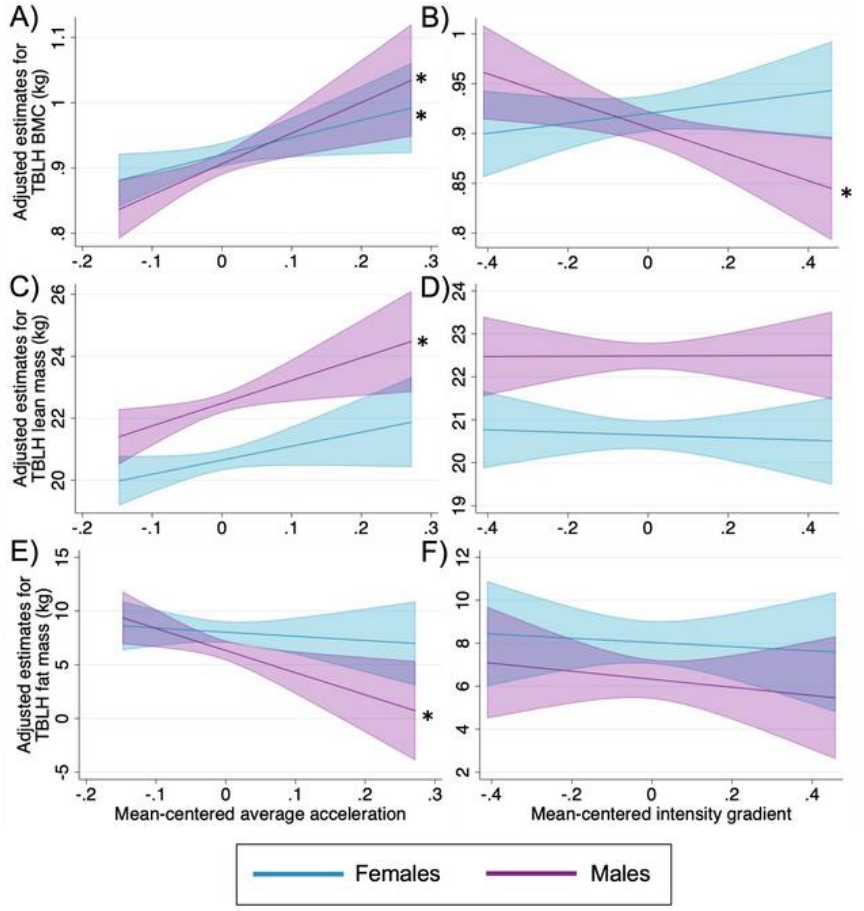

Figure 1. Main associations between PA volume (average-acceleration) and PA intensity (intensity-gradient) with TBLH BMC, lean mass and fat mass.
*indicates statistical significance ($p < 0.05$)
Values (95% confidence intervals) are predicted for a female and a male with mean levels of covariates. All models are adjusted for age, stature, pubertal status, wear time, alternate activity metric and the product term for average acceleration by intensity gradient. A and B are additionally adjusted for lean mass and fat mass, C and D are additionally adjusted for fat mass, and E and F are additionally adjusted for lean mass.

OC5.1

The low-density lipoprotein receptor-related protein 1 is essential for skeletal development.

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Abstract

The low-density lipoprotein receptor-related protein 1 (LRP1) is a cell-surface receptor expressed in various tissues. LRP1 mediates clathrin-dependent endocytosis of a variety of molecules and modulates cellular signalling pathways. Global deletion of the *LRP1* gene in mice results in early embryonic lethality indicating a critical, but as yet undefined role of LRP1 in development. We have knocked out LRP1 in progenitor cells in early limb bud and in a subset of craniofacial mesenchyme. The homozygote (KO) mice were born but showed alteration in both intramembranous and endochondral bone formation, the most obvious is "crawling" gait and reduced locomotor activity. Strikingly, *in vivo* microCT scan imaging of 2-weeks old mice revealed that the KO long bones were markedly thicker, shorter and twisted with much delayed secondary centres (Fig 1A). The KO did not present a defined acetabulum sockets nor patella formation compared with WT mice. The 14-weeks old KO mice

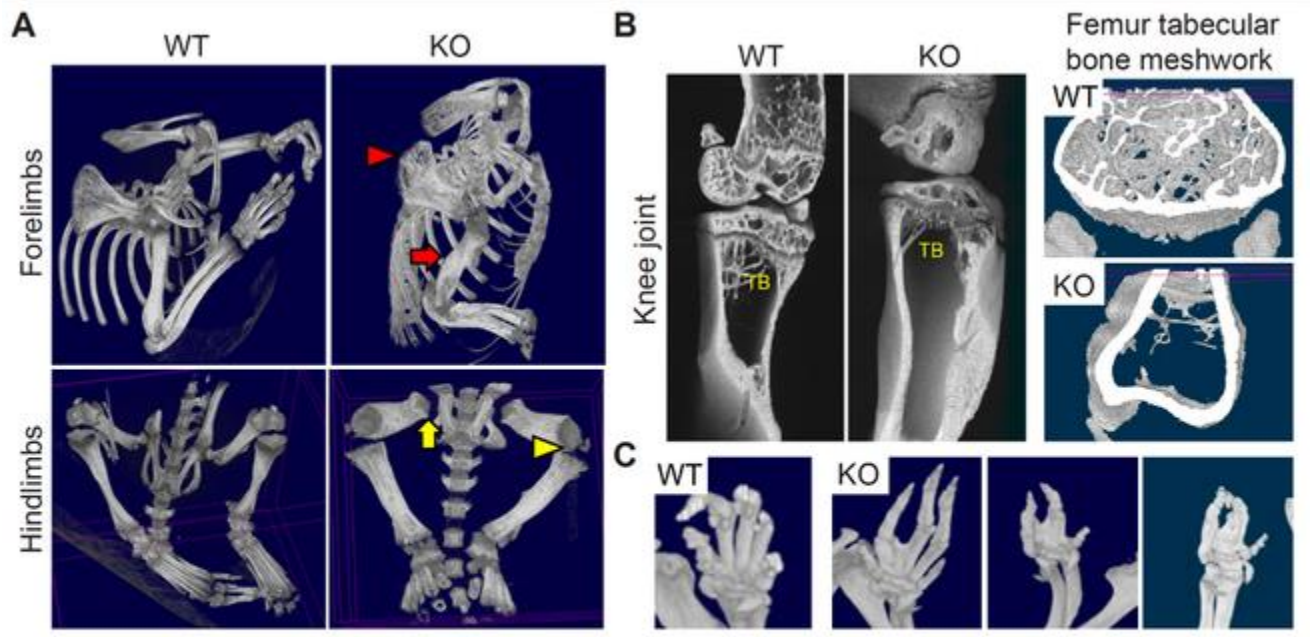

Figure 1. Striking skeletal abnormalities in postnatal LRP1Prx1 mice.
A and C, Representative images of in vivo X-ray analysis of 2-weeks old limbs (*A*) or 14-weeks old hand phalanges. Red arrow heads, red arrow, yellow arrow heads and yellow arrows indicate shoulder blades, long bones, knee and hip joints in KO mice, respectively. *B*, Representative images of high resolution X-ray analysis of 14-weeks old tibia.

exhibit a substantial reduction in trabecular bone density with virtual absence of primary spongiosa (Fig 1B). In some KO mice, we found fused phalanges (Fig 1C). These defects still were persisted up to 14-

weeks old KO mice. Histological analysis further revealed that impaired cartilage articulation and cavitation with much wider growth plate, unorganised columnar chondrocytes and altered proteoglycan contents in KO mice. On the other hand, our recent secretome and co-immunoprecipitation studies uncovered the chondrocyte specific LRP1 ligandome consisting of more than 50 novel ligands including WNT5A and WNT11, key players for the non-canonical Wnt pathway. Together, these results show that LRP1 plays a critical role in skeletal formation possibly by regulating Wnt signalling through endocytosis of the WNTs.

OC5.2

Targeting osteoblasts enhances Karonudib toxicity in myeloma

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Abstract

Rationale and hypothesis: Multiple myeloma is an incurable bone marrow cancer. Osteoblasts promote myeloma dormancy, and this involves the receptor tyrosine kinase AXL. Dormant myeloma cells are mitotically quiescent, making most chemotherapeutics ineffective. We hypothesised that the MTH1 inhibitor, Karonudib, would be an effective anti-myeloma treatment and toxicity would be enhanced by targeting osteoblasts.

Objectives: To determine if Karonudib is an effective myeloma treatment and if toxicity is improved with osteoblast-targeting agents.

Methodology: Myeloma cell lines were treated with Karonudib (1nM-5µM) alone or with standard-of-care (SOC) chemotherapeutics, viability was assessed by resazurin assays. DiD-labelled U266-GFP-luc myeloma cells were co-cultured with MC3T3-E1 osteoblasts and treated with Karonudib ± SD-208 (bone anabolic) or AXL inhibitors (Carbozantinib, R428). After 14 days, apoptosis (Annexin V/PI) and DiD retention (identifying mitotically quiescent cells) were analysed by flow cytometry. Mice with aggressive JJN3-GFP-luc tumours, were treated with Karonudib 1 week post-inoculation for 2 weeks. Mice with DiD-labelled U266-GFP-luc tumours were treated with SOC±Karonudib from 8 weeks (after development of high tumour burden). Tumours were analysed by bioluminescence and FACS.

Results: Karonudib reduced myeloma cell viability at nM concentrations and had an additive effect with SOC. In myeloma-osteoblast co-cultures, Karonudib reduced myeloma viability and induced apoptosis. SD-208 and AXL inhibitors had no effect on myeloma cells alone, but when combined with Karonudib viability was further reduced and virtually all remaining cells were apoptotic. Furthermore, apoptosis was induced in DiD+ cells with Karonudib+SD-208/Cabozantinib/R428, suggesting combined therapy killed dormant cells. In mice, Karonudib reduced or completely regressed JJN3-GFP-luc tumours. In U266-GFP-luc-bearing mice, Karonudib delayed relapse beyond SOC and reduced DiD+ dormant myeloma cells.

Conclusion: Karonudib is a potent anti-myeloma agent and osteoblast-targeting agents enhance toxicity in vitro. Thus, targeting osteoblasts may be an effective strategy for eradicating myeloma in a step towards finding curative treatment for myeloma patients.

OCCA1

Automated measurement of size of spinal curve in population-based cohorts: validation of a method based on total body dual energy X-ray absorptiometry scans

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Abstract

Background: Scoliosis may progress (requiring surgery) but risk factors for progression are little understood due to a lack of population-based research as radiographs cannot be performed on entire populations due to high levels of radiation. To address this we have developed, validated and automated a method for identifying scoliosis from total body dual energy X-ray absorptiometry (DXA) scans for research purposes. Our method is accurate and valid for identification of the presence or absence of scoliosis. To allow full utilisation of population-based research cohorts with clinical applicability, automation of curve size measurement is required to identify those whose spinal curves are progressing and clinical predictors of this.

Aim: To automate the measurement of size of spinal curvature from total body DXA scans using machine learning techniques.

Methods: We utilised total body DXA scans from 4657 participants from the Avon Longitudinal Study of Parents and Children (ALSPAC) aged 15. The manually annotated scans were randomised into a training, validation and test set and automation developed. Statistical validation of the output included the coefficient of variation and Bland Altman Limits of Agreement. Automation was run on 3680 non-annotated scans from ALSPAC aged 17. Clinical validation of the aged 17 output included assessment of association with physical activity, body composition and pain, followed by comparison to previous literature.

Results: At age 15, the mean difference between manual curve size measurement and the automation was less than1o, and 61.3% of manual vs automated readings fell within 5o and 90.4% within 10o. Coefficient of variation was 25.4%. At age 17, the automation identified 8.9% of females and 5.1% of males with scoliosis. Expected direction of associations were seen between physical activity, height, serum adiponectin and scoliosis curve size. In females, the expected negative associations were seen between body weight/BMI and size of curve. The opposite direction of association was seen in males. In females, positive associations were identified between automated curve size and current pain intensity.

Conclusion: We have developed a fully automated method of measurement of the size of scoliotic curve from total body DXA scans for research purposes. Machine learning technique has reasonable agreement with manual methods for curve size quantification, and appears valid. It can process 100,000 scans quickly, and is likely to revolutionise scoliosis research, allowing identification of clinical and

genetic predictors of curve initiation and progression through enabling the scoliosis phenotype to be inserted into population-based research cohorts worldwide.

OCCA2

Computer vision and machine learning of bone microarchitecture can improve the fracture risk prediction provided by DXA and clinical risk factors

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Abstract

Background

Traditional analysis of High Resolution peripheral Quantitative Computed Tomography (HR-pQCT) images results in a multitude of cortical and trabecular parameters which would be potentially cumbersome to interpret for clinicians compared to user-friendly tools such as FRAX®. A computer vision approach, where the entire scan is 'read' by a computer algorithm to ascertain fracture risk, would be far simpler. Thus, we investigated whether a computer vision and machine learning technique could improve the current methods of assessing fracture risk.

Methods

This study was nested in the Hertfordshire Cohort Study, a group of community-dwelling older adults. Participants attended research visits at which height and weight were measured; fracture history was determined via self-report and vertebral fracture assessment. Bone microarchitecture was assessed via HR-pQCT scans of the non-dominant distal tibia (Scanco XtremeCT) and bone mineral density measurement and lateral vertebral assessment were performed using dual X-ray absorptiometry (DXA) (Lunar Prodigy Advanced). Images were cropped and pre-processed and texture analysis was performed using a 3-dimensional local binary patterns method. These analyses, together with age, sex, height, weight, BMI, and dietary calcium, were used in the random-forest classification algorithm. Receiver operating characteristic (ROC) analysis was used to compare fracture risk identification methods.

Results

Overall, 247 males and 149 females were included in this study with a mean age of approximately 76 years. Using clinical risk factors alone gave an area under the curve (AUC) of 0.70 (95% CI: 0.56-0.84), which improved to 0.71 (0.57-0.85) with the addition of DXA-measured BMD. The addition of the machine learning classifier to clinical risk factors and DXA-measured BMD lead to an improved AUC of 0.90 (0.83-0.96) with a sensitivity of 0.83 and specificity of 0.74.

Conclusions

The results of this preliminary work demonstrate that using a 3-dimensional computer vision method to HR-pQCT scanning can enhance the identification of those at risk of fracture beyond that afforded by clinical risk factors and DXA-measured BMD. This approach has the potential to make the information offered by HR-pQCT more accessible and applicable to healthcare professionals in the clinic if the technology becomes more widely available. Whilst these findings require replication in other cohorts, they are an early indicator that the application of a machine learning technique to bone microarchitecture could improve fracture prediction and osteoporosis care.

OCCA3

Multiple factors predict skeletal maturity deviation in Zimbabwean children and adolescents living with HIV

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Abstract

Introduction

Skeletal maturity (SM) is measured as bone age (BA) on hand radiographs. Chronological age (CA) exceeding BA may indicate growth impairment and is common in HIV infection. We aimed to describe skeletal maturity deviation (SMD), defined as the offset between CA and BA, and determine its risk factors in Zimbabwean children/adolescents.

Methods

A cross-sectional study of children aged 8-16 years living with and without HIV was conducted. Children with HIV were recruited from HIV clinics at Parirenyatwa Hospital in Harare and children without HIV from schools in the same catchment area. Hand-wrist radiographs of the non-dominant hand were independently assessed by two observers, using the Tanner Whitehouse3 BA method. Paired sample student t-tests for continuous data and chi squared tests for categorical data compared participants' characteristics. Multivariate linear regression was used to examine associations between risk factors and SMD, stratified by HIV and sex.

Results

CA for boys with HIV (n=145) was 12.6±2.5years and for girls (n=134) was 12.5±2.5years, and for boys without HIV (n=147) was 12.4±2.5years and 12.7±2.5years for girls without HIV (n=144). Mean SMD±SD in children with HIV was 1.4±1.4years in boys and 1.1±1.3years in girls, and in those without HIV was 0.4±1.1years in boys and 0.2±1.2years in girls. BA was less than CA in 72% of participants.

In multivariate analyses, underweight was associated with SMD in children with HIV and boys without HIV, compared to those with normal BMI. Amongst girls with HIV, shorter durations of anti-retroviral therapy (ART) (<2 years) were associated with SMD 1.10 years (95%CI:0.14,2.05), as was unsuppressed

viral load (>1000 RNA copies/ml), SMD 0.59 (95%CI:0.01,1.17). Whilst amongst boys, older age at ART initiation was associated with SMD; those starting ART at age 8+ years had mean 1.22 years [95%CI:0.33,2.10) SMD. Furthermore, low socio-economic status was associated with SMD 0.59 (95%CI:0.14,1.04) compared to being in middle socio-economic position. In both boys with HIV and girls without HIV, very low dietary calcium intake (<150 mg/day) was associated with SMD of 0.67(95%CI:0.08,1.25) and 0.53 (95%CI:0.01,1.04) respectively, compared against those with low intake (150-299 mg/day).

Conclusion

In children with HIV, low calcium intake, delayed ART initiation, shorter ART duration, underweight and unsuppressed viral load were all independently associated with SMD, which may be indicative of delayed skeletal maturity. Underweight, low calcium intake and socio-economic deprivation similarly predict SMD in HIV negative children.

OCCA4

Up-to-date costs of hip fracture care in England and Wales identify substantial variation between hospitals; the REDUCE Study

Dr Petra Baji[1,2], Professor Celia L Gregson[1], Dr Rita Patel[1], Dr Muhammad K Javaid[3], Professor Yoav Ben-Shlomo[4], Professor Antony Johansen[5,6], Professor Andrew Judge[1,3,7], Dr Elsa M R Marques[1]

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Abstract

Background: Accurate and up-to-date costing of key healthcare indictors is important to inform cost-effectiveness analyses underpinning health policy decisions.

Objectives: To calculate NHS inpatient care costs in the first 120 days and the year following an index hip fracture, and understand regional variations in these costs.

Methods: The REDUCE study (REducing unwarranted variation in the Delivery of high qUality hip fraCture services) included all hip fracture admissions in England and Wales between 01/04/2016 and 31/03/2019, retrieving finished consultant episode (FCE) records from English Hospital Episodes Statistics and Patient Episode Database Wales in the year before and after an index fracture date. Healthcare Resource Groups (HRGs) were derived for all FCEs via the HRG4+2018/19 Reference Costs Grouper, and FCEs were valued using 2019–2020 reference costs imputing costs for codes invalid for grouping. Patient inpatient costs were estimated for the 120-days and 1-year before and after fracture and compared by hospital and region.

Results: Overall 178,757 patients in England and Wales had 288,032 FCEs in the year before and 647,257 in the year after an index hip fracture. Costs for 32,721 FCEs (3.5%) were imputed. Inpatient costs after index fracture were, on average, £14,465 per patient (95%CI £14,424 to £14,506) in the year after hip fracture; 86.7% of these costs occur within the first 120 days (mean 120 days cost=£12,547; 95%CI £12,516 to £12,578). One-year costs were higher in Wales (mean cost=£17,596; 95%CI £17,344 to £17,848) than in England (mean cost=£14,272; 95%CI £14,231 to £14,312). Mean costs varied by region, from average £13,580 (95%CI 13,442 to 13,718) in the East Midlands to £15,960 (95%CI £15,811 to £16,110) in London. Average costs varied substantially between hospitals from £10,787 to £22,721. The mean inpatient cost in the year prior to the index hip fracture was £2,560 (95%CI £2,535 to £2,585), hence the incremental cost after the hip fracture was on average £11,905 per patient (95%CI 11,862 to 11,949). According to our estimates, the annual NHS inpatient burden of hip fracture was £639 million to £664 million in England and £50 million to £55 million in Wales from 2016/7 to 2018/9.

Conclusion: Inpatient costs in the year following a hip fracture are higher than previously reported and vary considerably between hospitals and regions within England and Wales. Such between-hospital variability is difficult to justify clinically and requires further investigation of organisational cost-drivers.

OCCA5

Is musculoskeletal hypermobility associated with adolescent idiopathic scoliosis? A cross-sectional study in the Avon Longitudinal Study of Parents and Children (ALSPAC)

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Abstract

Background

Adolescent idiopathic scoliosis (AIS) is most often diagnosed during puberty and patients are followed up until spinal curvature stabilises or progresses enough to require intervention. Understanding causes and predicting progression of scoliosis will strongly influence this management pathway. One potential predictor of progression is musculoskeletal hypermobility, but a recent systematic review (In Press) has highlighted a lack of population-based studies, use of non-validated measures of hypermobility and lack of adjustment for potential confounders such as BMI.

Methods

We utilised a population-based birth cohort (the Avon Longitudinal Study of Parents and Children, ALSPAC) to investigate the association between musculoskeletal hypermobility at aged 14 and scoliosis at aged 15 in 4225 individuals. Musculoskeletal hypermobility, the primary exposure, was measured using the Beighton score. Spinal curvature was identified using a validated DXA-based method. The primary outcome was yes/no scoliosis. Logistic regression was used to assess the relationship between musculoskeletal hypermobility and scoliosis. We investigated the influence of BMI, as ongoing work by another group suggests a sex difference in the association between adiposity and scoliosis.

Results

The prevalence of musculoskeletal hypermobility was 19.6%, and the prevalence of scoliosis was 5.0%. Both hypermobility and scoliosis were more common among females. Hypermobile individuals were 1.48x more likely to have scoliosis compared to those without musculoskeletal hypermobility (OR 1.48 (95% CI 1.08,2.03), p=0.02). The strength of association did not differ between males and females, although separate analysis for males and females was limited by lack of power (for males OR 1.23 (0.58, 2.62), p=0.60; for females OR 1.22 (0.85, 1.75), p=0.28). We stratified by BMI categories in males and females due to differing directions of association between BMI and hypermobility and BMI and scoliosis. This suggested that in females the relationship between hypermobility and scoliosis is strongest in underweight individuals, whereas in males is strongest in obese individuals.

Conclusions

These results indicate a positive association between musculoskeletal hypermobility and AIS, with BMI influencing this association differently between sexes. Even in this large cohort there is limited power, highlighting a need to combine datasets for future analyses. This would allow robust analysis of factors influencing the association between hypermobility and scoliosis, including BMI as we hypothesise. The next step is investigating the association between hypermobility and curve progression using scoliosis data at aged 17 and 24 in the ALSPAC cohort. Delineating this complex relationship could help to identify patients at greater risk of progression.

OCCA6

Case series of patients with rheumatic diseases on long-term methotrexate who developed multiple lower limb insufficiency fractures

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Abstract

Background: Methotrexate associated insufficiency fractures were originally described in the 1980s in paediatric patients receiving high-dose Methotrexate for leukaemia. Since then about 80 cases of insufficiency fractures in adult patients on long-term, low-dose Methotrexate have been reported and labelled as "Methotrexate osteopathy" (MTXO). Our aim is to expand the pool of reported cases, to characterise the clinical and radiological features and to assess the impact of Methotrexate discontinuation or continuation after the initial fracture.

Methods: Retrospective case note review of patients who were identified by searching the MRI reports for the term 'insufficiency fracture' in a large tertiary Rheumatology centre and of referred suspected MTXO cases. Patient demographics, clinical and radiological features including DXA scans were analysed.

Results: We identified 24 patients with insufficiency fractures taking long-term Methotrexate. The mean age at presentation with MTXO was 68 ± 9 years. Most patients (96%) were women and had rheumatoid arthritis (88%). The average BMI of the cohort was 25.6 ± 5.1 kg/m2. The average duration of Methotrexate use was 7.9 ± 5.3 years and only 2 of the patients were on long-term steroids at the time of diagnosis. The most common sites of fracture were the distal tibia (63%) and calcaneus (63%). Other frequent fracture sites included the proximal tibia, talus and distal femur. The majority of patients (71%) had more than one fracture and 14/18 patients who had bilateral imaging, were found to have bilateral fractures. 14 of the patients (58%) required an MRI to identify the fracture and there was an average delay of 5 ± 4 months between the initial presenting symptoms and imaging confirming a fracture. Osteoporosis was found in 82% (18/22) of patients and interestingly the average BMD T-score of the femoral neck (-2.8±0.9) was lower than that of the spine (-2.2±1.1). 12/15 (80%) patients who had not stopped Methotrexate after the initial fracture sustained another insufficiency fracture whilst continuing Methotrexate, as opposed to 3/16 (19%) patients in whom Methotrexate was stopped.

Conclusions: This is one of the largest case series of patients with MTXO with detailed information on the fracture location and underlying bone health. Methotrexate related insufficiency fractures most commonly occur in women with rheumatoid arthritis and underlying osteoporosis. MRI imaging is often

required to identify incomplete fractures. It is important to recognise Methotrexate as likely causative factor of these fractures as 80% of patients in whom Methotrexate was continued developed a subsequent insufficiency fracture.

GOC1

Lowering of circulating sclerostin may increase risk of atherosclerosis and its risk factors: evidence from a genome-wide association meta-analysis followed by Mendelian randomization

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Abstract

Background: Sclerostin inhibition is a new therapeutic approach for increasing bone mineral density (BMD) and lowering fracture risk in patients with osteoporosis. However, phase III trials of romosozumab, a first-in-class monoclonal anti-sclerostin antibody, provide inconsistent evidence regarding cardiovascular safety. This study aims to establish the propensity of sclerostin inhibition on atherosclerosis and its risk factors using genetic evidence.

Methods: A genome-wide association study (GWAS) meta-analysis was performed of circulating sclerostin levels in 33,961 European samples. Two-sample MR was used to examine causal effects of sclerostin lowering on 15 atherosclerosis-related diseases and risk factors, based on available GWASs. To provide valid genetic instruments for therapeutic inhibition of sclerostin, sclerostin GWAS signals were selected which were associated with opposing effects on circulating sclerostin and estimated bone mineral density (eBMD).

Results: 18 conditional independent variants within 15 genomic loci were associated with circulating sclerostin. These included four directionally opposite signals for sclerostin levels and eBMD, namely a novel cis signal in SOST, rs66838809; a trans signal, rs215223, in B4GALNT3 reported in our previous sclerostin GWAS; rs7143806 in the RIN3 region, a reported BMD gene; and rs28929474 in the SERPINA1 region, a SNP reported to be associated with alpha-1 anti-trypsin deficiency. MR combining cis (SOST) and trans (B4GALNT3, RIN3 and SERPINA1) SNPs suggested that lower sclerostin increased hypertension risk (odds ratio [OR]=1.09, 95%CI=1.04 to 1.15). In contrast, bi-directional analyses revealed little effect of hypertension on sclerostin (b=0.08, 95%CI=-0.10 to 0.25). MR restricted to cis (SOST) SNPs also suggested sclerostin inhibition increased hypertension risk (OR=1.08, 95%CI=1.01 to 1.15), as well as risk of type 2 diabetes (T2DM) (OR=1.26; 95%CI=1.08 to 1.48),extent of coronary artery calcification

(CAC) (b=0.74, 95%CI=0.33 to 1.15), levels of apoB (b=0.07; 95%CI=0.04 to 0.10; this result driven by rs4793023) and triglycerides (b=0.18; 95%CI=0.13 to 0.24), and reduced HDL-C (b=-0.14; 95%CI=-0.17 to -0.10).

Conclusions: This study provides genetic evidence to support the causal effect of sclerostin inhibition on increased hypertension risk, in the absence of any evidence of reverse causality. Cis-only analyses additionally provided evidence to support causal effects of sclerostin inhibition on increasing risk of diabetes, CAC, and adverse lipid profiles. We conclude that genetic evidence suggests sclerostin inhibition may increase risk of atherosclerosis and levels of its risk factors.

GOC2

Osteoporosis and prevalent fracture are common and remain untreated in mid-life Zimbabwean women living with HIV: a cross-sectional study

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Abstract

Background: With an estimated HIV prevalence of 16% among adult Zimbabwean women, antiretroviral therapy (ART) rollout has improved life-expectancy, hence more women now live post-menopause. We aimed to quantify the prevalence of osteoporosis and fracture among urban-dwelling Zimbabwean mid-life women living with and without HIV.

Methods: A cross-sectional study recruited women age 40-60years from a public hospital HIV clinic and local communities in Harare, collecting demographic and clinical data, measuring anthropometry and BMD [Total body (TB), lumbar spine (LS), total hip (TH), femoral neck (FN)]. NHANES-III reference values enabled calculation of BMD T-scores. Women were classified as pre-, peri- (within 1-year of final menstrual period [FMP]) and post-menopause (>1-year from FMP). The association between HIV and BMD, and BMD by fracture was determined using linear regression, whilst logistic regression determined prevalence differences.

Results: Overall, 193(49.1%) and 200(50.9%) women living with and without HIV were recruited respectively; median (interquartile-range) age was 49(45-54) years. In total, 170(43.3%), 51(13%) and 172(43.8%) women were pre-, peri- and post-menopausal respectively, with no difference by HIV status. Although most women (271/393; 69%) reported food insecurity, obesity was common, particularly in those without HIV (102/200 [51% HIV-] vs. 55/193 [28.5% HIV+]). Most women with HIV were taking ART (n=184/193; 95.3%), with 161/184; 88% on tenofovir disoproxil fumarate; most were virally-suppressed (182/193 [94.3%]).

HIV was strongly associated with lower BMD at all measured sites. In those age 50-60years (n=196), 43 (21.9%) had a T-score≤-2.5 at one or more sites. Amongst women with HIV, 32/96 (33.3%) had a LS T-score≤-2.5, and 15/96 (15.6%) a FN T-score≤-2.5. A T-score≤-2.5 was more common in women with HIV than without (n=34/96 [35%] vs. 9/100 [9%], OR=5.54 [95%CI 2.49-12.49]; p<0.001). Self-reported prevalent adult fracture was more common amongst women with HIV (27/193 [14%] vs. 7/200 [7%]; OR=2.16 [95%CI 1.10-4.26]; p<0.001), as were major osteoporotic fractures (14/193 [7.3%] vs. 5/200 [2.5%]; OR=3.05 [95%CI 1.08-8.64]; p<0.001). In women with HIV, prevalent-fracture was associated with lower FN-BMD (mean-difference 0.047 [95%CI: 0.001-0.092]g/cm2; p=0.046). The association between HIV and prevalent-fracture was attenuated when adjusted for FN-BMD (OR=1.86 [95%CI 0.91-3.78]; p=0.087). No woman reported use of anti-resorptive medication.

Conclusion: Osteoporosis is common in mid-life women living with HIV in Zimbabwe, which in part explains increased fracture-risk. Yet no women are accessing treatment to reduce fracture risk. HIV services should consider bone health assessment for older women living with HIV.

GOC3

Prevalence of osteoporosis and osteopenia in men and women from sub-Saharan Africa, the UK and US: a global problem

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Abstract

Objective

There is a perception that osteoporosis prevalence in sub-Saharan Africa countries is low and does not pose a health risk to populations. However, recent evidence from across The Gambia, Zimbabwe and South Africa suggests otherwise. We aimed to collate multiple worldwide cohorts to compare total hip bone mineral density (BMD) T-scores and osteoporosis prevalence.

Methods

Data from the Gambian Bone and Muscle Ageing Study (40-94 years), The Zimbabwean Menopause study (40-60 years and HIV negative), The South African Agincourt Health and Socio-Demographic Surveillance System (21-80 years and HIV negative) the UK Hertfordshire Cohort Study (59-87 years), and the US Health, Aging and Body Composition Study (68-89 years) were compared. DXA-BMD T-scores (presented as mean (standard deviation)) were calculated for all study participants age >50 years using NHANES III reference data, and prevalence of osteopenia and osteoporosis were determined as T-score <-1 and ≥2.5, and ≤2.5, respectively.

Results

BMD T-scores in men, i.e., osteoporosis prevalence, were low across all cohorts (Table 1). However, marked differences in osteoporosis prevalence were seen in women. Interestingly, Black African women in GamBAS and White American women in HealthABC had a similarly high prevalence of osteoporosis (Table 1).

Conclusion

Contrary to common perceptions, osteoporosis and osteopenia exist in sub-Saharan African men and women, in some populations similar to the US prevalence. Immediate action is required to understand risk factors and outcomes and to prevent growing fragility fracture rates in resource-limited countries.

Table 1: Mean total hip T-scores, and prevalence of osteoporosis (T-score <-2.5) and osteopenia (T-score >-2.5 & <-1) in all those aged over 50 years

Study			Men			Women		
	Country	N (men/women)	Mean T-score (SD)	% (n) Osteoporosis	% (n) Osteopenia	Mean T-score (SD)	% (n) Osteoporosis	% (n) Osteopeni
GamBAS	Gambia	314 / 364	-0.16 (1.20)	1.6 (5)	22.6 (71)	-1.72 (1.19)	28.3 (103)	47.3 (172)
HCS	UK	1067 / 1049	0.55 (1.23)	0.7 (7)	9.7 (104)	-0.74 (1.16)	5.3 (56)	38.1 (400)
HealthABC Black	US	1738 / 2457	0.30 (1.37)	1.7 (30)	13.7 (238)	-1.07 (1.38)	14.6 (359)	38.9 (955)
HealthABC White	US	3509 / 3304	-0.32 (1.30)	3.9 (137)	27.4 (961)	-1.83 (1.12)	27.1 (896)	50.1 (1656
ARK	South Africa	224 / 418	-0.13 (1.30)	3.6 (3)	22.9 (19)	-0.32 (1.22)	4.2 (7)	23.5 (39)
Menopause study	Zimbabwe	0 / 200	-	-	-	0.34 (1.41)	0	17.0 (17)

GOC4

Opposing Extremes of Bone Mineral Density seen in Individuals with Structurally Distinct Variants in PIEZO1

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Abstract

Introduction
We aimed to identify novel monogenic causes of unexplained High Bone Mass (HBM), defined as a total hip and/or first lumbar vertebral bone mineral density (BMD) Z-score of ≥+3.2.

Methods
Whole exome sequencing (WES) was performed in 23 pedigrees from the UK HBM cohort, with data analysed for carriage of at least one novel or rare (MAF <0.005) nonsynonymous single nucleotide variant (SNV) or indel in a highly conserved region of a gene, segregating with HBM within the pedigree. Data were filtered by functional prediction using SIFT and Polyphen-2. WES data from a further 366 UK HBM cases and 126 HBM cases in the Anglo-Australasian Osteoporosis Genetics Consortium (AOGC) were then interrogated to identify additional individuals carrying either the same or another rare variant within the same gene. Further WES data from 493 low bone mass (LBM) cases in AOGC (hip Z-Score <-1.5) were analysed to ensure absence of the variant. Protein homology modelling was conducted using the SwissModel server.

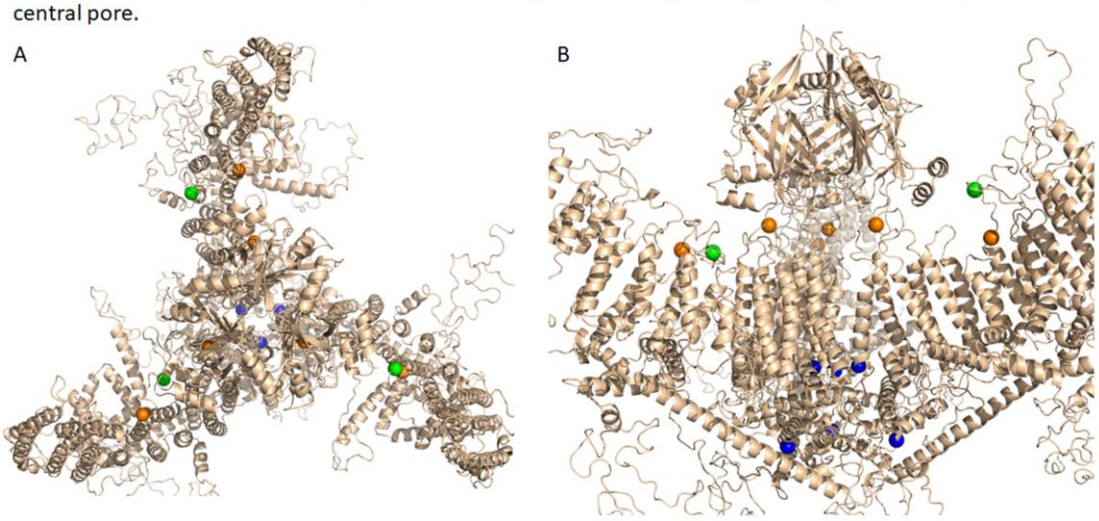
Results
WES identified a rare (MAF 0.00044) heterozygous deleterious missense variant (GRCh37/hg 19 16-88786797-A/G, p.Pro1771Leu) in PIEZO1 segregating with the HBM phenotype, in a pedigree from the UK HBM cohort with a case of unexplained HBM. Analysis of WES data from further HBM cases replicated the same variant in another HBM case. Subsequently, distinct rare (all MAF <0.0005) heterozygous deleterious missense PIEZO1 variants (16-88790325-T/A p.Glu1430Val, 16-88788010-T/A p.Asp1780Val and 16-88786666-G/A p.Thr1992Met) were identified in three further HBM cases. These HBM variants were not observed following interrogation of WES data from 493 LBM cases; however, distinct rare deleterious PIEZO1 variants were identified in two individuals with LBM (16-88782153-G/A p.Arg2476Cys [MAF 0.00067] and 16-88782205-G/C p.Phe2458Leu [MAF 0.001057]).

PIEZO1 encodes a large mechanosensitive cation channel. All 4 HBM variants affect extracellular residues within the mechano-sensing blades of PIEZO1, whereas the 2 LBM variants affect intracellular residues close to the central pore (Fig. 1).

Conclusion
Recently, a PIEZO1 agonist has been shown to increase bone mass in mice, while targeted deletion of PIEZO1 in murine osteoblasts and osteocytes causes osteoporosis. Similarly, in humans, rare variants in PIEZO1 may cause opposing extremes of BMD, such that activating mutations of the mechano-sensing blades result in HBM, whereas loss of function mutations affecting the central pore region lead to

reduced BMD. Further studies are currently underway to evaluate the impact of identified mutations on PIEZO1 function.

Homology model of the homotrimeric human PIEZO1 protein using PDB ID 6LQI as a template with top (A) and side (B) views. Residue affected by index HBM mutation depicted in green (p.P1771) with remaining HBM mutations in orange (p.E1430, p.D1780 and p.T1992), all located along extracellular aspect of the mechano-sensing blades. Residues affected by LBM mutations (p. R2476 and p.F2458) are depicted in blue, close to the central pore.



LBOC1.1

Treadmill running inhibited tumour growth in the femur but not the tibia of a mouse breast cancer bone metastatic model

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Abstract

Rationale: Breast cancer (BC) bone metastasis often leads to progressive bone damage as a result of heightened osteoclastic bone resorption. Our previous in vivo study showed treadmill running (TR) significantly decreased pro-osteoclastic cytokines levels in mouse serum, potentially contributing to the prevention of BC bone metastasis. **Objective**: To investigate whether TR could inhibit the development of BC bone metastasis in a syngeneic mouse model. **Methodology**: Twenty eight-week-old female BALB/c mice were randomised into TR and control groups (10 mice/group). Mice in TR group were subjected to a 3-week treadmill exercise regimen at 12m/min, 5° incline, 30 min/day, 5 days/week. One week into TR exercise, mice in both groups were intracardially injected with murine 4T1-Luc2 cells (5x104 cells/100µl PBS) and the TR group continued exercise for another 2 weeks. Tumour growth was monitored twice weekly by bioluminescence imaging. After euthanization, the tibias and femurs were microCT scanned for quantifying lytic lesion and subsequently sectioned for H&E staining to determine tumour load. **Results**: At the 2-week post inoculation endpoint, more mice in the TR group survived compared to the control group (67% vs. 33%, Kaplan Meier survival p=0.1814). Tumour incidence was lower in both the femur (72% vs. 100%, p=0.0455) and tibia (72% vs 94%, p=0.1774) of TR group. In tumour-bearing femurs, tumour load was significantly lower (35% lower, p=0.0019) in the TR group compared to controls. The lytic lesion count (20%, p=0.1896), total area (37%, p=0.1067) and percentage area (37%, p=0.1123) were also reduced although this was not statistically significant. However, these effects were not seen in the tumour-bearing tibias of TR group. **Conclusion**: TR exercise for 3 weeks may potentially improve BC survival and inhibit BC bone metastases, but the effect is site-dependent. Further study is warranted to investigate this site-specific effect.

LBOC1.2

5TGM1-GFP model can be prolonged with melphalan salvage; a window of opportunity for exploration of bone-targeted treatments for myeloma induced osteolytic lesions

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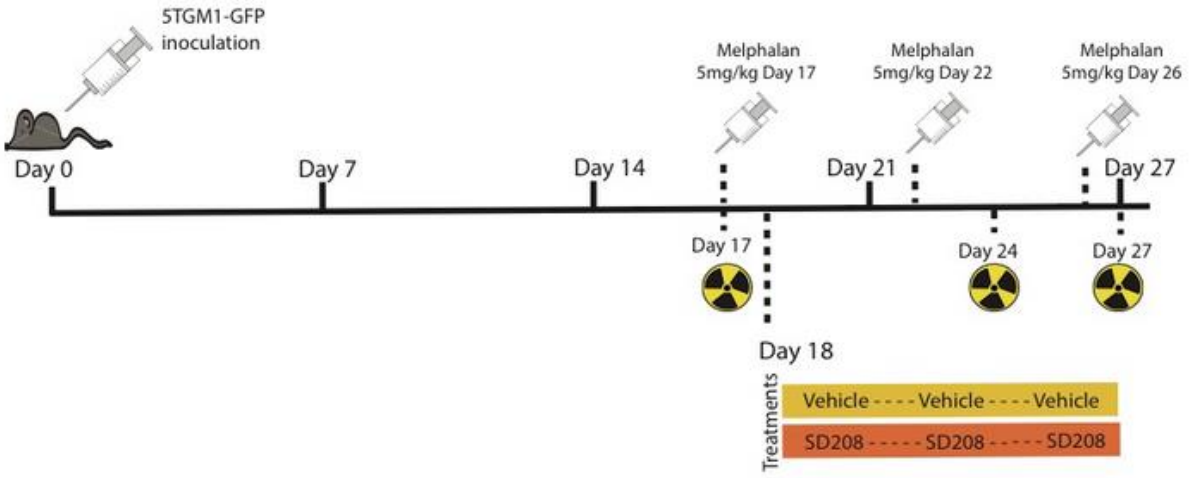
Abstract

Background: Myeloma bone disease (MBD) affects approximately 90% of myeloma sufferers, causing chronic pain, increased fracture risk, immobility and reduced quality of life. There is increasing interest in novel treatments to improve outcomes for patients. Our previous work demonstrated that SD208 (a small-molecule inhibitor to TGF-β) can heal lesions in the xenograft U266-NSG model. 5TGM1-GFP is an aggressive 3-week syngeneic model, but due to MBD appearing late, it has not previously been used to assess longitudinal benefit of therapy on osteolytic lesions.

Aims: We hypothesise that the 5TGM1-GFP model can be salvaged with melphalan chemotherapy, to allow for a period of bone-targeted treatments on established bone disease. This pilot study will also explore whether SD208 can improve outcomes when treating osteolytic lesions in an immunocompetent model.

Methods: C57BL/KaLwRij male mice (n=5/group) were inoculated via tail vein with $2x10^6$ 5TGM1-GFP cells. MBD was monitored by *in-vivo* microCT imaging (left tibia). When MBD was established, mice commenced melphalan chemotherapy treatment (5mg/kg every 4-5 days). Animals were randomised into 2 groups; melphalan or melphalan and SD208 (60mg/kg daily).



Results: MBD was demonstrated on day 17 post-tumour inoculation. Treatment with melphalan from day 17 prolonged the model to day 27 (compared to day 21 in mice not receiving melphalan in a parallel experiment). No significant differences in tumour burden were observed between the groups when assessing spleen weight (p=0.15) or *ex-vivo* flow cytometry (p=0.55). Analysis of total lesion surface area demonstrated a 131% increase between day 17 and 27 (endpoint) with SD208 compared to 192% (p=0.0079) with melphalan monotherapy. Endpoint lesion surface area was lower in mice receiving melphalan with SD208 (5.2% vs. 7.6% (p=0.016)). *Ex-vivo* analysis of right tibia suggest SD208 increases trabecular bone volume (4.2% (SD208) vs 3.5% (vehicle) (p=0.16)) and trabecular number (0.86mm^{-1} vs 0.71mm^{-1} (p=0.07)).

Conclusions: This pilot study demonstrates that melphalan can slow tumour progression in the 5TGM1-GFP model and allow preclinical assessment of bone-targeted treatments in established MBD. Use of *in-vivo* microCT can allow confirmation of lesions prior to salvage treatment. There is scope to increase the melphalan dose to attempt to prolong the model further still, in the hope of observing bone lesion repair as evidenced in immunosuppressed models. Preliminary data suggest that SD208 improves MBD outcomes in this immunocompetent model, but analysis is ongoing to assess bone histology and quality.

Funding: Weston Park Cancer Charity and Sheffield Hospitals Charity.

Poster Abstracts

P01

ANALYSIS OF BIOMIMETIC MATERIALS *IN VITRO* AND *IN VIVO* FOR APPLICATION IN BONE TISSUE ENGINEERING

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Abstract

Bone healing is scarless and uncomplicated in most cases, however, 5-10% of fractures fail to heal due to the extent of the bone defect, trauma to surrounding tissues or comorbidities such as cancer. This orchestrated healing process relies on various cell types including skeletal stem cells, functional vasculature and growth factors in the microenvironment. The current work examined the biocompatibility and efficacy of innovative, acellular, biodegradable scaffolds, based on an octetruss[1] configuration, coated with biomimetic protein and/or growth factors, to augment bone formation by human bone marrow stromal cells (HBMSCs). Material biocompatiblity and functionality to induce bone formation by HBMSCs in response to the scaffold material or surrounding coating was confirmed. Coatings found to be most promising, applied individually or concurrently, were: i) an elastin-like protein (ELP) coating developed at the University of Nottingham[2] and, ii) a bone-inducing growth factor-based coating of fibronectin (FN) and bone morphogenetic protein-2 (BMP-2), attached to the scaffold by polyethylacrylate (PEA), developed at the University of Glasgow[3]. Current focus has centred on polycaprolactone (PCL) scaffold compositions as the biocompatible, biodegradable material for clinical translation. HBMSCs were seeded onto PCL scaffolds and displayed extensive proliferation on ELP coating and/or growth factor coated PCL scaffolds over a 14 day period indicating excellent cytocompatibility. Thereafter, the chorioallantoic membrane (CAM) assay confirmed biocompatibility of the PCL material, ELP coating and growth factor coating, and the ability of the coatings to support blood vessel formation ($P<0.05$) in a dynamic biological environment[4] (Figure 1). Optimised coatings applied in a murine subcutaneous implantation model proved inconclusive. Current in vivo work is focussed on application in a standard preclinical murine segmental bone defect model prior to scaling up the material constructs in a large animal (ovine) bone defect model. Novel innovative biodegradable and biocompatible scaffolds have been identified with potential application in the bone reparative process subject to validation in large preclinical bone formation models.

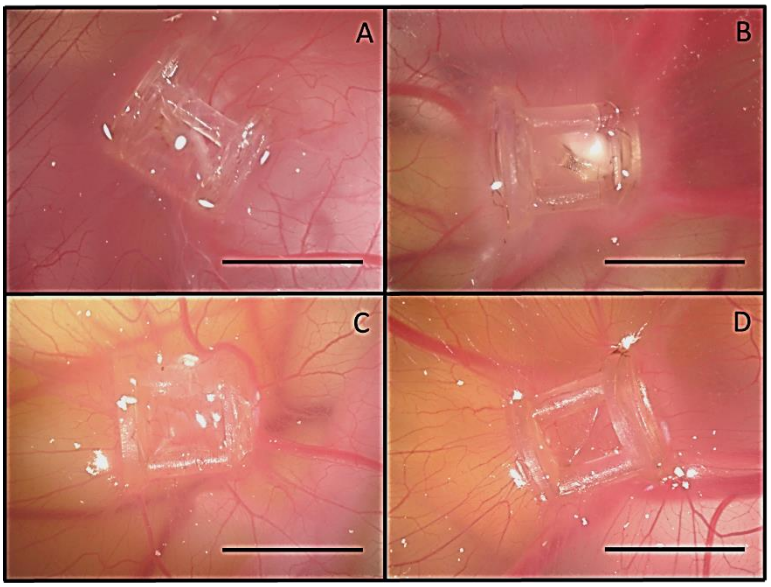

Figure 1: PCL scaffolds integrated with the chorioallantoic membrane (CAM) in the CAM assay (A) Plain uncoated PCL (B) ELP (C) PEA/FN/BMP-2 (D) ELP/PEA/FN/BMP-2 coated PCL. Scale bar 4mm.

Key words: bone, biomaterials, preclinical animal models

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P02

Understanding the balance between osteogenesis and adipogenesis in a mouse model of chronic kidney disease

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Abstract

Objectives

Chronic kidney disease (CKD) is a progressive chronic disease typified by structural changes and gradual loss of kidney function. Altered calcium and phosphorous homeostasis lead to hormonal changes and severe skeletal complications; a condition known as renal osteodystrophy (ROD). Previous studies have reported an increase of bone marrow adipose tissue (BMAT) in clinical and animal models of CKD but the mechanisms driving BMAT accumulation and the implications on bone health are unclear. The aim of this study was to evaluate the bone phenotype, BMAT accumulation and the expression of osteogenic and adipogenic genes in a mouse model of ROD.

Methods

Seven-week-old male C57BL/6 mice were fed a diet supplemented with 0.2% adenine for 1, 3, and 5 weeks to induce early, mid and advanced CKD. Control mice received the same diet without adenine. Serum analysis were quantified by ELISA and a biochemistry analyser. The structure of the left tibia at each time point was assessed by micro computed tomography (μCT). Thereafter the bones were decalcified, stained with osmium tetroxide for evaluation of BMAT by μCT. Cortical bone and bone marrow from the right tibiae were evaluated for osteogenic and adipogenic gene expression.

Results

CKD mice had elevated creatinine and blood urea nitrogen at 3 and 5 weeks whereas there was higher parathyroid hormone and fibroblast growth factor-23 levels at all CKD stages. Changes in serum levels of adiponectin and corticosterone (both higher) and leptin (lower) in CKD mice are consistent with loss of body fat and possibly an increase in BMAT. Trabecular bone loss occurred in the CKD mice after 5 weeks as indicated by decreased bone volume, trabecular thickness and number, and connectivity density. Ongoing studies to quantify BMAT and analyse gene expression changes will determine the differentiation potential of adipocytes and osteoblasts from their common mesenchymal precursors during CKD and ROD development.

Conclusion

This study has shown that CKD mice exhibited bone loss together with increased factors associated with BMAT development. Further analysis is required, but the results suggest that the accumulation of BMAT may be at the expense of structural bone leading to the development of ROD in CKD.

P03

Signaling through Notch 1 decreases osteoclast progenitor activity in the mouse model of rheumatoid arthritis

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Abstract

Background: Periarticular and systemic bone loss in rheumatoid arthritis is mediated by increased osteoclast activity. Osteoclast progenitor cells (OCPs) derived from the myeloid lineage are susceptible to regulation through Notch signaling. Murine bone marrow and splenic OCPs, identified as $CD45^+Ly6G^-CD3^-B220^-NK1.1^-CD11b^{lo/+}CD115^+$ cells, are specifically increased in arthritis. We aimed to determine the effects of OCP Notch signaling inhibition and Notch 1 signal activation on OCP activity and arthritis-induced bone resorption in murine collagen-induced arthritis (CIA).

Methods: Male C57Bl/6, CX3CR1CreERT2xRBP-J (Notch inhibition) and CX3CR1CreERT2xNICD1 (Notch 1 constitutive activity) mice were immunized with chicken type II collagen, treated with i.p. injections of tamoxifen (75 mg/kg) to induce Cre-mediated recombination and sacrificed at day 35 following immunization. Cre negative littermates were used as controls. Expression of Notch receptors 1 through 4 on OCPs was analyzed by flow cytometry in periarticular bone marrow (PBM) and spleen (SPL). FACS sorted OCPs were stimulated by osteoclastogenic factors (M-CSF and RANKL) and stained for TRAP expression. Murine hindpaws were scanned with Bruker SkyScan 1076 µCT and talar bones were analyzed. Research was approved by the Ethics Committee.

Results: We confirmed the expression of Notch receptors on OCPs by flow cytometry with Notch 1 and 2 being most abundantly expressed (around 45% and 60% positive OCPs in PBM and 35% and 20% in SPL respectively), with a significant increase of Notch 2 expression in arthritis. Notch 1 signal activation in OCPs differentiating in vitro leads to reduced numbers of TRAP+ osteoclasts while Notch deletion stimulates osteoclastogenesis. Arthritic $CX3CR1CreERT2^+$xNICD1 mice had lower OCP numbers and an increase in expression of all four Notch receptors on OCPs, while arthritic $CX3CR1CreERT2^+$xRBP-J mice had decreased OCP expression of Notch 1 through 3. Talar bone volume was reduced in arthritic $CX3CR1CreERT2^+$xRBP-J mice.

Conclusion: Our results confirm that Notch signaling may represent an important therapeutic target for the regulation of osteoclast activity in arthritis. Both in vitro and in vivo Notch 1 constitutive signal activation suppressed while Notch deletion enhanced osteoclastogenesis in CIA model. Taken together with our previous results of enhanced osteoclast formation by using neutralizing Notch 1 antibodies we confirmed an inhibitory role of Notch 1 signaling in osteoclast differentiation during arthritis.

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P04

Gendering bones: Improving transparency of sex reporting to address bias within preclinical studies

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Abstract

Introduction

Despite the knowledge that sexually dimorphic mechanisms regulate bone homeostasis, sex often remains unreported or unconsidered in preclinical experimental design. Failure to report animal sex could lead to inappropriate generalisations of research findings, and less effective translation into clinical practice. Sex bias preclinically has already been identified in articles related to neuroscience, cardiovascular biology and immunology. Herein, we have sought to determine the culture of reporting murine sex in manuscript titles or abstracts in the skeletal field and whether any bias in sex reporting exists.

Methodology

We undertook a systematic literature review of murine skeletal research articles published between 1999-2020 and assessed how often murine sex is included either in the article title or abstract. Since gonadectomy (either ovariectomy (OVX) in females, and orchidectomy (ORX) in males) are commonly used within bone research, with sex not typically reported in conjunction with these procedures, we also investigated reporting of OVX and ORX representing female and male mice respectively to inform our studies into sex bias.

Results

Between 1999 - 2020 we found that inclusion of sex in article title or abstracts is low in murine skeletal studies (2.6% - 4.06%). Reporting of OVX and ORX in the abstract or title in conjunction with sex was uncommon i.e. female and OVX and male and ORX (0.4%-0.3%) with use of OVX/ORX terms without sex more prevalent (1.44% - 2.64%).

In studies reporting female/male and/or OVX/ORX in the abstract or title, there was a bias towards reporting use of female mice in skeletal studies. Between 2001-2010, 1998 papers (61.08%) reported female and/or OVX in title or abstract; 1033 papers (30.41%) reported male and/or ORX and 241 (7.37%) papers reported male, female, OVX and ORX together. However, when the terms OVX and

ORX are removed, leaving murine skeletal studies not focussed on sex hormones (e.g. transgenics), a bias in reporting use of male mice was revealed with 983 papers (55.19%) reporting male, 629 papers (35.32%) reporting female and 169 papers (9.48%) including both.

Conclusion

A collective approach towards more transparent and accessible reporting of murine sex in research manuscripts (e.g stating sex in journal titles and abstracts) will allow for more effective monitoring of sex bias which exists and alignment with global research funding mandates focussed on improving health equality.

P05

Collagen (I) homotrimer potentiates the osteogenesis imperfecta (oim) mutant allele and reduces survival in male mice

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Abstract

Type I collagen is the major structural component of bone where it exists as an $(\alpha1)_2(\alpha2)_1$ heterotrimer in all vertebrates. The osteogenesis imperfecta (oim) mouse model comprising solely homotrimeric $(\alpha1)_3$ type I collagen, due to a dysfunctional α2 chain, has a brittle bone phenotype implying that the heterotrimeric form is required for physiological bone function. However, humans with rare null alleles preventing synthesis of the α2 chain have connective tissue and cardiovascular abnormalities (cardiac valvular Ehlers Danlos Syndrome), without evident bone fragility. Conversely a prevalent human single nucleotide polymorphism leading to increased homotrimer synthesis is associated with osteoporosis. Whilst the oim line is well-studied, whether homotrimeric type I collagen is functionally equivalent to the heterotrimeric form in bone has not been demonstrated. Col1a2 null and oim mouse lines were used in this study and bones analysed by microCT and 3-point bending. RNA was also extracted from heterozygote tissues and allelic discrimination analyses performed using qRT-PCR. Here we comprehensively show for the first time that mice lacking the α2(I) chain do not have impaired bone biomechanical or structural properties, unlike oim homozygous mice. However Mendelian inheritance was affected in male mice of both lines and male mice null for the α2 chain exhibited age-related loss of condition. The brittle bone phenotype of oim homozygotes could result from detrimental effects of the oim mutant allele, however, the phenotype of oim heterozygotes is known to be less severe. We used allelic discrimination to show that the oim mutant allele is not downregulated in heterozygotes. We then tested whether gene dosage was responsible for the less severe phenotype of oim heterozygotes by generating compound heterozygotes. Data showed that compound heterozygotes had impaired bone structural properties as compared to oim heterozygotes, albeit to a lesser extent than oim homozygotes. Hence, we concluded that the presence of heterotrimeric collagen-1 in oim heterozygotes alleviates the effect of the oim mutant allele but a genetic interaction between homotrimeric collagen-1 and the oim mutant allele leads to bone fragility.

P06

Using zebrafish elasmoid scales to study the effect of ageing on bone regeneration and repair

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Abstract

Osteoporosis is an age-related disease that can have a great detrimental impact on quality of life. Due to the ageing of society, the forecast financial costs on health care systems and society are expected to increase substantially. There is a great need to discover new osteo-anabolic factors that could become drug targets to improve bone quality in the clinic.

Zebrafish offer an attractive alternative to study bone metabolism and formation with their genetic tractability, allowing the generation of mutant and transgenic fluorescent reporter lines. They have an extensive mineralized exoskeleton located superficially that is easily accessible and can fully regenerate once removed. The elasmoid scales that cover their entire body are flat bony plates that are composed of both bone-building osteoblasts and bone-degrading osteoclasts that reside in a bone matrix, allowing the potential to study dynamic cell processes. Our previous work has shown that regenerating scales express genes that are involved in the formation of new bone and that these genes are associated with both rare and common skeletal diseases in humans. Thus, regenerating scales offer a resource of an evolutionarily conserved pool of osteo-anabolic factors.

In this study, we will present data showing the response to skeletal regeneration and injury throughout the lifespan of the zebrafish and include examples of lines carrying skeletal mutations. We will also present data showing fracture healing on cultured scales. With this ex vivo culture technique, we can offer a way to test new compounds on both ontogenetic and regenerating scales. In conclusion, zebrafish scales represent an attractive alternative model for dynamically following the behaviour of skeletal cells in their native environment that respond to perturbations that include age, genotype, or treatment with compounds.

P07

The development of epigenetically-activated extracellular vesicles to promote bone formation.

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Abstract

Introduction

Extracellular vesicles (EVs) are emerging as promising instructive acellular tools to recapacitate the natural bone healing process, circumvent many limitations with the translation of cell-based therapies. Although these cell-derived nanoparticles have shown promise, there is a tremendous need to enhance their therapeutic efficacy to promote clinical adoption. It has become increasingly apparent that epigenetic regulation plays a pivotal role in osteogenic differentiation. Several studies have shown that hyperacetylation and hypomethylation promotes the differentiation capacity of cells. Therefore, this study aimed to develop epigenetically-activated extracellular vesicles to promote human bone marrow stromal cells (hBMSCs) efficacy for bone formation.

Material and Methods

The effects of DNA methyltransferase inhibitor - 5-azacytidine (AZT) and histones deacetylase inhibitor - trichostatin A (TSA) on osteoblast viability, epigenetic functionality and osteogenic differentiation was evaluated. EVs were isolated from mineralising osteoblasts treated with AZT, TSA or AZT/TSA over a 2-week period. EV size and concentration were defined using nanoparticle tracking analysis and transmission electron microscopy. Osteogenic differentiation of hBMSCs cultured with EVs-derived from untreated (MO-EVs), AZT treated (AZT-EVs), TSA treated (TSA-EVs) and AZT/TSA (AZT/TSA-EVs) treated osteoblasts was evaluated by qPCR, biochemistry and histological analysis.

Results

AZT treatment significantly reduced osteoblast histone and DNA methylation levels, whilst TSA treatment significantly enhanced histone hyperacetylation. Combined AZT/TSA treatment further augmented osteoblast epigenetic functionality and increased mineralisation capacity when compared to AZT and TSA treatments alone. AZT-EVs, TSA-EVs and AZT/TSA-EVs treatment significantly promoted hBMSCs proliferation and migration when compared to untreated cells. Moreover, AZT/TSA-EVs substantially enhanced hBMSCs transcriptionally permissiveness through inducing decreased methylation and enhanced acetylation levels compared to the AZT-EVs treated, TSA-EVs treated and untreated controls. Importantly, AZT/TSA-EVs significantly accelerated hBMSCs osteogenic differentiation and extracellular matrix mineralisation when compared to the AZT-EVs treated, TSA-EVs treated and untreated cells in a dose-dependent manner.

Conclusion

Taken together, we have demonstrated the development of epigenetically-enhanced EVs as a novel acellular tool to promoted hBMSCs mineralisation through transcriptional activation.

P08

Regional differences in tibial vascular canal arrangement in men and women during arduous military training

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Abstract

Introduction

There are sex differences in bone vasculature of mice (Goring et al., 2019). Heterogeneity in strain distribution has been identified in murine tibiae and spatially links to regional differences in vascular canal arrangement (Núñez et al., 2018). Following military training, tibial strain distribution is also heterogeneous, with higher strains in the posterior region that is most prone to injury (Izard et al., 2016). We have investigated whether sexual dimorphism of cortical canals in humans is spatially regulated, and links to bone stress injury risk differences in men and women.

Materials and Methods

Tibiae of age-matched male (n = 5) and female (n = 6) British Army Officer Cadets were scanned at 30% using HR-pQCT (61 µm) at 1 week (baseline), 14, 28, and 44 weeks of a 44-week training course; global cross sections were divided into anterior, medial, lateral, and posterior regions with an automated method in ImageJ (Fig. 1). Cortical tissue volume (Ct.TV), canal number density (Ca.Dn), canal volume density (Ca.V/Ct.TV), canal volume (Ca.V), and canal diameter (Ca.Dm) were extracted and compared between men (M) and women (W) and time points per region using two-way ANOVA.

Results

In all regions, Ct.TV ($p < 0.001$, M: 3636±562 mm^3; W: 2545±120 mm^3 globally at baseline) and Ca.Dn ($p = 0.001$, M: 0.1718±0.073 mm^{-3}; W: 0.0658±0.028 mm^{-3} globally at baseline) were higher in men than women. Within the posterior region, Ca.V/Ct.TV was further found to be higher in men than women ($p < 0.001$, M: 0.3362±0.183 %; W: 0.0791±0.048 % at baseline). Additionally, within the lateral region, Ca.V/Ct.TV ($p < 0.001$, M: 0.1755±0.1200 %; W: 0.0150±0.0164 % at baseline), Ca.V ($p < 0.001$, M: 0.0265±0.013 mm^3; W: 0.0062±0.003 mm^3 at baseline), and Ca.Dm ($p = 0.049$, M: 0.1380±0.008 mm; W: 0.1310±0.0054 mm at baseline) were higher in men than women. There was no effect of time or interaction of sex*time for Ct.TV, Ca.Dn, Ca.V/Ct.TV, Ca.V, or Ca.Dm in any region.

Conclusion

These data revealed that sex differences in tibial vascular canals are not uniform across the section; regional sexual dimorphism was shown largely for the lateral segment. Sex-specific spatial arrangement

of cortical vascular canals could link to enhanced bone stress injury prevalence in women within the military and provide a novel biomarker for fracture risk.

References

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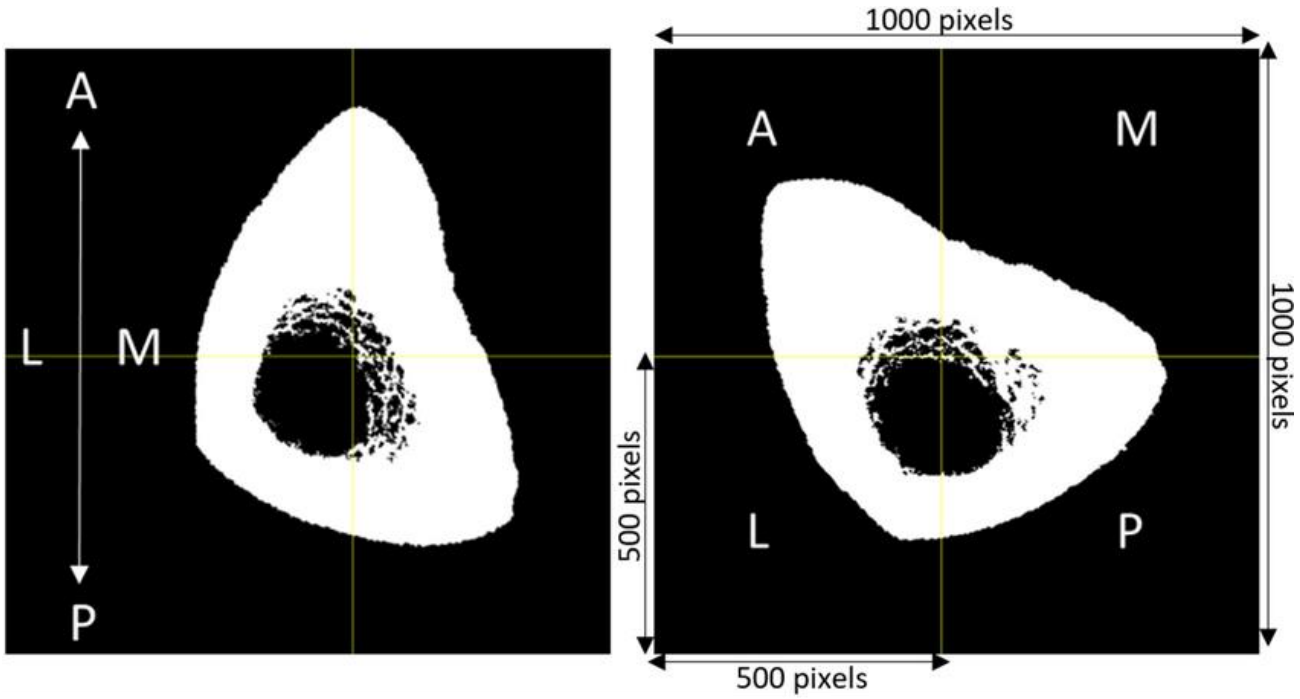

Figure 1. Regionalisation of human tibial HR-pQCT scans
The anterior crest was positioned upwards and the medullary cavity in the middle using 'Moment of Inertia' in Image J (left); followed by 45-degree tilting so that anterior (A), medial (M), lateral (L), and posterior (P) regions would fall in a quadrant (right).

P09

Evidence for a role of G-protein coupled receptor signalling in mediating the functional effects of pyrophosphate on osteoblasts and vascular smooth muscle cells

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Abstract

Extracellular pyrophosphate (PP_i) is well known for its fundamental role as a physiochemical mineralisation inhibitor. However, information describing the direct actions on cells remains limited. This study used primary osteoblasts and calcifying vascular smooth muscle cells (VSMCs) to investigate the effects of physiological levels of PP_i (1-100μM), and the cellular mechanisms underpinning any observed actions. In osteoblasts, PP_i (≥1μM) for the whole (0-21d) or latter stages of culture (7-21/14-21d) reduced bone mineralisation by ≤95%. However, PP_i for the differentiation phase only (0-7/0-14d) increased bone formation by ≤70% ($p<0.001$). Prolonged treatment with PP_i resulted in earlier matrix deposition and increased soluble collagen levels (≤2.3-fold, $p<0.01$). Expression of osteoblast (*RUNX2, Bglap*) and early osteocyte (*E11, Dmp1*) genes along with the mineralisation inhibitors (*Spp1, Mgp*) was increased by PP_i (≤3-fold, $p<0.05$). These effects were reflected at the protein level. PP_i levels are regulated by tissue non-specific alkaline phosphatase (TNSALP) and ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1). PP_i reduced NPP1 expression but increased TNSALP expression (≤2.5-fold) and activity (≤35%, $p<0.05$). Breakdown of extracellular ATP by NPP1 represents a key source of PP_i. ATP release from osteoblasts was decreased ≤60% by PP_i and a selective TNSALP inhibitor ($p<0.001$). In VSMCs, PP_i (≥10μM) decreased calcification and cell death by ≤90% and ≤40%, respectively ($p<0.001$). This treatment also increased TNSALP activity (65%, $p<0.01$), reduced NPP1 expression and inhibited ATP release (80%, $p<0.001$). Pertussis toxin, which prevents $G_{\alpha i}$ subunit activation, was used to investigate whether G-protein coupled receptor (GPCR) signalling mediates the effects of PP_i. The actions of PP_i on bone mineralisation, collagen production, ATP release, osteoblast gene/protein expression and VSMC calcification were abolished or attenuated by pertussis. PP_i also causes a decrease in intracellular cAMP levels (≤35%, $p<0.001$). Methylene diphosphonate (≥1μM), a PP_i analogue where the central oxygen is replaced with carbon, also inhibited bone mineralisation and VSMC calcification by ≤95% and ≤80%, respectively ($p<0.001$). However, its other functional effects differ to those of PP_i e.g. it has no effect on TNSALP activity in either cell type and inhibits the expression of key osteoblast genes (e.g. *Bglap, Spp1, Col1α1*). Together these findings show that PP_i selectively modulates cell differentiation and function, actions that are not mimicked by a chemical analogue. The ability of PP_i to alter ATP release and NPP1/TNSALP expression and activity suggests that cells can detect PP_i levels and respond accordingly. Finally, these data suggest that these actions are mediated by a currently unidentified, G_i-linked GPCR.

P10

Intermittent loading induces an increased bone formation marker response in mouse pre-osteoblasts compared to continuous loading

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Abstract

Introduction

Bone formation is an important process throughout life that responds to internal and external stimuli. Certain weight-bearing exercise has proven effective at inducing bone formation. Rest periods between exercise bouts may have a role in optimising bone mechanosensitivity and could be an important consideration when designing exercise regimes for osteogenic effects [1-2]. It is not clear how pre-osteoblast/osteoblast activity reacts when loading intensity and duration are matched, but the mode of load application is altered. The aim of this study was to assess the pre-osteoblast response to a loading regime with and without periods of unloading.

Methods

Mouse pre-osteoblast cells (MC3T3, ATCC) were cultured under cyclic loading conditions (non-loaded, continuous, intermittent) using a Flexcell bioreactor for tension (n=3). Loading conditions were matched for duration under strain (5 hrs) and intensity of strain (5000 μS at 1 Hz). The mode of applying continuous load was 5 hrs of strain followed by 19 hrs of rest whereas intermittent loading was 1 hr of strain followed by 3 hrs 48 mins of rest every 24 hrs for 1, 3 and 12 days. Alizarin Red (ARS), Alkaline Phosphatase (ALP) and total procollagen type 1 N-terminal propeptide (P1NP) analyses were performed. A one-way repeated measures ANOVA compared differences between loading conditions for ARS, ALP and P1NP for each timepoint.

Results

There were no differences between loading conditions in ARS ($p>0.235$). ALP activity was greater following intermittent loading compared to non-loaded and continuous conditions on days 1, 3 and 12 (non-loaded 0.258 ± 0.028; continuous 0.313 ± 0.031; intermittent 0.440 ± 0.054 umol/min/mL; $p<0.05$; Figure 1). ALP concentrations were greater in the continuous condition than in the non-loaded condition on day 1 ($p<0.05$; Figure 1). P1NP was greater in the intermittent condition at day 12 (ctrl 151 ± 39; conex 121 ± 61; intex 279 ± 116 ng/ml; $p<0.05$) but lower at 3 day (ctrl 46 ± 12; conex 66 ± 9; intex 32 ± 9 ng/ml; $p<0.05$) than in the non-loaded and continuous conditions.

Conclusion

Intermittent loading resulted in higher ALP and P1NP activity on days 1, 3 and 12. This may be due to rest periods between the bouts of loading restoring the mechanosensitivity of the pre-osteoblasts, and thus speculatively enhancing the activation of bone formation [1]. Our findings suggest that intermittent loading is important for re-sensitising pre-osteoblast cells and should be considered when attempting to maximise the osteogenic effects from loading.

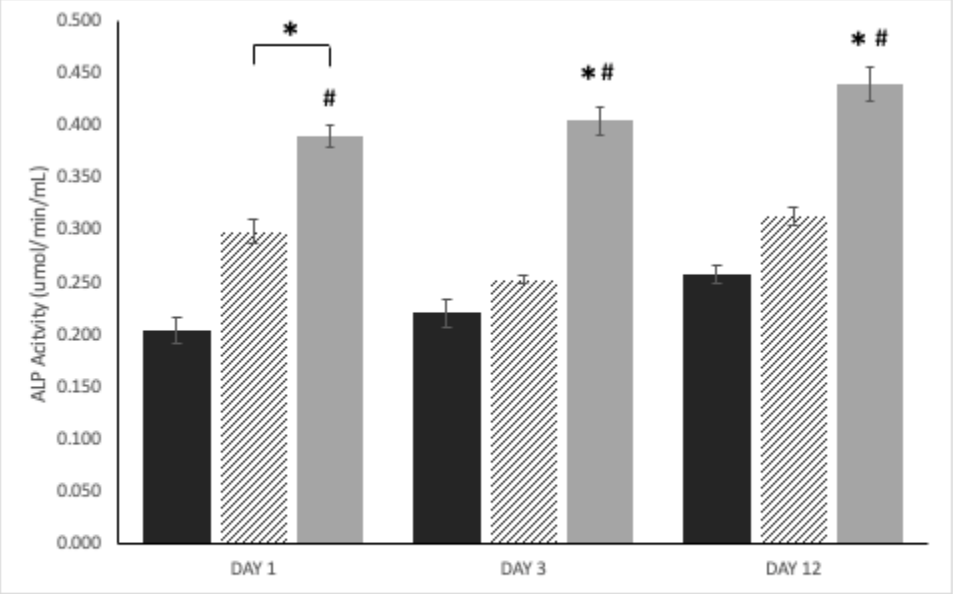

Figure 1. ALP activity across loading conditions. ■ denotes non-loaded; ▩ denotes continuous loading and; ▭ denotes intermittent loading. * <0.05 compared to non-loaded. # <0.05 compared to continuous loading.

P11

Transcriptomic profiling of osteoclasts exposed to extracellular acidification

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Abstract

Extracellular pH modulates osteoclast function, whereby bone resorption is near-maximally activated at pH7.0 but is limited at ≥pH7.4. This study investigated the transcriptomic differences of osteoclasts in response to low pH. Mouse bone marrow-derived osteoclasts were cultured on dentine discs at pH7.4 or pH7.0. At day 5, osteoclasts originally cultured at pH7.4 were also acidified to pH7.0. The effect of pH on osteoclast formation, activity, protein, and differential gene expression was investigated 4-hours post-acidification by image analysis of TRAP-stained discs, western blotting and RNAseq, respectively. Short-term exposure to low pH reduced osteoclast size by ~24% and increased osteoclast number 1.2-fold ($p<0.05$), but resorptive activity remained limited. A reduction in extracellular pH was also associated with increased cathepsin K ($p<0.01$) but decreased cleaved osteopontin protein expression ($p<0.01$). Principal component analysis of RNAseq data revealed three transcriptionally distinct populations of osteoclasts at different pH conditions (pH7.4, pH7.0 and pH7.4 to pH7.0 for 4-hours). Generally, low pH-treated osteoclasts, irrespective of exposure duration, shared more variance than those cultured at pH7.4. The expression of over 2000 genes was found to be differentially regulated by pH. Functional analysis with Reactome pathway gene sets suggest that Kit and AMPK signalling is activated in osteoclasts continually cultured at pH7.4, relative to those at pH7.0 ($p<0.05$). Consistent with the limited resorption observed, energy metabolism and amino acid transport pathways were down-regulated at pH7.4 relative to cells grown at low pH ($p<0.05$). Short-term acid-activated osteoclasts were enriched in amino acid transporter and energy metabolism activities, suggesting preparedness for resorptive activity initiation. Reactive oxygen species pathways were down-regulated 4-hours post-acidification relative to continuous culture at pH7.0 ($p<0.05$), suggesting that protection against oxidative stress is required under longer exposure to low pH. Together, these data suggest that small reductions in pH transcriptionally and functionally modulate osteoclast activity in as little as 4 hours.

P12

Metformin protects against vascular calcification through the selective degradation of Runx2 by the p62 autophagy receptor.

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Abstract

Vascular calcification is associated with aging, type 2 diabetes and atherosclerosis, and increases the risk of cardiovascular morbidity and mortality. It is an active, highly regulated process that resembles physiological bone formation. It has previously been established that pharmacological doses of metformin alleviate arterial calcification through AMPK-activated autophagy, however the specific pathway remains elusive. In the present study we hypothesized that metformin protects against arterial calcification through the direct autophagic degradation of Runx2.

Calcification was blunted in VSMCs by metformin in a dose-dependent manner (0.5mM - 1.5mM) compared to control cells (2.0 fold, $P<0.01$). VSMCs cultured under high-phosphate (Pi) conditions in the presence of metformin (1mM) showed a significant increase in LC3 puncta following bafilomycin-A1 (Baf-A; 5nM) treatment compared to control cells ($P<0.001$). Furthermore, reduced expression of Runx2 was observed in the nuclei of metformin-treated calcifying VSMCs (2.0 fold, $P<0.0001$).

Evaluation of the functional role of autophagy through Atg3 knockdown in VSMCs showed aggravated Pi-induced calcification (3.0 fold, $P<0.0001$), failure to induce autophagy (punctate LC3) (2.0 fold, $P<0.001$) and increased nuclear Runx2 expression (3.0 fold, $p<0.0001$) in VSMCs cultured under high Pi conditions in the presence of metformin (1mM). Mechanistic studies employing three-way co-immunoprecipitation (co-IP) with Runx2, p62 and LC3 revealed that p62 binds to both LC3 and Runx2 upon metformin treatment in VSMCs. Furthermore, immunoblotting with LC3 revealed that Runx2 specifically binds with p62 and LC3-II in metformin-treated calcified VSMCs.

Lastly, we investigated the importance of the autophagy pathway in vascular calcification in a clinical setting. *Ex vivo* clinical analyses of calcified diabetic lower limb artery tissues highlighted a negative association between Runx2 and LC3 in the vascular calcification process. These studies suggest that exploitation of metformin and its analogues may represent a novel therapeutic strategy for clinical intervention through the induction of AMPK/Autophagy Related 3 (Atg3)-dependent autophagy and the subsequent p62-mediated autophagic degradation of Runx2.

P13

Migratory bodies express markers of tumour initiating cells and may represent an early stage of osteosarcoma sarcosphere initiation and metastasis

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Abstract

Osteosarcoma is a primary malignant tumour of bone. There are many subtypes but the most common, although still rare, is the central (medullary) osteosarcoma. Survival rate in osteosarcoma improved considerably (20% to 60%) from early 1970s to 1980s with the introduction of effective multiagent chemotherapy regimens. Unfortunately, this figure has reached a plateau over the past few decades, partly due to a paucity of research into the metastatic mechanisms involved in tumour initiation and metastasis, as well as the lack of new effective treatments. Metastatic disease is one of the major factors affecting prognosis, as seen by the dramatic decline in overall survival of patients who present metastasis 20% to 30% compared to up to 80% observed in nonmetastatic patients.

Preliminary data from our group has shown that migratory body formation can be modulated by co-culturing osteosarcoma cells with bone-marrow derived mesenchymal stem cells. We hypothesise that these migratory bodies are early sarcospheres or sarcosphere precursors. Two osteosarcoma cell lines; MG-63 and HOS-143B were used to develop a sphere formation assay to study the formation of migratory bodies under normal monolayer culture conditions, with additional supplementation of the medium with sarcosphere promoting growth factors; epidermal growth factor (EGF) and fibroblast growth factor (FGF). Osteosarcoma cells cultured under specific conditions form migratory bodies with increased numbers of spheroids per field of view observed at a concentration of 20ng/ml of FGF and 40ng/ml EGF; MG-63 (increase $p \leq 0.01$) and HOS-143B (increase $p = 0.11$). These migratory bodies can be enzymatically dispersed and regrown in monolayer culture. Additionally, RT-qPCR data showed an upregulated gene expression of stemness markers Oct3/4 (10-fold increase $p \leq 0.01$) and Nanog (8-fold increase $p \leq 0.01$) in the migratory bodies found in suspension compared to adherent monolayer cells, in the highly metastatic cell line HOS-143B. Thus, the modulated response with the addition of EGF and FGF in combination with an increase in the gene expression signature of stemness markers are indicative that these migratory bodies contain tumour initiating (cancer stem) cells (TICs). This is also supportive of our hypothesis that these migratory bodies are indeed a precursor to sarcospheres, and potentially mimic the metastatic process. In conclusion, these data support the existence of a subpopulation of TICs in osteosarcoma and provides an indication of how the metastatic process may be initiated.

P14

ATR inhibition increases sensitivity of a highly aggressive osteosarcoma cell line to cisplatin treatment

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Abstract

Since the introduction of chemotherapy, the survival rate of osteosarcoma patients has greatly improved. However, chemoresistance remains a major clinical problem, often leading to metastatic disease. Our group has previously shown that deregulation of autophagy is closely associated with this phenomenon. Indeed, inhibition of autophagy through CRISPR/Cas9 knockout (KO) of the key autophagy gene ATG7 in HOS-143B (highly metastatic/aggressive) osteosarcoma cells increases sensitivity to cisplatin. We now present data to define kinase activity in ATG7 deficient HOS-143B cells, and in response to cisplatin, to identify novel pharmacological targets that mimic ATG7 KO and sensitise cells to cisplatin

The level of phosphorylation of 43 kinase phosphorylation sites was determined using the Proteome Profiler Human Phospho-Kinase Array. Identified kinase target site phosphorylation was validated by Western blot. Subsequently, 4 kinase/kinase targets were identified as potential mediators of autophagy-associated cisplatin sensitivity. A dose response assay was carried out to determine the 50% inhibitory concentration (IC50) of HOS-143B cells treated with these inhibitors in combination with cisplatin.

The kinase array indicated that phosphorylation of Akt1/2/3, WNK1 and p53 (on specific sites) was decreased in ATG7 KO cells and further reduced following cisplatin treatment. Interestingly, this was only partially recapitulated when treated with doxorubicin. This led to the identification of 4 small molecule inhibitors for further investigation. From these inhibitors, treatment of wild-type HOS-143B cells with 1μM and 5μM VE-821 (ATR inhibitor, ATR phosphorylates p53 at serine (S)15) in combination with cisplatin resulted in a 1.9-fold and 3.3-fold decrease in IC50 respectively, compared to controls (p-value = 0.002 and 0.001). Additionally, Western blot analysis confirmed that ATR inhibition combined with cisplatin decreased phosphorylation of p53 and blocked autophagy (characterised by the accumulation of LC3-I and p62).

In summary, ATR/p53 may be a novel mediator of autophagy in osteosarcoma, which mimics ATG7 loss. Furthermore, as p53 S15 is a key target of the DNA damage response (DDR), these data suggest that VE-821 may enhance cisplatin efficacy through reversal of the DDR. Taken together, inhibition of ATR with VE-821 may have a dual effect and represents a potential adjuvant therapy for osteosarcoma patients.

P15

CRISPR/Cas9-mediated ATG7 knockout results in enhanced osteosarcoma chemosensitivity

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Abstract

Due to the development of chemotherapy, the survival rate for osteosarcoma (OS) has improved immensely in patients with localised disease. Despite this, long-term survival rates for OS patients with metastatic or recurrent disease remains unchanged. One of the main reasons for this is chemoresistance to anti-cancer therapies such as doxorubicin (Dox) or cisplatin (Cis), which has become a major obstacle to improving OS treatment. Increasing evidence from our group and others implicates autophagy (a 'self-degradation' cell survival pathway) as a potential mechanism, helping cancer cells thrive under stress from anti-cancer therapies.

In the current study, CRISPR/Cas9 was used to knockout ATG7, a key gene in the autophagy process, in HOS-143B (highly metastatic/aggressive) osteosarcoma cells. Successful knockout of ATG7, and the autophagy process, was validated by qPCR and Western blotting. Furthermore, a colony-forming assay was used to examine the effect of ATG7 KO on cell survival following treatment with Dox or Cis. *In vitro* metastatic potential following chemotherapy treatment was investigated through assessment of cell migration in monolayer and by the formation of migratory bodies from monolayer cultures.

Gene expression of ATG7 was significantly reduced in CRISPR/Cas9 edited HOS-143B cells, with a 14.5-fold decrease compared to control cultures ($p<0.01$). Western blotting confirmed abrogation of the autophagy pathway, with a significant decrease in LC3BII/I (2.9-fold; $p<0.001$), and accumulation of p62 (2.7-fold; $p<0.001$). Clonogenicity assays revealed a 1.4-fold reduction in clone formation in ATG7 KO cells compared to controls ($p<0.05$), and no colonies were observed in KO cells treated with Dox or Cis (15.6-fold and 4-fold reduction versus drug-treated controls, respectively; $p<0.05$). No difference in migration was observed in ATG7 KO cells compared to controls, however, Dox- and Cis- treated KO cells displayed a 93.2% and 97.2% decrease in migration, respectively ($p<0.05$). Interestingly, formation of migratory bodies was reduced by 48.9% in ATG7 KO cells compared to HOS-143B controls ($p<0.05$) and was comparable to the reduction of migratory bodies observed in MG63 cells (77.0%; $p<0.05$), which have low metastatic potential.

In conclusion, we have shown that the KO of ATG7 in osteosarcoma cells results in increased sensitivity to chemotherapy. Despite these data indicating the importance of autophagy in chemotherapeutic efficacy, targeting this pathway *in vivo* poses many questions. Not least the mechanism of action, potential patient stratification and tumour targeting. Nevertheless, this work highlights the importance of this cell survival pathway in the susceptibility of osteosarcoma to currently used chemotherapeutic agents.

P16

Molecular mechanism of intravenous iron therapy induced hypophosphatemia and osteomalacia

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Abstract

Background: Osteomalacia is often caused by severe vitamin D or calcium deficiency, elicited by malnutrition or genetic predisposition. Mutated forms of fibroblast growth factor 23 (FGF23), the osteoblast-derived peptide hormone controlling phosphate homeostasis, or of dentin matrix protein 1 (DMP1), a protein regulating FGF23, are known genetic causes. However, some patients who require repeated intravenous (IV) iron supplementation present with osteomalacia after receiving repeated doses of ferric carboxymaltose (FCM). After administration of FCM over 50% of patients suffer from transient hypophosphatemia, an effect caused by high serum FGF23 concentrations. We aim to identify the molecular mechanism causing hypophosphatemia and osteomalacia after IV iron therapy.

Methods: FCM, ferric derisomaltose (FDI) and iron dextran (ID) were investigated for differences in charge and phosphate binding properties by isoelectric focusing, ion exchange chromatography and size exclusion chromatography (SEC). The impact of different IV iron formulations on DMP1 binding to its cell surface receptor αVβ3 integrin was assessed by ELISA and radio-ligand binding studies. The DMP1 initiated signaling pathway was investigated in osteoblastic precursor MC3T3-E1 cells by LC-MS/MS for phosphoproteomics and western blotting. In an iron deficiency anemia (IDA) mouse model iron quantification in bone was performed with atomic absorption spectroscopy (AAS) after IV iron administration.

Results: FCM was the only positively charged IV iron formulation, while FDI and ID were negatively charged. Moreover, only FCM changes its charge when co-incubated with phosphate buffer. High-affinity phosphate binding by FCM was confirmed by SEC followed by iron and phosphate quantification. Phosphoproteomics analysis of MC3T3-E1 cell extracts revealed that treatment of cells with DMP1 causes strong cytoskeletal regulation by Rho GTPase and activation of the integrin signaling pathway via the MAP kinases. FCM but neither FDI nor ID, strongly reduced DMP1 binding to αVβ3 integrin, as well as ERK phosphorylation as indicator for MAP kinase activity. Four days after IV injection, bone iron concentrations were higher in FCM treated mice when compared with FDI treatment.

Conclusion: We show that FCM exhibits a stronger inhibition of DMP1 binding to its receptor and αVβ3 integrin signaling than FDI and ID. This potentially causes high FGF23 serum concentrations after FCM

treatment. The high affinity of FCM to phosphate and inhibition of DMP1, as well as its enhanced location in the bone could be a possible explanation for FCM-induced hypophosphatemia.

P17

At your fingertips: characterisation of extrinsic signalling in mammalian digit tip regeneration using single cell and spatial transcriptomics

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Abstract

In mammals, the distal digit tip is one of the only complex multi-tissue structures capable of spontaneously regenerating following amputation. Digit tip regeneration is dependent on the formation of a proliferative mass of heterogeneous progenitor cells known as the blastema. However, the cellular mechanisms which drive blastema development and control its subsequent differentiation as tissues are regenerated remain unclear. We hypothesise that extrinsic signalling arising from putative signalling centres surrounding the site of blastema development drive its formation.

Distal digit tips were amputated in 6-8 week female C57BL/6 mice and blastemas allowed to form over 10 days. Regenerating digits were collected and prepared for Visium spatial transcriptomics or 10X Genomics single cell RNA sequencing. For single cell sequencing, blastemas were micro-dissected out prior to dissociation. Subsequent to data processing and quality control, single cell data were mapped to spatial transcriptomics data using bioinformatics packages including STUtility and Seurat. Results of statistical testing are given as Bonferroni-corrected p values.

UMAP clustering of single cell data revealed several distinct cell populations corresponding to blastema, blastema-like, immune and other cell types. Spatial mapping of single cell transcriptomics data was performed using an anchor-based integration approach against spatial data. These analyses were successful in spatially resolving blastema cells (k scores 0.25-1), along with surrounding structures including a subset of blastema-like cells resident within the bone marrow cavity caudal to the blastema (k scores 0.25-0.7) and an immune cell-rich capsule surrounding the blastema (k scores 0.25-0.8).

Functional annotation clustering of differentially expressed genes revealed blastema and bone marrow resident cells (BMRCs) were both enriched in genes annotated as developmental markers (enrichment scores 13.07 and 9.87 respectively). These include members of the BMP, Notch, IGF and Wnt signalling pathways. Differential expression analysis between these two specific cell clusters revealed interesting differences, however. *Notch2* was significantly upregulated in blastema cells ($p = 2.5e-03$) whereas *Notch1* and *-3* were significantly upregulated in BMRCs ($p < 3.8e-03$). Several growth factor receptors are also selectively expressed by blastema cells, including *Fgfr1* and *Bmpr2*. Furthermore, the canonical Wnt signalling pathway receptor *Fzd1* is upregulated in blastema cells ($p < 0.01$).

These data indicate that BMRCs represent a potential novel signalling centre regulating blastema formation and subsequent differentiation during digit tip regeneration through activation of developmental signalling pathways including the BMP, Notch and Wnt pathways. Future studies will selectively ablate aspects of these pathways to investigate their specific roles in blastema formation.

P18

73-Deoxychondropsin A: A novel marine sponge derived V-ATPase inhibitor disrupts osteoclast differentiation and bone resorption.

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Abstract

The vacuolar ATPase (V-ATPase) is an essential proton pump responsible for acidifying intracellular vesicles. During bone resorption, the V-ATPase becomes localized to the osteoclast ruffled border, where it mediates the acidification of the extracellular resorption pit, thus regulating bone resorption. Specific V-ATPase subunit isoforms are strongly upregulated during osteoclast differentiation, which regulate the activity and localization of the ruffled border V-ATPase. Targeting these V-ATPase subunit isoforms could be used to specifically inhibit osteoclast bone resorption for therapeutic use. However, despite decades of discovery, no V-ATPase inhibitor has progressed to clinical trials, largely due to a lack of specificity and subsequent toxicity.

Here, we describe our investigations into 73-Deoxychondropsin A (73-DOC), a novel marine sponge derived V-ATPase inhibitor. We treated differentiating osteoclasts with low nanomolar (≤10nM) concentrations of 73-DOC before or after the addition of RANKL. We found 73-DOC potently restricts the formation of mature osteoclasts in a dose-dependent manner independent of time-of-addition. However, the culture viability was not affected when treatment occurred after RANKL addition, in contrast to other potent V-ATPase inhibitors. Treatment of mature, resorbing osteoclasts in the same concentration range inhibited resorption pit formation.

Low nanomolar dosages of 73-DOC may specifically inhibit osteoclast differentiation without perturbing viability, an effect unique amongst known V-ATPase inhibitors. Ongoing experiments seek to establish a mechanism for this effect by investigating the biochemistry of V-ATPase inhibition, potential subunit specificity, and the effect of 73-DOC on osteoclastogenic signaling. This will provide insight into the therapeutic potential of 73-DOC for diseases characterized by bone loss.

P19

New markers of bone resorption, including lysyl-hydroxyproline and glycl-prolyl-hydoxyproline, identified by in vitro and in vivo metabolomic analysis

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Abstract

Biomarkers can play an important role in the accurate diagnosis of disease and in the monitoring of responses to therapies. There is a limited repertoire of biomarkers of bone turnover and their value in diagnosing and treating bone diseases and has been debated. Furthermore the biology of the most widely used clinical biomarker of bone resorption, CTX 1, is not fully understood.

In this study, we aimed to identify new potential biomarkers of bone resorption using a metabolomic approach. Initially we analysed conditioned medium from resorbing osteoclast cultures and then searched for identified metabolites in mouse and human urine and plasma.

Precursors of human osteoclasts were purchased from Lonza. They were seeded on to walrus dentine slices and induced to differentiate by addition of MCSF (33ng/ml), plus or minus RANKL (66ng/ml) (n=6 per group). After 19 days medium was harvested and resorption quantified by reflected light microscopy. Conditioned medium was analysed by liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS). Metabolomic profiles were compared between +RANKL on dentine and three control groups: -RANKL on dentine, + or - RANKL on plastic and a panel of analytes was identified. To test correlation of analytes with resorption, osteoclast cultures were treated with zoledronate. The presence of analytes was subsequently investigated in plasma and urine.

A panel of metabolites comprising 28 distinct entities, ranging in molecular weight from 144 to 1,541 daltons, was upregulated in +RANKL on dentine compared with control groups ($p<0.001$). These included the dipeptide lysyl-hydroxyproline (L-Hyp), the tripeptides glycl-prolyl-hydoxyproline (G-P-Hyp), and several oligopeptides derived from type I collagen. Addition of zoledronate from10^{-10} to 10^{-4}M led to a dose dependent inhibition of resorption ($p<0.001$). Comparative dose-response curves were observed for the release of all of the collagen breakdown products ($R^2=0.82$). 16 of the entities were detected in mouse and human plasma and/or urine, and their concentrations varied with age for example in mice, plasma L-Hyp and urine G-P-Hyp were negatively correlated with age (-0.88 and -0.78).

In conclusion, we have identified several novel products of bone resorption that can be used to quantify bone resorption in vitro, simply and reliably. Some of these products are present in serum and urine, and their concentrations are altered in physiological and pathological conditions thus potentially expanding the range of bone turnover biomarkers.

P20

Osteoporosis in ankylosing spondyloarthritis and its relationship to disease activity and duration

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Abstract

Osteoporosis is common in spondyloarthritis, due to reduced spinal mobility, and inflammation.

Purposes of the study : The study the prevalence of osteoporosis in patients with ankylosing spondyloarthritis (AS), as well as its relationship to the duration and activity of the disease.

Methods :

This is a retrospective descriptive study carried out in the Rheumatology Department on patients with ankylosing spondyloarthritis meeting the modified New York criteria, having had a measurement of bone mineral density by double energy x-ray absorptiometry (DXA). The clinical, biological and densitometric data were collected and analyzed.

Resulats:

We included 36 patients with AS. There is a male predominance (75%) with a sex ration F / M = 0.33. The mean age (SD) was 36.6 (± 14.8) years. The mean body mass index (SD) was 21.52 (± 3.73) kg / m. 20.5% of patients were on long-term corticosteroid therapy of more than 7.5 mg / day for at least 3 consecutive months.The mean duration of AS was 14.7 ± 9.74 years. The mean sedimentation rate was 34.4 ± 26.6 mm at the 1st hour, the mean C reactive protein was 28.6 ± 33.1 mg / l. HLA B27 was positive in 22.2% of cases. Disease activity was moderate with a mean ASDAS VS score of 2.37 ± 1.02, an ASDAS CRP score of 2.45 ± 1.16, and a mean BASDAI score of 2.41 ± 1.46. The mean bone mineral density of the lumbar spine was 1.071 g / cm2 (mean T score = -0.69) and that of the femoral neck, the mean bone mineral density was 0.847 g / cm2 (mean T score = -1.31).). Osteoporosis in the lumber spine and femoral neck was noted in 25% and 11.1 respectively, and osteopenia was observed in 25% of our patients in the lumbar spine and 36% in the femoral neck. . In addition, bone mineral density was normal in 50% of cases in the lumber spine and 18% in the cervix. No significant correlation was observed between osteoporosis and elevated disease activity (r = 0.11, p = 4.26). In contrast, oseoporosis was significantly associated with advanced disease duration (p = 0.0009)

Conclusion

Our study shows that osteoporosis is responded to in Ankylosing Spondyloarthritis, and it is most marked in the lumbar spine. In our study, we found that the risk of osteoporosis is more related to the duration of the disease and not to the high activity of the disease.

P21

(withdrawn)

P22

Effect of anti-TNF treatments on bone mineral density in ankylosing spondylorthritis

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Abstract

Background:
Osteoporosis is common in spondyloarthritis due to reduced spinal mobility and inflammation. Anti-inflammatory treatments have a beneficial bone effect, and there is a significant increase in bone density during treatment with anti-TNF alpha. The objective of our study is to evaluate bone mineral density in patients with ankylosing spondyloarthritis (AS) treated with anti-TNF alpha.

MATERIALS & METHODS:
This is a retrospective descriptive study of patients with AS meeting the modified New York criteria. The bone mineral density, assessed by dual-energy X-ray absorptiometry (DXA), of AS patients treated with anti-TNF alpha agents was compared with that of a control group of AS patients not treated with anti-TNF alpha agents.
Inclusion criteria: male patients, patients without an anomaly disturbing phosphocalcic and bone metabolism.
- For Patients on anti-TNF alpha: the treatment must be received for more than 6 months.

RESULTS:
A total of 22 patients were included, including 11 patients on anti-TNF alpha and 11 patients not treated with anti-TNF. The mean (SD) age was 28 (±7.2) years and 41 (±14.8) in cases and controls respectively. The mean body mass index in the SA group on anti-TNF was 22.16 kg/m2 and in the control group was 19.64 kg/m2. In the anti-TNF alpha AS group, the mean bone mineral density of the spine was 1.092 g/cm2 (mean T-score = -0.63) and that of the femoral neck, the mean bone mineral density was 0.888g/cm2 (mean T-score = -1.04). In the control group, the mean bone mineral density of the spine was 0.959 g/cm2 (mean T-score = -1.91) and the mean bone mineral density of the femoral neck was 0.774 g/cm2 (mean T-score = -1.99). Bone mineral density in the spine and neck was higher in the group receiving anti-TNF alpha (p=0.09, p=0.173 respectively).
CONCLUSION:
Our study shows the increase, although not statistically significant, of bone mineral density in patients with AS receiving anti-TNF alpha compared to controls. Our results joined with those of the literature which support the bone protective effect of anti-TNF alpha. The non-significant difference can be explained by the delay in the introduction of biotherapy at the advanced stage of the structural evolution of AS. The best solution is to start anti-TNF drugs at the early inflammatory stage of AS.

P23

Effects of vitamin D on disease activity in rheumatoid arthritis

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Abstract

Introduction : Vitamin D deficiency is common in autoimmune diseases, including rheumatoid arthritis (RA).

Objectives: determine the vitamin D status in patients with rheumatoid arthritis and their association with disease activity.

Methods :

This is a descriptive retrospective study carried out in the Rheumatology department over a period of 1 year in patients with RA meeting the criteria of the ACR / EULAR 2010, and having had a measurement of the serum vitamin D level. The activity of the disease is evaluated by the DAS score 28. The clinical and biological data were collected and analyzed. Exclusion criteria: Any patient who has been supplemented with vitamin D in the previous six months.

Materiel :

We included 34 patients, their mean age was 54.3 ± 11.7 years. The mean duration of RA was 14.4 years. A normal vitamin D level was found in 11.7%, vitamin D insufficiency (serum vitamin D level between 10 and 30 ng / ml) in 58.8%, and vitamin D deficiency (level serum vitamin D <10 ng / ml) in 14.7%. Disease activity was moderate with a DAS 28 score of 3.85 ± 1.29. There is no correlation between vitamin D levels and disease activity ($p = 0.004$; $r = -0.16$).

CONCLUSION:

Our study suggests that hypovitaminosis D is widespread in patients with RA, and that the serum 25 (OH) vitamin D level is not associated with disease activity.

P24

Prospective observational study on tolerability of buffered soluble alendronate in patients who were unable to adhere to oral bisphosphonate tablets

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Abstract

Background: Oral bisphosphonates (BP) such as alendronate and risedronate are the mainstay of treatment for osteoporosis. Both SIGN and NICE guidelines recommend oral BP as first line treatment for most patients with osteoporosis. Long-term adherence to oral bisphosphonates however is poor and the most common reason for discontinuation are gastrointestinal (GI) side effects. A novel buffered soluble alendronate effervescent formulation (ALN-EFF), Binosto® has strong buffering properties and was developed with the aim to improve GI tolerability.

Objective(s): To assess tolerability and adherence to ALN-EFF in patients who had reported side effects to weekly oral bisphosphonates (BP) in tablet form in "real-world".

Material and Methods: Single-centre observational study of patients with osteoporosis who were referred to a specialist osteoporosis clinic as they had reported side effects to either alendronate, risedronate or both types of oral BP in tablet form. Patients were offered a trial of ALN-EFF, 70 mg weekly or to commence yearly parenteral bisphosphonate treatment (Zoledronate). As per routine care, patients who started ALN-EFF were assessed via telephone 2-3 months after commencement.

Results: We have included 102 patients with a mean age of 72 ± 9 years, the majority were women (89%). Most patients (94%) had tried both alendronate and risedronate and reported side effects to either of them. The most common side effects were gastrointestinal side effects; 9 patients had confirmed gastroesophageal reflux disease, 5 patients a hiatus hernia and 4 patients Barret's Oesophagus at baseline. Out of 102 patients, 38 patients were willing to commence a trial of ALN-EFF. After 10 weeks 24/38 (63%) patients reported good tolerability and they were agreeable to continue the medication long-term. The majority of reported side effects in patients who did not tolerate ALN-EFF were of gastrointestinal nature such as gastroesophageal reflux, abdominal bloating and diarrhoea. In a subset of patients, long-term compliance with ALN-EFF was assessed after 19 ± 4 months and 7/13 (54%) patients reported ongoing treatment tolerability and adherence to ALN-EFF.

Conclusion: Over half of the patients who had reported side effects to risedronate and alendronate in tablet form tolerated ALN-EFF when assessed after 10 weeks. The effervescent form of ALN might be a good alternative option for patients who develop side effects to bisphosphonates in tablet form however long-term tolerability and adherence will need to be studied further.

P25

Electrically stimulated synergistic Recruitment of Antagonist Muscle Pairs (RAMP-ES), to maximise bone stimulation in the paralysed limbs of people with spinal cord injury

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Abstract

People with complete spinal cord injury (SCI) experience dramatic bone loss in their paralysed limbs, leading to increased risk of fragility fractures and associated medical complications. Different electrically-stimulated interventions have been developed to try to attenuate bone loss. However, the low muscle forces produced by atrophied muscles have limited their effectiveness.

In this project, electrical stimulation (ES) was used to recruit knee flexors and extensors simultaneously, to maximise the muscle forces produced and minimise limb movement. Three participants (aged 54-59 years) with chronic complete SCI sustained for more than a year (level of injury C6-T4) were recruited from the Queen Elizabeth National Spinal Injuries Unit. Participants undertook 4 weeks of muscle conditioning with ES for each muscle group individually before starting the intervention phase using simultaneous recruitment of antagonist muscle pairs ('RAMP-ES'). Bone and muscle scans were obtained before and after 4 months of RAMP-ES intervention using peripheral quantitative computed tomography (pQCT) and MRI, respectively. Bone scans were obtained at the distal femur (DF) and proximal tibia (PT), and muscle scans were taken at mid-thigh. Effect on muscle strength was assessed by measuring the knee torques produced at 100 mA current intensity using a Biodex dynamometer.

The total cross-sectional area of all thigh muscles in the trained limb increased in all participants (between 7.3% to 19.8%) but decreased in the untrained limb in one and slightly increased in two (-2.7 and 2.2%). Muscle fat fraction decreased in all participants in the trained limb (-10.8% to -5.2%) and showed slight changes in the untrained limb (-1.1% to 2%). Electrically-induced knee extension and flexion torque increased compared to those measured during week 2 of the muscle conditioning (46.2% to 428.6% and 70.8% to 262.5%, respectively). Bone mineral density changes at the DF did not show a clear pattern (-6.9% to -1% and -4.5% to 0.5% in the trained and untrained limbs, respectively) following the RAMP-ES intervention phase.

The results of this pilot study show that 4 months of RAMP-ES training helped improve muscle size and reduce fat fraction in the paralysed limbs of people with SCI. However, there was no evidence of a positive effect on bone outcomes at fracture-prone sites. Implementing training for a longer duration and on a larger population may be required to ascertain whether RAMP-ES can improve bone health after SCI.

P26

The role of accelerated growth plate fusion in the absence of SOCS2 on osteoarthritis vulnerability

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Abstract

Background
Osteoarthritis is the most prevalent systemic musculoskeletal disorder characterised by articular cartilage degeneration and subchondral bone (SCB) sclerosis.
Purpose
Here we sought to examine the contribution of accelerated growth to osteoarthritis development using a murine model of excessive longitudinal growth. Suppressor of cytokine signalling 2 (SOCS2) is a negative regulator of growth hormone (GH) signalling, thus mice deficient in SOCS2 (Socs2-/-) display accelerated bone growth.
Methods
We examined vulnerability of C57BL/6 16-week-old wild-type (WT; n=6) and Socs2-/- male mice knee joints to osteoarthritis following surgical induction of disease (destabilisation of the medial meniscus (DMM) or sham operated), and with ageing (12-13-month-old, n=6) by histology and micro-CT. All experimental protocols were approved by Roslin Institute's Animal Users Committee and mice were maintained in accordance with UK Home Office guidelines for the care and use of laboratory animals.
Results
We observed a significant increase in density of growth plate bridges in Socs2-/- mice in comparison to WT mice (WT DMM: 2.2±0.9; WT sham: 1.2±0.5; KO DMM: 13.0±0.5; KO sham: 14.4±0.7; $P<0.01$) and with ageing (WT: 8.6 ± 2.2; KO: 21.3 ± 1.4 $P<0.001$). Histological examination of WT and Socs2-/- knees revealed articular cartilage damage with DMM in comparison to sham. Articular cartilage lesion severity scores (mean and maximum) were similar in WT and Socs2-/- mice with either DMM, or with ageing. Micro-CT analysis revealed decreases in SCB thickness (WT: 0.15mm ± 0.003; KO: 0.11mm ± 0.003; $P<0.001$), epiphyseal trabecular number, and thickness in the medial compartment of Socs2-/-, in comparison to WT mice ($P<0.001$). DMM had no effect on the SCB thickness in comparison to sham in either genotype.
Conclusions
These data suggest that enhanced GH signalling through SOCS2 deletion accelerates growth plate fusion, however this has no effect on osteoarthritis vulnerability in this model.

P27

Irisin improves trabecular bone microarchitecture in type 1 diabetes

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Abstract

Osteoporosis is a significant co-morbidity of type 1 diabetes mellitus (DM1) leading to increased fracture risk. Exercise-induced hormone 'irisin' in low dosage is shown to have a beneficial effect on bone metabolism by increasing osteoblast differentiation and reducing osteoclast maturation; and inhibiting apoptosis and inflammation. We hypothesized that irisin would help improve diabetic osteopathy in DM1.

Irisin is known to improve cortical bone mass and strength. We investigated the role of irisin in ameliorating bone fragility associated with DM1 by observing its effect on trabecular bone. DM1 was induced by intraperitoneal injection of streptozotocin 60 mg/kg body weight. Irisin in low dosage (5 µg twice a week for 6 weeks I/P) was injected into half of the control and 4-week diabetic male Wistar rats. Animals were sacrificed after six months of induction of diabetes. Trabecular bone in the femoral head and neck was analysed using a micro-CT technique. Bone turnover markers were measured using ELISA, Western blot, and RT-PCR techniques. It was found that DM1 deteriorates the trabecular bone microstructure by increasing trabecular separation (Tb-Sp) and decreasing trabecular thickness (Tb-Th), bone volume fraction (BV/TV), and bone mineral density (BMD). Irisin treatment positively affects bone quality by increasing trabecular number $p < 0.05$, and improves the BMD, Tb-Sp, and BV/TV by 27-28%. The deterioration in bone microarchitecture is mainly attributed to decreased bone formation observed as low osteocalcin and high sclerostin levels in diabetic bone samples $p < 0.001$. The irisin treatment significantly suppressed the serum and bone sclerostin levels $p < 0.001$, increased the serum CTX1 levels $p < 0.05$, and also showed non-significant improvement in osteocalcin levels.

The data obtained from our study corroborates that DM1 deteriorates the trabecular bone microstructure in the proximal end of the femur which is partially improved by irisin.

P28

The association between hip geometry and hip osteoarthritis: findings from 40,000 UK Biobank participants

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Abstract

Introduction:

Previous studies found measures of hip geometry (such as narrowest neck width (NNW), diameter of the femoral head (DFH), neck shaft angle (NSA) and hip axis length (HAL)) to be associated with increased risk of hip osteoarthritis (HOA). However, these measures are related to each other and body size, and it is unclear whether observed associations represent independent effects. The aim of this study was to investigate the association between four geometric parameters (GPs) and radiographic HOA (rHOA), hip pain and hospital diagnosed HOA (HESOA) in a large sample of hip dual-energy x-ray absorptiometry (DXA) scans in UK Biobank (UKB), providing good power to elucidate independent associations.

Methods:

Left hip DXAs from UKB were assessed for NSA, NNW, DFH and HAL (Figure 1). These measures were derived automatically in Python, based on an 85-point template applied to each image. Correlation matrices were used to display the inter-relationships between GPs, height, weight, and age. Logistic regression was used to examine the relationship between each GP with grade ≥2 rHOA, hip pain and HESOA. Analyses were adjusted for sex, age, height, weight, and GPs.

Results:

40,313 left hip DXAs were examined (47.9%/52.1%, male/female). Mean (range) NSA was 133.0° (104.9-152.8)/ 135.2° (112.1-161.7) in males/females, NNW 34.5mm (22.9-45.8)/ 29.0mm (21.4-37.8), DFH 49.0mm (34.7-64.40)/ 43.0mm (33.4-53.7) and HAL 103.1mm (76.9-127.1)/ 90.8mm (68.1-115.5). Height, NNW, DFH and HAL were strongly correlated with each other ($r2 \geq 0.75$). In adjusted models accounting for age, sex, height, weight, and GP inter-relationships, all four parameters showed associations with grade ≥2 rHOA [NSA OR 0.78 (95% CI 0.75-0.81), NNW 2.38 (2.18-2.59), DFH 0.55 (0.50-0.61) and HAL 1.25 (1.15-1.36)]. Only NNW showed an association with hip pain [1.19 (1.10-1.30)] and HESOA [2.19 (1.80-2.67)]. Similar results were seen in sex-stratified analyses, and sex interaction terms were seen most strongly for NNW and DFH.

Conclusions:

Geometric parameters are strongly correlated with height and each other and differ between the sexes. We observed the strongest associations between NNW and rHOA, hip pain and HESOA. Although geometric parameters are intrinsically related, when considered collectively they show independent associations with rHOA. For example, a relatively small DFH is associated with increased risk of rHOA after controlling for NNW. Further studies are justified to determine whether these associations reflect causal relationships, and if so to examine the underlying mechanisms responsible.

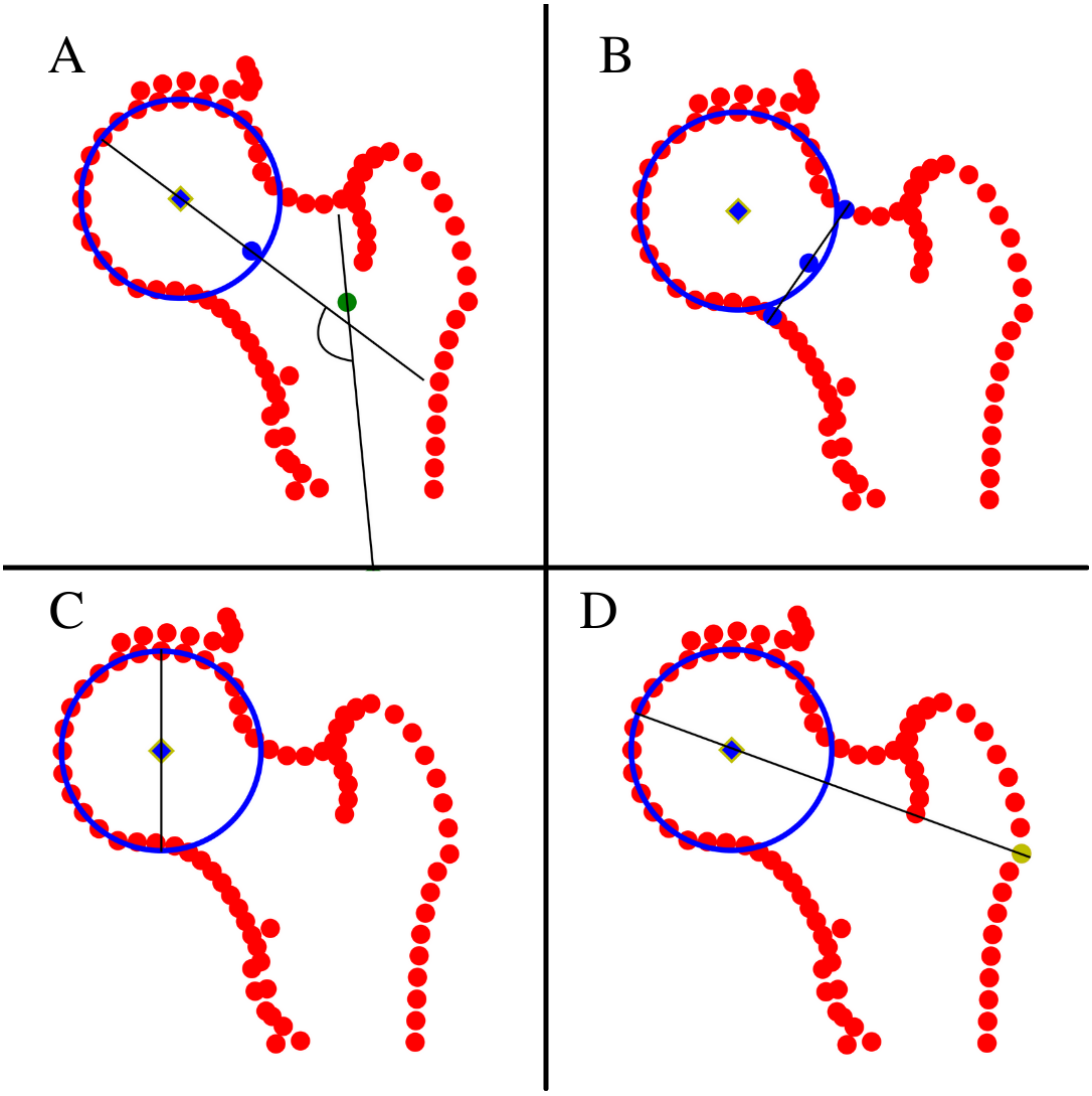

Figure 1: Geometric parameter measurement: A – NSA, B- NNW, C- DFH, D – HAL.

P29

Picking up Hidden Osteoporosis Effectivelyduring Normal CT Imaging without additional X-rays - PHOENIX

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Abstract

Background

Up to 40% of all diagnostic computed tomography (CT) scans include views of the spine or hips. Among older people, osteoporosis or vertebral fractures have been found in 30% of such CT scans. Our 'PHOENIX' intervention repurposes CT scans taken for other reasons to identify fractures and measure bone density as an 'added extra'. Early detection and treatment of osteoporosis in CT-attending patients could improve health outcomes.

Objectives

To determine the feasibility and efficacy of PHOENIX versus usual care in a multi-centre, randomised, pragmatic study conducted in Eastern England involving our Cambridge Specialist Hospital 'hub' and four regional General Hospital 'spokes'.

Methods

Women ≥65 and men ≥75 years attending for routine diagnostic CT scans were invited to participate via a novel consent form incorporating FRAX Fracture Risk Assessment questions. After calculating their FRAX 10-year risk score, higher risk patients were block randomised (1:1:1) to Group 1) PHOENIX intervention, 2) Active Control, where the GP was sent the patients' FRAX answers only, or 3) Usual Care where data were only analysed after 13 months had elapsed. The CT scans of high FRAX risk patients in Group 1 were retrieved by the Cambridge team using NHS Connecting for Health (Burnbank, UK). The team performed vertebral fracture assessment and measured bone density using QCT Pro (Mindways, USA). Results were reviewed by a physician, authorised and sent to general practitioners (GPs). Baseline CT scans from groups 2 and 3 were assessed in the same way after 13 months to ensure no patient with

osteoporosis/fractures was neglected long term. Assuming 25% attrition, the study was powered to find a superior osteoporosis treatment rate in Group 1 (estimated 20%) versus 16% (Active Control) and 5% (Usual Care). Co-primary feasibility endpoints were the ability to a) randomise 375 patients within 10 months and b) retain 75% of survivors able to complete a 1-year bone health outcome questionnaire. Secondary outcomes included osteoporosis/vertebral fracture identification rates and osteoporosis treatment rates. Stakeholder acceptability and economic aspects will be reported separately.

Results and Conclusion

Results will be presented at the meeting

Osteoporosis treatment rates were almost tripled by screening patients attending for routine diagnostic CT scans with waiting room FRAX, CT-bone densitometry and vertebral fracture analysis.

P30

Regional and temporal variation in bone loss during the first year following spinal cord injury

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Abstract

Osteoporosis is a common secondary consequence of spinal cord injury (SCI) that leads to fragility fractures. Visual assessment of cross-sectional bone scans suggests that there is regional variation in bone loss, but this has not been objectively characterised. In addition, substantial inter-individual variation in bone loss following SCI has been reported but to date it is unclear how to identify individuals most at risk of rapid bone loss.

Therefore, to examine regional bone loss and the utility of early-stage bone assessments in predicting individuals at risk of rapid bone loss, tibial bone parameters were assessed in 13 individuals with SCI (aged 16-76 years). Peripheral quantitative computed tomography scans at 4% and 66% (distal-proximal) tibia length were acquired within 5 weeks (baseline), 4 months and at 12 months of injury. Changes in total bone mineral content (BMC), bone cross-sectional area (CSA) and bone mineral density (BMD) were assessed in ten concentric rings at the 4% site, and regional changes in total BMC, cortical BMD and periosteal and endocortical circumferences were analysed in thirty-six polar sectors at the 66% site using paired T-tests. In addition, relationships between regional and total loss in BMC and BMD at 4-month and 12-month timepoints were assessed using Pearson correlation.

At the 4% site, total BMC ($P = 0.001$) but not total CSA ($P = 0.28$) decreased with time. Absolute loss in BMD was greater in the outermost ring than other rings (all $P < 0.001$), but relative (percentage) losses were equal across rings (all $P > 0.1$). At the 66% site, both BMC and cortical BMD absolute losses were similar (all $P > 0.3$ and $P > 0.05$, respectively) across polar sectors, but relative loss was greatest in the posterior region (all $P<0.01$). At the 4% and 66% sites, total BMC loss at 4 months was strongly positively associated with the total loss at 12 months ($r=0.84$ and $r=0.82$ respectively, both $P< 0.001$). This correlation was stronger than those observed with 4-month BMD loss in several individual sectors ($r=0.56$-0.77, $P < 0.05$).

These results confirm that bone loss following SCI varies regionally in the tibial diaphysis. In addition, total but not regional bone loss at 4-months is a strong predictor of total loss after 12-months postinjury. More studies on larger populations are required to verify the validity of early-stage bone assessments in identifying individuals at risk of rapid bone loss, in order to ensure timely treatments to minimise future fracture risk.

P31

Musculoskeletal health in active ambulatory men with Cerebral Palsy and the role of vitamin D.

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Abstract

Individuals with Cerebral Palsy (CP) show impairment in ambulation, gait and range of motion. It is possible that the severity of musculoskeletal impairments in individuals with CP is exacerbated by low vitamin D caused by poor diet and living in northern latitudes such as the UK, who experience negligible sun exposure during the winter months. Where previous research in controls has shown associations between vitamin D with strength and bone health. A cross-sectional comparison study conducted according to the guidelines of the Declaration of Helsinki, where 24 active, ambulant men with CP aged 21.0±1.4 years (Gross Motor Function Classification Score (I–II) and 24 healthy controls aged 25.3±3.1 years completed in vivo assessment of musculoskeletal health, including: *vastus lateralis* anatomical cross-sectional area (VL ACSA), isometric knee extension maximal voluntary contraction (KE iMVC), and radius and tibia bone ultrasound (US) T(us) and Z(us) scores to assess bone dimensions. Assessments of body mass and body fat (BF)% was used to determine lean body mass (LBM), vitamin D status was measured through venous samples of serum 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone, dietary vitamin D intake from food diary, and total sun exposure via questionnaire were also taken. The results showed that men with CP had 40.5% weaker KE iMVC, 23.7% smaller VL ACSA, 22.2% lower VJ, 22.4% lower KE iMVC/body mass (BM) ratio, and 25.1% lower KE iMVC/LBM ratio (all $p<0.05$). Radius T(us) and Z(us) scores were 1.75 and 1.57 standard deviations lower than controls, respectively ($p<0.05$), whereas neither tibia T(us) nor Z(us) scores showed any difference compared to controls ($p>0.05$). The 25(OH)D was not different between groups, and 90.9% of men with CP and 91.7% of controls had low 25(OH)D levels when compared to current UK recommendations. The 25(OH)D was positively associated with KE iMVC/LBM ratio in men with CP (r=0.500, $p=0.020$) but not in controls (r=0.281, $p=0.104$). Overall this study shows that musculoskeletal outcomes in men with CP were lower than controls, and despite there being no difference in levels of 25(OH)D between the groups, 25(OH)D was associated with strength (KE iMVC/LBM) in the CP group but not controls. The findings suggest that vitamin D deficiency can accentuate some of the condition-specific impairments to musculoskeletal out-comes.

P32

Disruption of bone development following progressive synaptic loss is sexually dimorphic

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Abstract

Background: Neurodegenerative diseases predominantly present as progressive cognitive decline underpinned by synaptic loss. Neurodegenerative patients are more susceptible to fragility fractures due to decreased bone mineral density (BMD)[(1)] suggestive of co-morbidities. Consistent with this, sexual dimorphisms exist in the pathogenesis of neurodegenerative disease[(2)] and osteoporosis[(3)]. Cysteine string protein alpha (CSPα) is a presynaptic co-chaperone protein; CSPα$^{-/-}$ mice are alike to CSPα$^{+/+}$ until postnatal day 23 (P23) whereby a progressive sensorimotor deficit develops[(4)]. We assessed tibial development during early postnatal growth in both sexes following CSPα deletion and we hypothesise that progressive synaptic loss and neurodegeneration will lead to subsequent bone deterioration.

Methodology: Tibiae from male and female mice (n=5) from each genotype (CSPα$^{+/+}$, CSPα$^{-/-}$) at 3 postnatal stages of progressive sensorimotor decline (P7, P14, P23) were CT scanned (17.77µm). Following reconstruction and segmentation, cortical bone was assessed for cortical thickness, periosteal and endosteal perimeters and bone mineral density (BMD).

Results: There were no significant differences between CSPα$^{+/+}$, CSPα$^{-/-}$ at P7 or 14 in cortical thickness, endosteal/periosteal perimeter or BMD in either sex. Differences were evident at P23 CSPα$^{-/-}$ with thinner cortices detected in CSPα+/+ mice in male (Fig 1A; $p<0.0001$) and females (Fig 1B; $p<0.0001$) across bone length. Further, at P23 endosteal perimeter was lower in CSPα-/- mice (male $p=0.031$ and female: $p=0.0054$). P23 CSPα$^{-/-}$ female mice possess reduced BMD across tibial length (Fig 1D, $p<0.0001$) whereas males do not (Fig 1C, $p=0.86$).

Conclusions: We describe a direct link between onset of sensorimotor deficit following synaptic dysfunction with regulation of cortical bone development. We have further exposed sexual divergence of synaptic loss on BMD suggestive of an uncoupling of cortical thickness with BMD specific to males. Our work begins to establish a link between neurodegeneration and bone health in both men and women.

References
1. DOI10.1186/s12883-014-0175-2
2. DOI:10.1016/j.jsbmb.2015.09.039
3. DOI:10.1007/s11999-011-1780-7.
4. DOI:10.1016/S0896-6273(04)00190-4

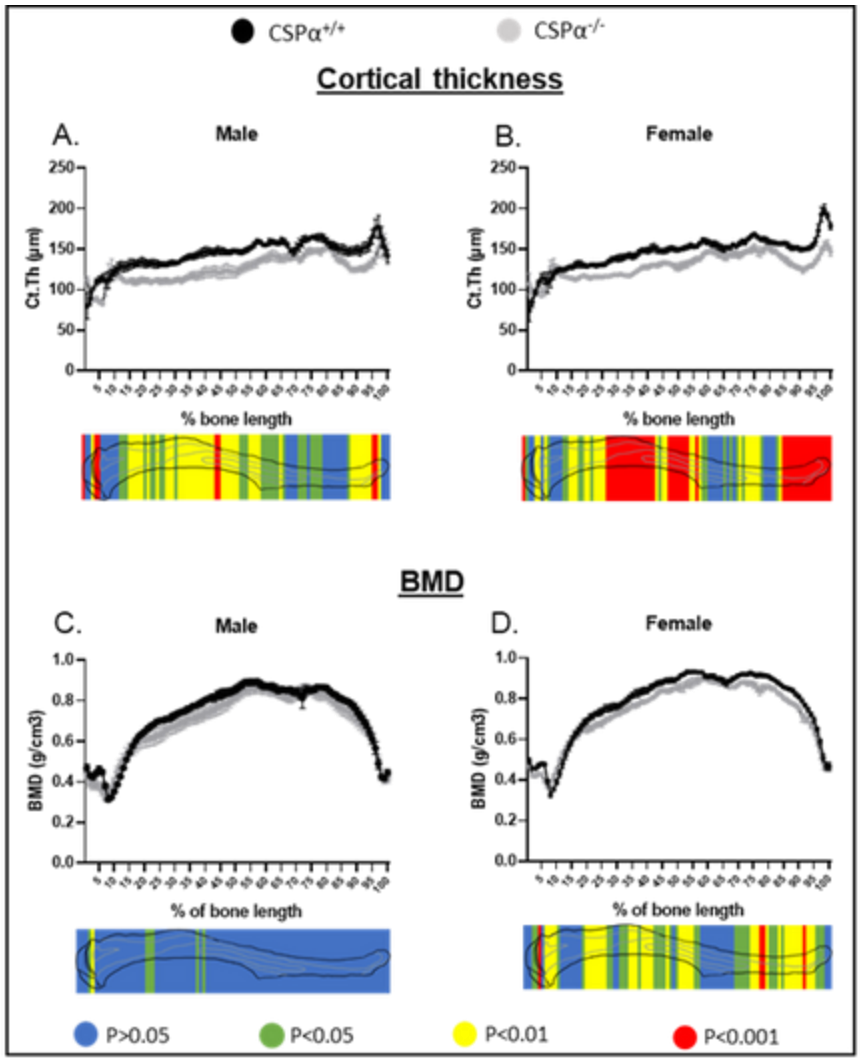

Fig 1. CSPα$^{-/-}$ impacts bone development. Cortical thickness is reduced in male (A) and female (B) CSPα$^{-/-}$ mice versus CSPα$^{+/+}$ across bone length, with graphical heat map summarising statistical differences at specific matched locations along tibial length representative of overall effect of genotype on cortical thickness. Red $p \leq 0.0001$, yellow $p \leq 0.001$–0.01, green $p \leq 0.01$–0.05, and blue $p \geq 0.05$. (C-D) Displays BMD mapped across bone length, with minimal differences observed in male CSPα$^{-/-}$ versus CSPα$^{+/+}$, whereas female mice possess deficits across the entire tibia. N=5 p/group. Data shown as mean ± SEM.

P33

Are Ethnic Differences in Muscle Mass and Grip Strength Explained by Markers of Adiposity or Inflammation in a Tri-Ethnic UK-Based Cohort?

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Abstract

Background

Declines in musculoskeletal health with age are of major concern. Global and ethnic variation in muscle mass and strength are known to exist; differences in body composition, including adiposity, a source of inflammation, may explain the differences.

Aim

To investigate whether ethnic differences in muscle mass or grip strength are explained by markers of central adiposity or inflammation in the Southall and Brent Revisited Study (SABRE), a tri-ethnic UK-based cohort.

Methods

SABRE consists of men and women of European, South Asian and African Caribbean origin. At baseline, SABRE included South Asian and African Caribbean migrants to the UK; at study visit 3, presented here, additionally recruited participants included partners of participants. The 422 men and 329 women included had measures of grip strength and DXA body composition. Linear regression was used to determine ethnic differences in appendicular lean mass (ALM) and grip strength, and whether body mass index (BMI), markers of central adiposity (visceral adipose tissue mass or android to gynoid fat mass ratio) or inflammation (log(IL-6) or log(CRP)) attenuated those differences (all models adjusted for age and height, except the model containing BMI which was adjusted for age only). Results are presented as age- and height-adjusted standardised beta coefficient (95% confidence interval) unless otherwise stated; European was the referent group.

Results

Mean (SD) age and BMI were 74.9(5.7) years and 27.3(3.9) kg/m2 in men and 70.7(6.9) years and 28.6(5.1) kg/m2 in women. ALM and grip strength were lower in South Asian men (ALM: -0.45(-0.59,-0.30); grip strength: -0.25(-0.43,-0.07)) and higher in African Caribbean men (ALM: 0.84(0.65,1.02); grip strength: 0.30(0.08,0.53)) compared to European men. In African Caribbean men the difference in grip strength was reduced after adjustment for BMI (0.21(-0.02,0.45)). ALM and grip strength were higher in

African Caribbean women (ALM: 0.99(0.81,1.17); grip strength: 0.53(0.33,0.73)) and, in South Asian women, ALM was similar and grip strength lower (ALM: -0.19(-0.39,0.02); grip strength: -0.28(-0.50,-0.06)) compared to European women. The markers of central adiposity or inflammation did not attenuate ethnic differences in ALM or grip strength in men or women.

Conclusions

Ethnic differences in ALM and grip strength were not explained by markers of central adiposity or inflammation. It is important to further understand how ethnic differences in muscle mass and strength impact falls and fracture in later life, and the role of muscle composition including intermuscular fat.

P34

Describing the genetic architecture of minimum joint space width at the hip joint.

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Abstract

Purpose

Cartilage is vital for joint function with its degeneration being a key feature of osteoarthritis (OA). Minimum joint space width measured on X-rays or dual-energy X-ray absorptiometry (DXA) images can provide a proxy for cartilage thickness. A previous genome-wide association study (GWAS) found four loci associated with $mJSW_{X\text{-}ray}$. The UK Biobank Study (UKB) has undertaken a large imaging study involving high resolution hip DXAs. This study aimed to (i) conduct a GWAS of $mJSW_{DXA}$ in UKB, (ii) meta-analyse this with previous GWAS of $mJSW_{X\text{-}ray}$ to identify novel loci, and (iii) better understand the genetic architecture of mJSW.

Methods

$mJSW_{DXA}$ was obtained from high resolution left hip DXA images in UKB using a novel automated method. $mJSW_{X\text{-}ray}$ was previously obtained in three additional cohorts: The Rotterdam Study, Osteoporotic Fracture in Men Study, and Study of Osteoporotic Fractures. GWASs of standardised mJSW residuals (adjusted for age, sex, genotyping array and ancestry principal components) were completed by each cohort and meta-analysed using METAL. Independent mJSW-associated single nucleotide polymorphisms (SNPs) were identified using GCTA-COJO and followed-up in a GWAS of height, estimated bone mineral density (eBMD), chronic hip pain and hip OA (HOA). Genetic correlations were examined using linkage disequilibrium score regression.

Results

50,745 individuals were included. 42 conditionally independent loci were identified (38 were novel), which together explained 4.6% of mJSW variance. mJSW was genetically correlated with height (rg 0.28 [95% CI -0.22-0.33]) and eBMD (0.10 [-0.04-0.17]), but not with HOA (0.09 [-0.03-0.20]) or chronic hip

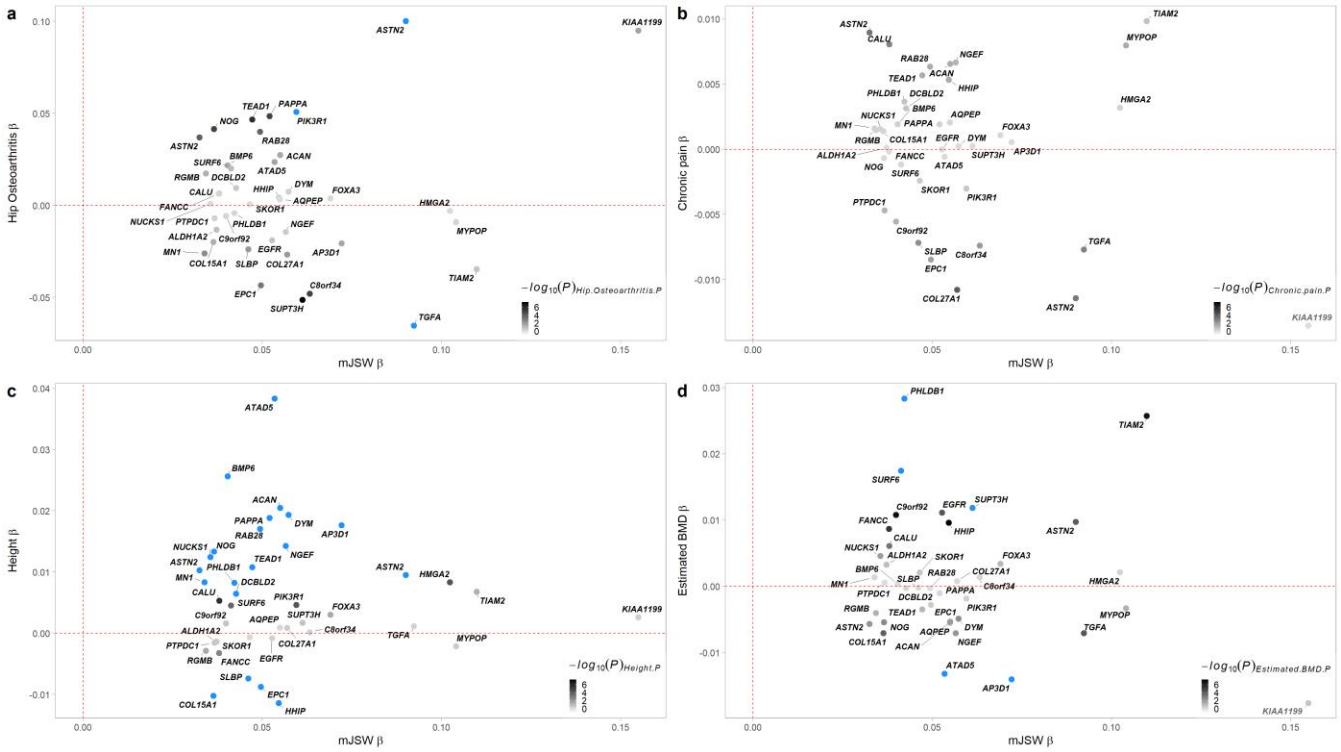
pain (0.00 [-0.07-0.06]). Several loci (*TGFA, SUPT3H, C8orf34, EPC1*) appeared to both increase mJSW, and protect against HOA and chronic hip pain (Figure 1). In contrast, other loci (

) which increased mJSW also increased HOA risk. Interestingly, the latter loci all showed positive associations with height.

Conclusions

In the largest mJSW GWAS meta-analysis to date we found 38 novel mJSW loci. Overall, mJSW showed little genetic correlation with HOA and chronic hip pain but rather stronger correlations with height and eBMD. Locus-based analyses suggested one group of mJSW loci reduces HOA risk via increased mJSW, suggesting possible utility as targets for chondroprotective therapies. A second group increases HOA risk despite increasing mJSW. The latter group is also associated with increased height, a recognised risk factor for HOA, suggesting this contributes to mJSW and HOA risk via an entirely separate, growth related pathway.

Figure 1. mJSW loci look up in other traits



P35

Development of Musculoskeletal Deficits in Children with Cystic Fibrosis Occur in Later Childhood

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Abstract

Cystic fibrosis (CF) is a genetic condition primarily affecting the respiratory system, with the associated progressive lung damage and loss of function resulting in reduced lifespan. Bone health is also impaired in CF, leading to much higher fracture risk even in adolescence. However, the development of these deficits during growth and contributions of pubertal development, body size and muscular loading remain relatively unexplored.

Therefore we recruited 25 children with CF (10 girls, age 11.3±2.9y) and 147 children without CF (75 girls, age 12.4±2.6y). Bone characteristics were assessed using peripheral quantitative computed tomography (pQCT) at 4% and 66% distal-proximal tibia - muscle cross-sectional area (CSA) and density (an indicator of muscle quality) were also assessed at the latter site. Tibia bone microstructure was assessed using high-resolution pQCT (HR-pQCT) at 8% distal-proximal tibia length. In addition, peak jump power and hop force were measured using mechanography. Group-by-age interactions and group differences in bone and muscle characteristics were examined using multiple linear regression, adjusted for age, sex and pubertal status and in additional models, height and muscle force – α-level was set at $P<0.05$.

In initial models, group-by-age interactions were observed for distal tibia total bone mineral content (BMC) and trabecular bone mineral density (BMD), with a lower rate of age-related accrual evident in CF. From HR-pQCT, similar patterns were observed for trabecular number and thickness, and cortical CSA. In the tibia shaft, group-by-age interactions indicating slower growth in CF were evident for total BMC and cortical CSA, whilst age-independent deficits in CF were observed for several other variables including peak jump power and hop force. Group-by-age interactions for bone were partially attenuated at distal tibia and fully attenuated at the tibia shaft by adjustment for muscle force.

These results suggest that bone and muscle deficits in children with CF develop throughout later childhood, independent of differences in pubertal development and body size. These diverging growth patterns appear to be mediated by differences in muscle function, particularly for the tibia shaft. Given high fracture risk in this population from childhood onwards, development of interventions to improve bone health would be of substantial clinical value.

P36

The Effects of Physical Activity on Bone Architecture in Adulthood: A Systematic Review of Peripheral Quantitative Computed Tomography Studies

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Abstract

Introduction: Physical activity (PA) has an important role in the prevention of osteoporosis and can improve and maintain bone mass in adulthood. Previous systemic reviews focused on the influence of PA on bone mineral content and bone mineral density, however, previous systemic reviews on PA and bone have largely focused on the influence of PA on bone mineral content and density assessed via DXA rather than bone architecture assessed by peripheral quantitative computed tomography (pQCT).

Purpose: The aim of this systematic review was to determine the effects of PA on bone architecture measured by pQCT in healthy adults.

Methods: The systematic review was undertaken in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines. Observational and interventional studies that evaluated the effects of PA on bone outcomes measured by pQCT among healthy adults aged 18 and older were included. Newcastle-Ottawa Scale (NOS) was used in the quality assessment of all included studies.

Results: After screening a total of 374 records, 24 articles met the inclusion criteria and the total number of participants included was 3402 (968 males). All studies included participants with a mean age between 19 and 78 years. The sample size varied from 20 to 1013 participants. From the 24 studies, 5 randomized controlled trials, 15 cross-sectional studies, and 4 longitudinal observation studies were included. According to the methodological quality assessment, 66.7% of studies (n=16) were rated as “high quality”. A positive relationship between PA and cortical thickness at loading sites was reported by 18 studies. Participants engaging in high-impact PA (such as running, jumping, and ball games) showed 8 -15% higher cortical thickness at the tibia for both young and elderly adults compared with inactive peers in both females and males. The correlations between PA and trabecular parameters were reported in 6 studies, participation in bone-specific PA, especially high-intensity exercises (such as combat training and soccer), was positively associated with the trabecular thickness and trabecular number in adults.

Conclusion: Participation in high-intensity PA is positively related to bone architecture outcomes in adults. There was large heterogeneity in the studies identified indicating that potential covariates, such as body composition, sex, and age, might affect the impacts of PA on bone microarchitecture outcomes in adults.

P37

Investigating the level of inter-practice variation in denosumab prescribing across primary care in the Buckinghamshire NHS Trust

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Abstract

Introduction

Within the Buckinghamshire NHS Trust, denosumab is recommended as a treatment for the prevention of osteoporosis, in accordance with NICE guidelines. Recently, there has been a significant shift towards primary care management of osteoporosis and administration of denosumab by practice staff once recommended by secondary care. Capturing inter-practice variation in denosumab prescription is crucial in identifying the potential inequities in osteoporosis prevention. Targeted interventions may be identified thereafter to ensure follow-up of these patients, with potential for regional scale-up.

Aims

To assess the level of inter-practice variation of denosumab prescribing across primary care practices within the Buckinghamshire NHS Trust.

Methods

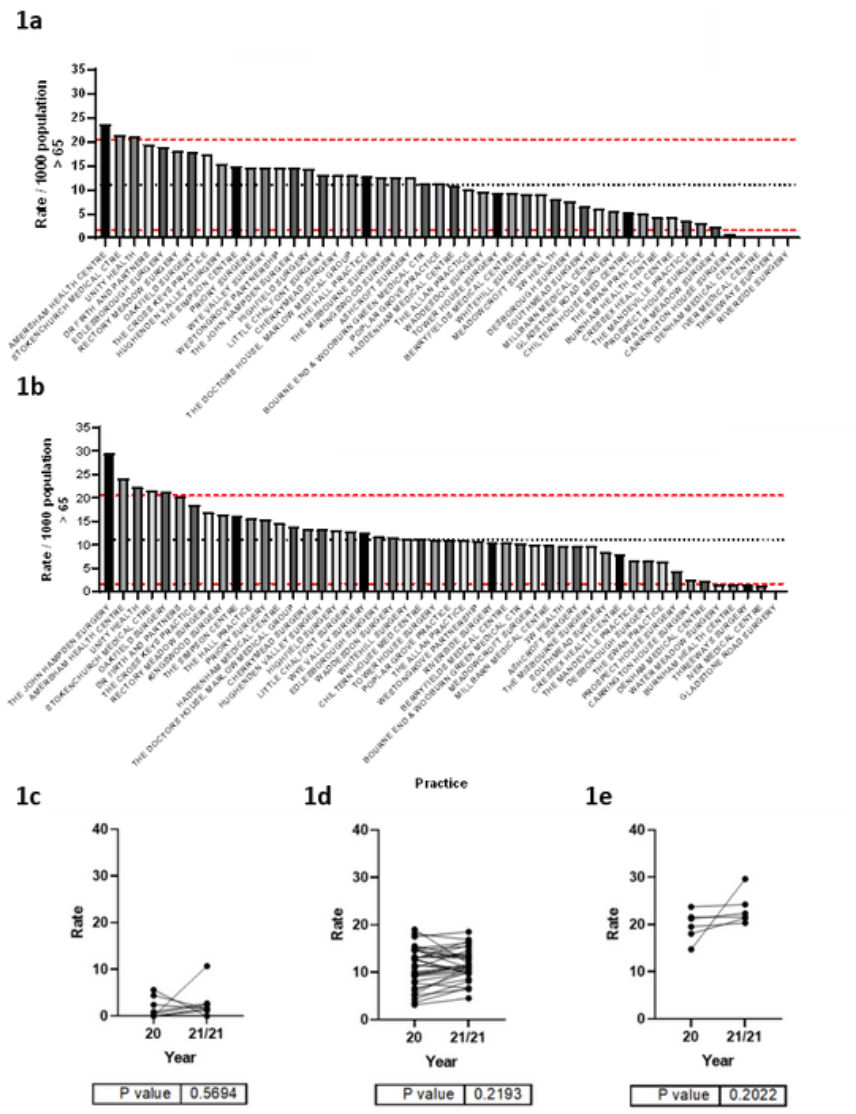
The *OpenPrescribing* database was used to collect crude prescription counts for denosumab and grouped by primary care practices within the Buckinghamshire NHS Trust. List size data was collected from NHS Digital and total population ≥65 years of age was generated. Descriptive statistics were employed to explore the inter-practice heterogeneity in denosumab prescribing.

Results

Between 2020 and 2021, there was a wide variation in the rates of denosumab prescribing across practices within the Buckinghamshire NHS Trust. The total mean (±1.5 SD) prescribing rate per 1000 patients across the Trust was 11.28 (±8.60) (fig. 1a) and 11.81 (±9.34) (fig. 1b) for 2020 and 2021, respectively. Grouping practices into those with rates of less than 2 per 1000 and greater than 20 per 1000 (in either 20/21) revealed largely stable prescribing practices between 2020 and 2021 (fig. 1c, d, e). Practice outliers with higher than the total mean prescribing rates were more likely to remain high yield prescribers year-on-year (fig. 1e, p=0.2022) with the same true for practices with the lowest prescription rates (fig. 1c, p=0.5694).

Discussion

This study demonstrates the variation that exists at the practice-level for denosumab administration. Further exploration into the factors related to this variation is warranted and subsequent solutions could be scaled up to incorporate other regions. Whilst this study can help to visualise the marked difference in denosumab prescribing across the Trust, the nature of this data provides little information on the patient- and practice-level factors which could be contributing to the observed variation. Further studies exploring denosumab follow-up post-initiation in secondary care are warranted.



P38

Current approaches to secondary prevention after hip fracture in England and Wales — results from the National Hip Fracture Database

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Abstract

INTRODUCTION

National clinical audit seeks to enhance the quality of care of the 75,000 people who break their hip in the UK each year. A key aim for the National Hip Fracture Database (NHFD) is to encourage secondary fracture prevention through bone health assessment and the appropriate provision of anti-osteoporosis medication (AOM). We set out to describe trends in anti-osteoporosis medication prescription, and to examine the types of oral and injectable AOM being prescribed both before and after a hip fracture.

METHODS

We used data freely available from the NHFD www.nhfd.co.uk to analyse trends in oral and injectable AOM prescription across a quarter of a million patients presenting between 2016 and 2020, and more detailed information on the individual type of AOM prescribed for 63,705 patients from 171 hospitals in England and Wales who presented in 2020.

RESULTS

Between January 2016 and December 2020, NHFD annual reports have presented data from a quarter of a million patients over the age of 60. Patients' mean age was 83 years and the majority (70.5%) were women. Most patients (88.2%) are not taking any AOM when they present with hip fracture. Half of all patients (49.9%) were prescribed AOM treatment by the time of discharge, but the proportion deemed 'inappropriate for AOM' varied hugely (0.2-83.6%) in different hospitals. Nearly two thirds (64%) of those who were previously taking an oral bisphosphonate were simply discharged on the same type of medication. The total number of patients started on oral medication fell by 11.4% over 5 years. The number started on injectable AOM almost doubled to 14.4% over the same period, but remains hugely variable across the country, with rates ranging 0-67% across different units.

CONCLUSION

A recent hip fracture is a strong risk factor for future fractures. If teams are to learn from each other's experience and patients are to be protected against further fragility fractures the huge variability in approaches, and in particular to the use of injectables, in different trauma units across England and Wales requires further investigation.

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Analysis of monitoring practices by Fracture Liaison Services using the IOF Best Practice Framework

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Abstract

Despite robust evidence for the effectiveness of secondary fracture prevention, translation in the real-world setting remains disappointing. One key aspect is improving medication adherence through monitoring. There is little guidance for the optimal methods for monitoring patients. Our objective is to describe the current delivery of monitoring in the FLS setting as a first step to inform a clinical best practice guide for monitoring patients.

We used the International Osteoporosis Foundation (IOF) Capture the Fracture Best Practice Framework questionnaires from 461 FLSs across forty-eight countries. The questionnaire includes how FLSs were undertaking patient monitoring in their setting. The reported monitoring components were reported globally and by comparing concordance within a country.

Of the 461 FLSs surveyed, 89% reported a plan for evaluating adherence to treatment recommendations. Approximately 63% of FLS used more than one method of monitoring. 17% of FLSs delivered monitoring alone, 9% conducted monitoring in conjunction with the GP, 20% in collaboration with the speciality doctor and in 16% of FLSs, both the GP and speciality doctor were involved in monitoring.

Only 13% (n=61) of FLSs monitored patients at both less than 12 months and more than a year after the fracture and so met the gold standard. Twenty percent (n=91) monitored patients only 12 months following a fracture and met the bronze standard. Sixty-seven percent (n=309) did not report meeting either of these targets.

FLSs used different modalities to follow patients, including clinic review (69%), DEXA (43%), telephone interview (43%), prescription review (41%) and novel methods, for example, use of social media (WhatsApp or Facebook) or postal questionnaires (16%). Re-evaluation included questions on medication adherence, unwanted effects, refractures, fracture risk factors, recurrent falls and other individualized questions by each FLS which included nutrition, height loss, use of functional scales and questionnaires such as the EQ-5D.

In twenty-four countries, there were more than five or more responses within a country. When analysed within the country, we observed marked variability across all aspects of monitoring within some countries and similarities within other countries.

The variation in monitoring practices by FLSs globally highlights the need for best practice guidelines that can be applied globally. Further work is needed to describe the effect of different monitoring pathways on patient adherence.

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Gender differences in osteoporosis screening: retrospective analysis

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Abstract

Background:

Osteoporosis is one of the most common diseases to affect the skeleton, especially in the elderly population. In this study, we retrospectively evaluate the patients' characteristics who underwent DXA scan in a tertiary hospital in the southern part of Saudi Arabia.

Methods:

The data of 189 patients with unknown underlying diseases who underwent DXA scan in the last year were collected. These data include ag, sex, weight, height, scanned area, aBMD, Z score, T score and the diagnosis. The data were analysed using SPSS.

Results:

There were 48 (25%) male and 141 (75%) female. The age of the patients ranged from 18 to 91 years and the median was 58 years. The DXA measurements were taken at the femoral head and/or the lumber spine. Among the 189, there were 62 (32%) normal scans (49 female), 74 (39%) osteopenia (57 female) and 53 (28%) osteoporotic (35 female). The median age for osteopenia group was 55 (52 for male, 58 for female). The median age for osteoporosis group was 57 (49 for male, 57 for female). BMI did not affect the diagnosis in this cohort.

Conclusion:

The prevalence of osteoporosis among our cohort is comparable to the osteoporosis prevalence among middle eastern ethnicities reported in the literature. Although osteoporosis affected smaller number of men compared to women in our cohort, men tend to get osteoporosis younger than women.

P41

Associations of anthropometry and lifestyle factors with bone mineral density of the skull are weaker than at other skeletal sites in children

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Abstract

Background

Whole-body-less-head (WBLH) is the recommended site for dual-energy X-ray absorptiometry (DXA) assessment of bone mineral density (BMD) in children due to the relatively large contribution of the skull to the overall size of the skeleton. Historically it has been suggested that skull BMD is less responsive to stimuli than BMD at other skeletal sites. However, there are few published data to support this notion. We therefore compared the associations of BMD at various skeletal regions of interest (ROI) with anthropometric, body composition, diet and activity variables in children.

Methods

Children from the Southampton Women's Survey (SWS) mother-offspring cohort participated at age 6-7 years, including measurement of height, weight and whole body and lumbar spine BMD by DXA (Hologic Discovery). Physical activity was assessed by accelerometry (Actiheart) and diet and fracture history by interviewer led questionnaire. Skeletal ROI examined were whole body, skull, WBLH and lower limbs (all derived from the whole body scan) and lumbar spine. Associations assessed by linear regression are presented as β (95% CI).

Results

1218 children participated (50.7% male, mean (SD) age 6.82 (0.32) years). Skull BMD was associated with height z-score (0.15 (0.10, 0.21) SD/SD), weight z-score (0.18 (0.13, 0.24) SD/SD), whole body lean mass (0.11 (0.08, 0.14) SD/kg) and daily milk intake (β=0.16 (0.01, 0.31) SD/pint). These associations with skull BMD were weaker than with BMD at the other ROI; for example, the association between lean mass and BMD were 0.32 (0.30, 0.34), 0.38 (0.37, 0.40) and 0.23 (0.21, 0.25) SD/kg for whole body, WBLH and lumbar spine BMD, compared with 0.11 (0.08, 0.14) SD/kg for skull BMD. Relationships with whole body BMD were attenuated compared to with WBLH BMD.

Conclusion

Skull BMD was less strongly associated with anthropometry, body composition and dietary variables than for other DXA sites. These findings support, and importantly provide a quantitative basis for, the recommendation that the skull should be excluded from whole-body DXA analyses in children.

P42

Associations between Trapeziometacarpal joint shape and Thumb Base Osteoarthritis: analysis of the Osteoarthritis Initiative

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Abstract

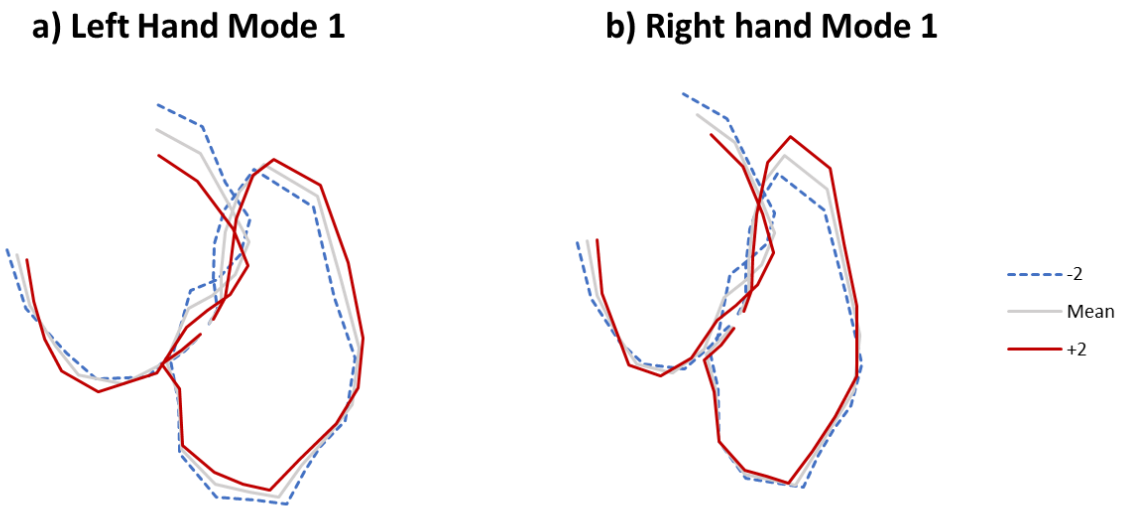
Background: Statistical shape modelling (SSM) has been extensively used to understand the associations between joint shape and osteoarthritis (OA) in both hip and knee. However, associations between trapeziometacarpal joint shape (TCMJ) and OA have not been widely investigated and are poorly understood. The thumb base is reported as being the single most affected joint in hand OA. The aim of this study was to establish a statistical shape model of the TCMJ using bilateral hand radiographs and assess the associations between clinical symptoms, radiographic severity and TCMJ shape.

Methods: 100 individuals with bilateral hand and wrist radiographs taken at baseline of the Osteoarthritis Initiative (OAI) study were selected at random. Clinical information was extracted from the publicly available data. An SSM consisting of 32 points was developed to characterise the TCMJ and applied to both hands. Independent modes of variation were identified using principal component analysis and radiographic severity was graded using the Kellgren-Lawrence (KL) scale. OA severity was categorised as no OA, mild OA and moderate to severe OA based on KL grade. One-way ANOVA with contrasts examined the relationship between mode score and OA severity. Spearmans's Rho correlations examined associations with symptoms such as pain and stiffness using SPSS v27.0. Left- and right-hand TMCJ models were assessed separately.

Results: The study population consisted of 99 participants (67.7% female) with a mean age of 60.9 years (SD ± 9.0 years). One image was withdrawn due to quality issues. The average height was 1671 mm (± 88.2 mm) and an average weight of 81.6 kg (± 17.9 kg). Ten modes of variation were identified in the right hand and nine modes in the left hand. In the left hand three modes were significantly associated with OA severity; modes 1, 6 and 7 (adjusted for age, sex, height and weight), compared to a single mode (mode 1) in the right hand. Mode 1(fig 1) described less prominent distal projection of the lateral trapezium joint surface between thumb and index metacarpals in both hands and was negatively associated pain and stiffness (Right: correlation coefficient -0.228, p=0.023; Left: correlation coefficient -0.186, p=0.066).

Conclusions: TCMJ shape and symptoms of hand OA (pain and stiffness) are correlated and that there are associations with radiographic severity. Joint shape analysis may have a role in the prediction of thumb base OA and further investigation is needed to establish this.

Figure 1: Hand shape mode 1



P43

Comparison between QCT and DEXA in assessing bone density in axial spondyloarthritis (axSpA)

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Abstract

Introduction

In axial spondyloarthritis (axSpA), osteoporosis, osteoproliferation and fragility fractures can occur (often simultaneously) in the same patient. Routinely, DXA scanning, has been used to assess osteoporosis and fracture risk but utility is limited by osteoproliferation, and sometimes osteosclerosis or aortic calcification in the lumbar spine (LSp) scanning field of view. Quantitative computerised tomography (QCT) has been proposed to provide a more accurate predictor of vertebral bone loss and may operate better than DXA, in predicting vertebral fracture in axSpA. This study aims to compare these two scanning modalities in assessing osteoporosis in axSpA patients

Methods

A 2 year retrospective analysis (2016-18) of axSpA patients at our centre who had both LSp quantitative computerised tomography (QCT) and spinal and/or hip DXA undertaken. Data collected included patient demographics, DXA-derived bone mineral density (BMD) measurements and (WHO-grade osteoporosis) classification, LSp QCT data [lumbar vertebra L1 to L4 inclusive (L1-L4)] and radiographic evidence of vertebral fracture. The primary outcome was detection of osteoporosis in each modality using conventional scanning mode definitions (i.e. BMD T-score ≤ -2.5 for DXA; BMD < 80 mg/cm^3 for QCT). In all patients, DXA and CT scans (for QCT analysis later) were completed on average by 26 ± 5 months.

Results

We identified 25 patients eligible for analysis (median age 62 years old; range 46-83; 16 males). There were 17/25 (68%) patients with a diagnosis of ankylosing spondylitis (AS; i.e. *radiographic* axSpA), and 8/25 (32%) with *non-radiographic* axSpA. Notably 13/25 (52%) of patients had QCT data retrieved by retrospectively applying QCT analysis to CT scans requested previously by other clinicians for various (non-musculoskeletal) reasons. Amongst analysed patients, 3/25 (12%) and 20/25 (80%) met osteoporosis diagnostic threshold through DXA scanning and QCT respectively - thus the prediction of osteoporosis, by the two scanning modalities, was significantly different (McNemar test, $P<0.001$). However, there was no significantly increased risk of vertebral fracture associated with osteoporosis diagnosis by DXA [$X(1) = 2.273$, $p<0.250$] or QCT [$X(1)=1.042$, $p<0.358$].

Conclusions

QCT may provide a better predictor of low bone mass in the LSp of axSpA patients than DXA scanning, however larger studies are required to interrogate the performance of scans in predicting vertebral fracture.

P44

Characterisation of Diagnostic Collagen Phenotypes in Human Osteosarcoma Biopsies Using Second Harmonic Generation Imaging

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Abstract

Intro/Aims: Osteosarcoma (OS), the most common primary cancer of bone, typically describes highly aggressive paediatric tumours characterised by an aberrant extracellular matrix (ECM)[2]. The poor prognosis is in part a result of delayed diagnosis. Current diagnostic modalities lack the sensitivity required for detection of micro-metastases – hugely problematic as chances of 5-year survival decrease drastically for individuals with metastatic disease (>20%)[1]. In recent years, novel, label-free approaches have been explored in the oncology field for rapid, non-invasive detection of tumours by identifying pathologically altered matrix components – such as collagen. Herein, we aim to investigate the potential of second harmonic generation (SHG) microscopy for distinction of osteosarcoma from healthy bone.

Method: SHG imaging was employed to examine paraffin embedded human bone and OS (stage IIB) biopsies (n=3 female, n=3 male). Image analysis by ImageJ and CTFIRE enabled quantification of a number of matrix-collagen parameters including fibre length- (mm) and fibre orientation-based (p.d.u) metrics.

Results: Optimisation of SHG imaging revealed diagnostic collagen phenotypes enabling differentiation between non-diseased bone and OS biopsies. Field of view (FOV) optimisation identified the longest collagen fibres in human cortical bone were optimally extracted with a FOV of 700 mm2 or more. Both female and male OS biopsies exhibited significantly shorter collagen fibres compared to normal bone counterparts, evidenced by average fibre length (female bone: 29.39 mm ± 1.25, female OS: 23.18 mm ± 0.33, p = 0.0006***; male bone: 27.87 mm ± 0.61, male OS: 24.39 mm ± 0.76, p = 0.016*), maximum fibre length (female bone: 301.36 mm ± 28.41, female OS: 109.64 mm ± 2.33, p = 0.0055**; male bone: 270.53 mm ± 54.66, male OS: 136.65 mm ± 37.14, p = 0.030*) as well as a number of curve fitting parameters quantified from the total distribution of fibre lengths exhibited. Additionally, orientation-J analysis of collagen fibres demonstrated an altered distribution of collagen fibre orientations in both female and male OS compared with non-diseased bone.

Conclusion: Our findings validate the potential for label-free diagnosis of OS using SHG microscopy and illuminate aberrant collagen metrics – namely fibre length and fibre orientation – as part of an

envisioned ‘diagnostic signature’ of OS that would enable label-free diagnosis of the cancer in clinical settings.

References:

1. Lindsey, Markel and Kleinerman (2016). doi: 10.1007/s40744-016-0050-2
2. Shapiro and Eyre (1982). doi: 10.3109/01913128309140786

P45

Severity of acute phase reaction in children receiving the first dose of Zoledronic acid – the impact of underlying condition

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Abstract

Introduction

Zoledronic acid (ZA), a third-generation intravenous bisphosphonate, is used for the treatment of children with primary and secondary osteoporosis at our institution. ZA has a recognised acute phase reaction (APR) with fever, myalgia, fatigue, or nausea, usually occurring within 48 hours of the first infusion. Hence, our institution's policy is to admit for monitoring all children for their first ZA infusion.

Objectives

1. To determine if the APR with the first dose of ZA warrants hospital-level care.
2. To determine if the severity of the APR correlates with the underlying condition.

Methods

Retrospective data of patients who received their first ZA infusion as inpatients between 2017 and 2021 was obtained from electronic records, including indications for infusion and relevant comorbidities. Severity of APR was assessed from the Early Warning Score (EWS) obtained from the nursing observations, need for critical care, and length of hospital stay. The EWS is a clinical observation tool for identification of patients at risk of deterioration, and measures respiratory, cardiovascular, and neurological parameters. A score of ≥4 requires senior medical review.

Results

Eighty-four patients were included, with mean age 9.6 years (range: 2-18 years). A peak EWS of ≤3 was found in 84% of children. Mean peak EWS by indication for ZA was 0.8 for osteogenesis imperfecta (OI; n=30), 0.9 for "other" primary bone disorders (n=8), 1.3 for immobilisation osteoporosis (n=17), 2.0 for idiopathic juvenile osteoporosis (n=8), 2.3 for steroid-induced osteoporosis and systemic inflammatory disorders (n=9), and 3.2 for Duchenne muscular dystrophy (DMD, n=12). The EWS differed significantly among the various groups (p=0.024) and the difference was most marked between the patients with OI and DMD (p=0.003). Only two patients required intravenous fluids, of whom one required critical care and prolonged hospital stay for 10 days. She had an underlying diagnosis of systemic juvenile idiopathic arthritis and received methotrexate and tocilizumab. These factors might have contributed to her exacerbated reaction. Three of 14 patients (21%) with epilepsy experienced increased seizures on the day of infusion.

Conclusion

Most patients receiving their first dose of ZA had a mild APR that did not require hospital-level care. However, patients with DMD in particular seem to suffer more severe APR and may warrant in-patient monitoring with the first infusion, as may children with brittle epilepsy. A change in the institution policy can thus be brought about to reduce the health expenditure.

P46

EVALUATION OF A MULTINATIONAL FLS MENTORSHIP PROGRAMME IN LATIN AMERICA

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Abstract

Despite effective treatments and service models to deliver secondary fracture prevention, there is a significant gap in the capacity and capability in many countries. We here describe the initial findings from a global activity to develop national communities of FLS mentors to support local FLS initiation and sustainable delivery in Latin America.

Resources for mentorship training around FLS knowledge, adult education and quality improvement were developed. In coordination with the IOF regional team and Capture the Fracture® Partnership (CTF-P), 3- 4 mentors were selected and trained per country using 90-minute online sessions. Following training, monthly support meetings were delivered. The programme was evaluated using pre-training needs assessment, post-training evaluation and a post-training annual survey.

Eleven mentors were successfully selected and trained in Mexico, Brazil, and Colombia. There was 100% attendance and completion of tasks. The impact of these communities of mentors across three countries has led to 42 new FLS getting mapped (vs 55 FLS at the end of 2019 in Latin America), 33 of which are getting started while 23 are becoming more effective. Post-training activities included best practice guidelines policy tools as well as other resources for FLSs in the local language.

Despite the COVID pandemic, CTF-P mentorship pillar has developed and implemented a learning community of FLS mentors and provides a scalable platform to develop mentors further across the globe.

P47

Mineralisation in the Ageing Skeleton: Development or Disease?

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Abstract

The developing skeleton undergoes significant changes in mineralisation as a child grows, but it is currently difficult to discern if these changes are associated with growth, or disease. The current, clinically available, technologies for analysing bones without surgery are limited to ionising imaging techniques which only observe the macroscopic structure. There is need for new, non-invasive, diagnostic tools that are capable of establishing the chemical composition of bones: to facilitate a better understanding of any chemical changes that occur as a result of mineralisation pathologies such as Osteogenesis imperfecta (OI) and X-linked hypophosphatemic rickets (XHPR).

Raman spectroscopy is a monochromatic, non-ionising, laser-based technology, which this study aims to develop for use as a diagnostic tool for children with mineralisation associated bone diseases. It determines bone's biochemical fingerprint by measuring the energy exchange between the Raman laser, and the molecular bonds of a sample. All chemicals have a unique energy exchange expressed as a wavenumber, thereby allowing the chemical composition to be determined. The initial stage of this research focused on establishing baseline measurements from archaeological human bone, before analysing clinical samples. The aim of this initial research was to detect changes in chemical composition with age.

Lower lumbar vertebrae (L3-L5) from nine adults of three age groups (18-25, 25-45 and 45+ years) were procured from Thornton Abbey. These samples were analysed using Raman spectroscopy at three areas of high biomechanical stress. Results demonstrated age-related changes in both carbonate (1070cm-1) and hydroxyapatite (960 cm-1); minerals essential to bone function. These results suggest that it is possible to detect age associated changes in bone mineral using Raman spectroscopy.

The next stage applies the technique to clinical samples provided by Royal Manchester Children's Hospital from juvenile patients with OI and XHPR, as well as patients with Cerebral Palsy: a condition known to impact skeletal development. These are being compared to healthy, control, juveniles whose samples are obtained when surgery is required for trauma. Showing age-related mineral changes in archaeological bone using Raman spectroscopy was an important first step before investigating the hypothesis that analyses of juvenile bone using Raman spectroscopy will reveal chemical differences between 'normal' juvenile bone growth and pathological changes in mineralisation. The overall aim of these studies being to improve understanding of bone chemistry patterns in both ageing and disease, and to begin to develop Raman spectroscopy as a diagnostic tool for such conditions.

P48

Radiological and histomorphometric characteristic of two peripubertal X-Linked Hypophosphatemic children treated with burosumab till the end of growth

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Abstract

Introduction: Burosumab heals rickets and osteomalacia in children and adolescents with X-linked hypophosphatemia (XLH) as demonstrated by improvement in biochemical markers and radiographic changes {Rickets severity score (RSS) and Radiographic Global Impression of Change (RGIC) scores}. Radiographic changes of lower limb deformities in peripubertal children treated with burosumab and histomorphometry when they reach the end of growth have not been previously described.

Objectives: In two peripubertal children treated with burosumab we report radiographic changes and histomorphometry at the end of growth.

Methods: Two peripubertal children {patient 1 (P1): male aged 10 years, Tanner stage 1 and patient 2 (P2): female aged 10.5 years, Tanner stage 2}, previously managed with oral phosphate and active vitamin D analogues since 1-year of age, were treated with burosumab for a duration of 6 years. Radiographs were assessed for improvement in rickets and deformities. Tetracycline labelled trans-iliac bone biopsy was performed at the end of growth, at the time of lower limb deformity correction surgery.

Results: Both patients, over 6 years of treatment, showed normalisation of biochemical markers of rickets on burosumab. RSS score improved from 4 to 0.5 on P1 and 3 to 0.5 in P2. Genu valgum worsened with an increase in intercondylar distance from 16 cm to 23.3 cm and 19 cm to 20 cm on P1&2 respectively. Histomorphometry demonstrated the size of the specimen was above the age-related mean (116% in P1 and 106% in P2); cortical width was increased by 197% on P1 and 125% on P2; osteoid thickness was marginally above the reference range (8.6mm on P1 and 8mm on P2 vs control 6.3±1.0) however, osteoid volume/bone volume was within the normal range in both patients (2.8 on P1 and 1.1 on P2 vs control 2.2±0.9) suggesting substantial healing of osteomalacia. Both patients had mild mineralization defects with the persistence of periosteocytic hypomineralized lesions as expected in XLH patients.

Conclusion: Burosumab commenced on peripubertal children led to substantial healing of rickets and osteomalacia, which was confirmed by bone histomorphometry. However, residual lower limb deformity required surgical correction.

P49

Lower limb bone geometry in adult individuals with X-linked hypophosphatemia: an observational study

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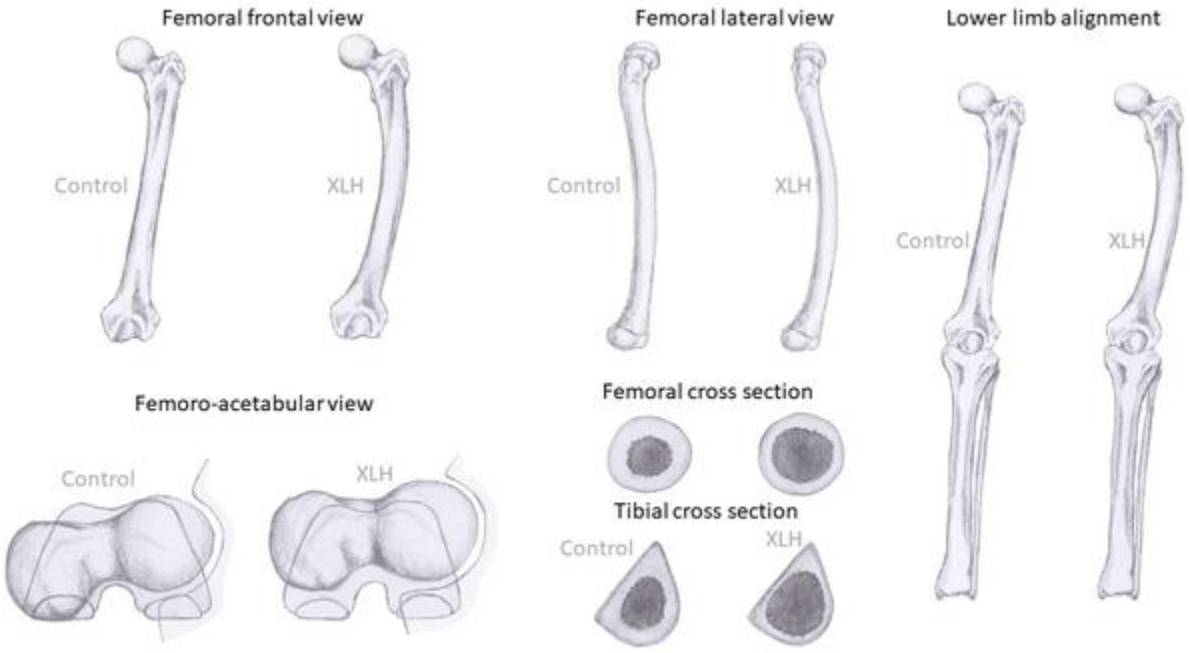
Abstract

Purpose: Individuals with X-linked hypophosphatemia (XLH) are at risk of lower limb deformities and early onset of osteoarthritis. These two factors may be linked, as altered biomechanics is a risk factor for osteoarthritis. This exploratory evaluation aims at providing clues and concepts for this association to facilitate future larger scale and longitudinal studies on that aspect.

Methods: For this observational study, 13 patients with XLH, aged 18-65yr (6 female), were compared with sex age and weight matched healthy individuals at a single German research centre. Femoral and hip joint geometry, including femoral and tibial torsion, femoral and tibial shaft bowing, bone cross-sectional area (CSA), acetabular version and coverage measured from magnetic resonance imaging (MRI) scans.

Results: Total femoral torsion was 29° lower in individuals with XLH than in controls ($p<0.001$), mainly resulting from lower intertrochanteric torsion (ITT) ($p<0.001$). Femoral lateral and frontal bowing, tibial frontal bowing, mechanical axis, femoral mechanical-anatomical angle, acetabular version and acetabular coverage were all greater and tibial torsion lower in individuals with XLH as compared to controls (all $p<0.05$). Greater femoral total and marrow cavity CSA, and greater tibial marrow cavity CSA and lower cortical CSA were observed in XLH (all $p<0.05$).

Discussion: We observed large differences in clinically-relevant measures of tibia and particularly femur bone geometry in individuals with XLH compared to controls. These differences may plausibly contribute to clinical manifestations of XLH such as early onset osteoarthritis, pseudofractures and altered gait and therefore should be considered when planning corrective surgeries.


Femoral frontal view
Control
XLH
Femoral lateral view
Control
XLH
Lower limb alignment
Control
XLH
Femoro-acetabular view
Control
XLH
Femoral cross section
Tibial cross section
Control
XLH

P50

Valuation of lost productivity for people with osteogenesis imperfecta: preliminary results from the RUDY cohort

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Abstract

Background

Osteogenesis imperfecta (OI) is a genetic condition that causes bone fragility due to abnormal Type 1 collagen. Depending on the severity, the condition might affect people's ability to work or impact their productivity if they do. There are no studies quantifying the loss of productivity due to ill health for people with OI in the UK.

Objective

To characterise and evaluate the cost of lost productivity for individuals with OI using the valuation of lost productivity (VOLP) questionnaire.

Methods

We extracted data from RUDY, a rare disease cohort study in the UK, from 2020-2021. Participants completed the VOLP questionnaire which measures absenteeism, presenteeism, and unemployment due to ill health, lost unpaid work, and the overall monetary cost of lost productivity to society over the three months prior. The sample was comprised of adults diagnosed with OI who had completed a VOLP questionnaire at baseline. RUDY participants were invited to complete a baseline questionnaire and then subsequently a follow-up questionnaire every three months.

Results

31 participants completed a VOLP questionnaire at baseline. Just under half were employed (48.4%) and of those unemployed, half reported being unemployed due to their ill-health.

Of those who completed a follow-up questionnaire, everyone who was employed at baseline remained employed at follow-up and everyone who was unemployed at baseline remained unemployed (see Figure 1).

At baseline, the estimated cost of paid work productivity loss over three months per participant was £2,397 (£799 per month on average). The main driver of costs was days lost due to unemployment caused by ill-health, which totalled £2,377 per participant over the period. Presenteeism accounted for a small part of the overall cost of productivity (£20). No absenteeism was reported at baseline.

The average cost of unpaid productivity loss in the previous three months was £353 per participant and the sample averaged a loss of 3.35 unpaid work hours per week.

Overall, the total cost of lost productivity due to ill health over three months was £2,750 (£917 per month on average) for participants diagnosed with OI.

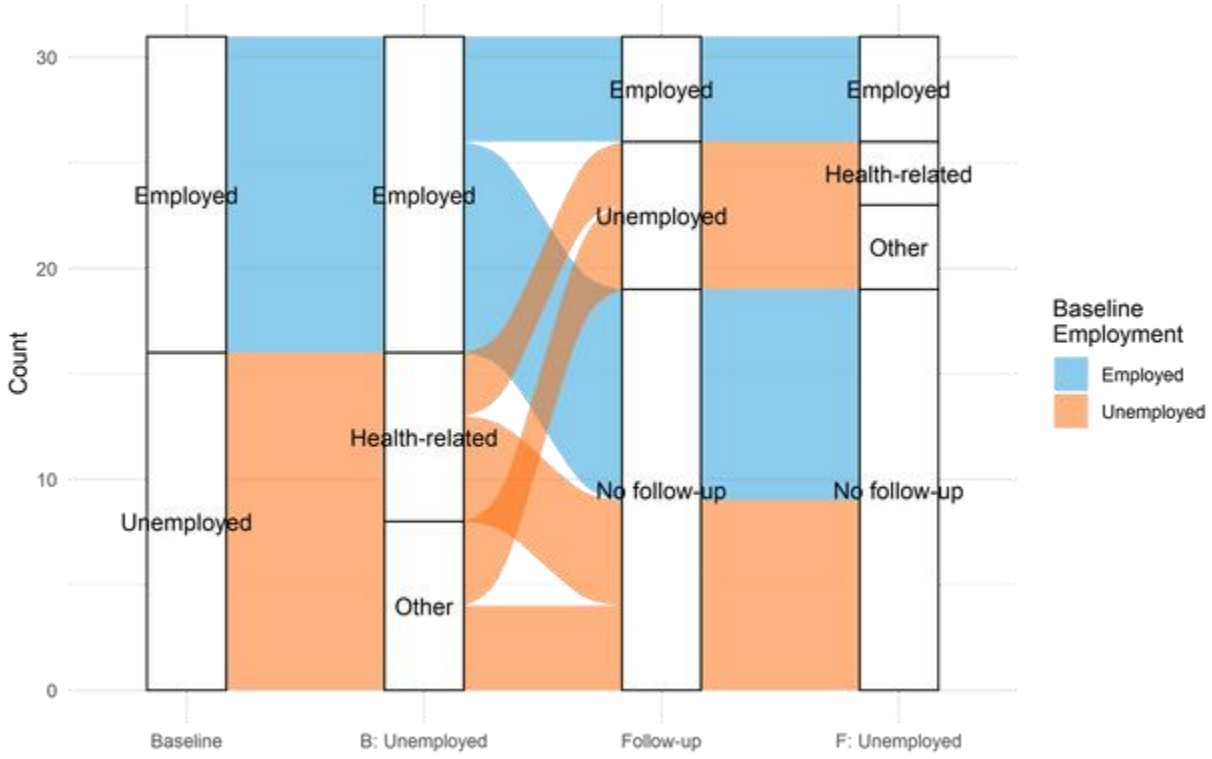
Conclusion

Many participants with OI did not report any absenteeism or presenteeism at baseline. Unemployment due to ill-health is the key driver of the cost of lost productivity for people with OI. Further research is needed to investigate how the cost of lost productivity for people with OI changes overtime.

Acknowledgement

We would like to thank the RUDY Study.

Figure 1.



P51

IPN60130 for the Treatment of Fibrodysplasia Ossificans Progressiva: Methodology of the Randomized, Double-Blind, Placebo-Controlled Phase II FALKON Trial

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Abstract

Objectives:

Fibrodysplasia ossificans progressiva (FOP) is an ultra-rare genetic disorder caused by *ALK2/ACVR1* mutation and characterized by heterotopic ossification (HO) and progressive disability. IPN60130 is a selective ALK2/ACVR1 inhibitor being investigated for FOP treatment.[1] Here, we describe the methodology of the FALKON trial (NCT05039515) designed to compare efficacy and safety of IPN60130 with placebo in patients (pts) with FOP.

Methods:

The FALKON trial is a two-year study whereby pts will be randomized to oral placebo, or low or high dose IPN60130 for the first 12 months. Pts receiving placebo will then transition to IPN60130 so that all pts will receive the active treatment for the second 12 months. Enrollment criteria include: ≥5 years old, FOP diagnosis with disease-causing mutation in the *ACVR1* gene, and either a flare-up, new HO or joint ankylosis, or increase in Cumulative Analogue Joint Involvement Scale (CAJIS) score in the prior year. Recruitment is ongoing to enroll 90 pts. The primary efficacy outcome will be annualized change from Baseline in HO volume to Month 12, assessed by low-dose whole-body computed tomography (WBCT) excluding the head. Secondary efficacy outcomes are presented in the **Table**. Safety will be assessed via adverse event (AE) and serious AE incidence over 25 months. Pts aged ≥15 years will be eligible for a sub-study assessing HO activity by [^{18}F]NaF positron emission tomography-computed tomography (PET-CT).

Summary:

Results from FALKON, estimated to end in August 2025, will allow evaluation of IPN60130 in FOP.

References:

1. Davis A et al. J Bone Miner Res 2019;34(Suppl 1):290

Table: Secondary efficacy outcomes

Timeframe, months[a]	Outcome	Comparison

12	Change from Baseline (CfB) in volume of new heterotopic ossification (HO) lesions[b]	IPN60130 vs placebo
	CfB in number of HO lesions[b]	
	Flare-up rate; number of flare-up days	
	Number of body regions with new HO	
	CfB in pain intensity	
	Proportion of patients with new HO	
24	CfB in HO volume[b]	IPN60130 vs placebo and untreated natural history study (NCT02322255) participants

[a]From Baseline up to the month given; [b]Assessed by low-dose whole-body computed tomography (excluding the head).

P52

Whole-body vibration training in addition to muscle-strengthening exercises alone in improving muscle function in children with Neurofibromatosis Type 1 – a randomised interventional trial

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Abstract

Introduction: Children with Neurofibromatosis Type 1 (NF1) have muscle weakness. Currently no evidence-based intervention exists for improving this. Whole-body vibration (WBV) therapy has been shown to improve muscle function in children with other neuromuscular disorders.

Objectives: This randomised trial investigated whether WBV therapy in combination with muscle-strengthening exercises would improve muscle function compared to muscle-strengthening exercises alone, in children with NF1 who have muscle weakness.

Methods: Children with NF1 aged 6-16 years with evidence of muscle weakness [grip force standard deviation score (SDS) <-1.0] were randomised to daily muscle-strengthening exercises for 6 months (EXER group), or these daily exercises plus a WBV therapy programme for 6 months (EXER+WBV group). The primary outcome was jumping power SDS measured using mechanography, with secondary and pre-determined exploratory outcomes including jumping efficiency, hopping force, grip force, 6-minute walk test, balance, physical activity intensity, perceived fatigue and quality of life. Qualitative data regarding safety, feasibility and compliance were also collected.

Results: Forty-four children were recruited (20 males; age range 6.1-16.5 years, mean age 10.6 years), with equal allocation to EXER group and EXER+WBV group. There was no effect noted on the primary outcome of jumping power SDS between the two groups at the end of the trial (absolute effect size=-0.1, 95% confidence interval -0.5 to +0.3, P=0.53). Similarly, no difference was detected with regards to secondary and exploratory outcomes. No significant changes in muscle function SDS were noted from baseline in either intervention group. Mean reported compliance to muscle-strengthening exercises was 57%, mean measured compliance to WBV therapy was 23%. Compliance was affected by the Covid-19 pandemic and waning of enthusiasm over time. No adverse events were reported.

Conclusion: Six months of daily muscle-strengthening exercises alone or in combination with a WBV therapy programme is safe, although compliance was an issue. No difference in muscle function, balance, physical activity, fatigue or quality of life was detected when WBV therapy was added to daily

muscle-strengthening exercises. However, this was a pilot study, not powered for moderate effect sizes, and therefore larger trials would be needed to determine the true effect.

P53

Does body mass index influence the risk of vertebral fractures in children with osteogenesis imperfecta?

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Abstract

Introduction: Children with osteogenesis imperfecta (OI) may have a tendency towards being overweight or having obesity due to reduced mobility and physical activity. This could theoretically increase the load on their vertebrae, compounding the risk of vertebral fractures, with literature already suggesting an increased risk of long bone fractures with a higher body mass index (BMI).

Objectives: This study investigates whether an association exists between standardised BMI and vertebral fractures in children with OI. With discrepancy in the little data available on mean body mass index standard deviation scores (BMI SDS) for children with OI, we also aimed to investigate the distribution of BMI SDS in this cohort.

Methods: Medical notes and imaging were reviewed of all children with OI known to the Paediatric Endocrinology team at Royal Manchester Children's Hospital. The number of vertebral fractures from the last dual-energy X-ray absorptiometry (DXA) scan or X-ray prior to the commencement of bisphosphonate therapy was recorded. The most recent scan was used if no treatment had been started. Auxological data were obtained from clinic or scan visits. BMI SDS was calculated using the UK population reference data. Overweight and obesity were defined as BMI exceeding the 91st and 98th centiles respectively.

Results: Eighty-six patients (age range 2.3-17.6 years, 52% female) with confirmed OI were included in data analysis. Twenty-six percent of children were classified as being overweight or having obesity. Fifty-seven percent had at least one vertebral fracture. Mean BMI SDS in this cohort was significantly greater than the normal population (0.43; 95% confidence intervals: 0.15–0.70; p=0.002). There was no correlation between mean BMI SDS and the number of vertebral fractures (rho=0.10, p=0.38). There was no association between being overweight or having obesity and having at least one vertebral fracture ($\chi 2$=0.96, p=0.33).

Conclusions: Children with OI have a higher BMI SDS than the general paediatric population. Being overweight or having obesity do not appear to be risk factors for sustaining vertebral fractures in children with OI, however there may be confounding factors, such as physical activity and severity of OI, which may be affecting the results.

LB1

Novel MRI quantitative scores from vertebral signal intensity on T2 correlates with T1 in female patients: Another possible indicator in osteoporosis detection

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Abstract

Background

On MRI, bone marrow signal enhancement represents fatty replacement in vertebral bodies and previous studies found that signal enhancement on T1-weighted images (T1WI) correlate with osteoporosis. MRI based quantitative scores can be calculated from signal intensity on T1WI while never calculated for T2-weighted images (T2WI) signal which is plausible representative of water contents. Our new method used patient's body subcutaneous fat and cerebrospinal fluid as signal control to optimize signal.

Purpose

To find correlation between new quantitative scores from MRI signal intensities and patient age

Materials and Methods

A retrospective study of 72 female low back pain patients with MRI lumbar spine scans from Mendeley data were included in this study based with specific scanning parameters. T1 and T2 sagittal images were acquired with 1.5 Telsa MRI Magnetom Essenza (Siemens). A total of 360 vertebrae from L1-L5 were assessed for signal quantification by two independent radiologists. F-score (fat) and W-score (water) were calculated using new formula. Pearson correlation, regression, graphs and descriptive statistics were calculated using SPSS 21.

Results

The mean patient age was 40.75 y. The F-score and patient age showed moderate positive Pearson correlation, $r=0.622$ ($p<0.01$). Correlation between F-score and W-score resulted moderate positive, $r=0.667$ and similar positive correlation of $r= 0.488$ was reported between W-score and patient age.

Conclusion

Novel MRI quantitative F-score and W-score is in direct correlation with age while increase W-score besides F-score is suggestive of increase in water contents in lumbar spine vertebrae besides fat content with increasing age. This MRI method may prove reliable in osteoporosis diagnosis.

LB2

Engineering hydroxyapatite-coated, topographically designed microparticles to provide bio-instructive 3D cues for in vitro culture of bone-residing cells.

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Abstract

Introduction:

The strategic combination of bioengineering and biomaterials provides a path towards the construction of bone models that are truly physiologically relevant. Such models are essential for the understanding of bone diseases and metastasis. Mechanical cues, including topography, chemistry, and stiffness, significantly influence behaviour and differentiation of cells, including mesenchymal stem cells (MSCs). Polymer microparticles can be designed to deliver specific 3D mechanical and chemical cues. The topography of microparticles has been shown to direct the differentiation of MSCs into osteoblasts[1]. Hydroxyapatite is the main inorganic component of bone; therefore, the functionalisation of cell-instructive, textured microparticles with hydroxyapatite is important to accurately recreate the microenvironment of bone-residing cells.

Methods:

Poly(lactic-co-glycolic acid) microparticles were prepared by an oil-in-water emulsion solvent evaporation method, with dimples created by exploiting the phase separation of fusidic acid[2]. Microparticles were mineralised with hydroxyapatite using the Polymer-Induced Liquid Precursor[3] method and characterised using scanning electron microscopy and Raman spectroscopy. MSCs and human monocytic cell line (THP-1)-derived macrophage attachment and viability was assessed using ActinGreen/DAPI staining on day 3 and LIVE/DEAD™ Viability/Cytotoxicity staining on day 5 post-culture.

Results:

The influence of the topographical features of the microparticles and mineralisation conditions on the formation of hydroxyapatite was studied. Hydroxyapatite crystals, with average dimensions of 42.4nm ± 47.3nm and ≤10nm in thickness, formed on the microparticle surfaces and displayed a biomimetic plate-like morphology (Fig. 1A).

MSCs and THP-1-derived macrophages attached to the mineralised microparticles, with actin filaments extending around the microparticles (Fig. 1B). Both cell types showed high viability when cultured on dimpled mineralised microparticles (as exemplified in Fig. 1C).

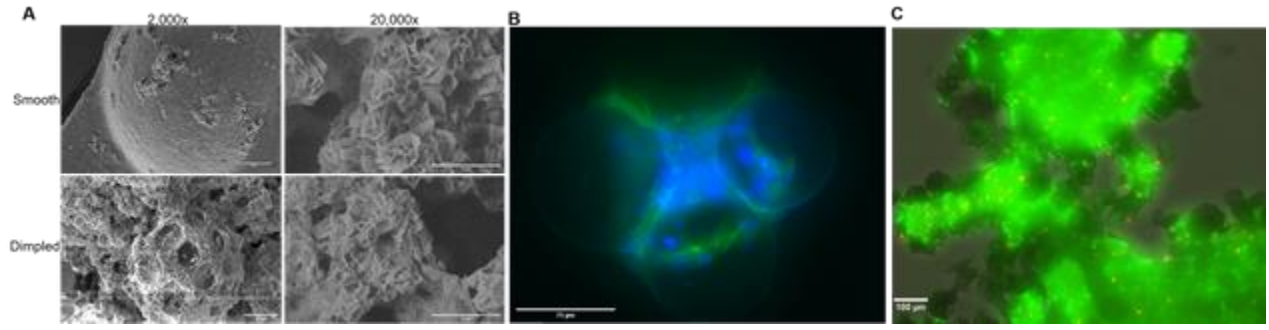

Fig. 1: (A) Hydroxyapatite crystals formed on smooth and dimpled PLGA microparticles. (B) Actin (green) and nuclear (blue) staining of MSCs cultured on surface-mineralised microparticles. (C) Viability of MSCs, confirmed using Live (green) and dead (red) staining, on mineralised microparticles.

Conclusions:

We have demonstrated that a thin layer of biotemplated hydroxyapatite can be created on topographically textured microparticles whilst maintaining their cell-instructive topographical features. These microparticles were shown to be biocompatible with MSCs and THP-1-derived cells (used as osteoclast precursor cells). Current work focuses on investigating the cell-instructive properties of these microparticles by studying the function and differentiation of these bone-residing cells.

1 Amer, et al. (2021) Biomaterials 266, 120450.

2 Gilchrist, et al. (2012) Mol. Pharm., 9 (5):1489-1501

3 Li, et al. (2015) ACS Appl Mater Interfaces. 7(46):25784-25792.

LB3

A Global Natural History Study of Fibrodysplasia Ossificans Progressiva (FOP): 36 Month Outcomes in Participants Aged <25 Years

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Abstract

Background: FOP is an ultra-rare, severely disabling genetic disorder characterised by progressive heterotopic ossification (HO) following flare-ups. Median age at diagnosis is 5 years; patients are managed by multiple specialties. No study to date has provided a longitudinal evaluation of FOP. Final data are presented for participants, aged <25 years, enrolled in the first 36-month, prospective, global natural history study of FOP (NCT02322255).

Methods: Individuals with FOP aged ≤65 years with a documented *ACVR1*^{R206H} mutation were enrolled in the study; this analysis includes participants aged <25 years at Baseline. HO volume was evaluated by low-dose whole-body computed tomography (WBCT), excluding head. Physical function was assessed using the Cumulative Analogue Joint Involvement Scale (CAJIS; total score 0-30 represents degree of ankylosis across joints) and the FOP Physical Function Questionnaire (FOP-PFQ; % total score); in both measures, higher scores indicate more severe limitations. Change from Baseline (CfB) at Month 36 in HO volume, CAJIS and FOP-PFQ are presented.

Results: Of 87 participants aged <25 years at Baseline, 39 had evaluable WBCT HO data at Baseline and Month 36. At Month 36, 84.6% of participants had new HO. CfB in mean [SD] total WBCT HO volume over 36 months was higher in those aged 8-<15 years (75.0×10^3 [102.6×10^3] mm^3; n=16) and 15-<25 years (77.5×10^3 [149.2×10^3] mm^3; n=15) versus those 2-<8 years (69.3×10^3 [60.2×10^3] mm^3; n=8). Mean (SD) number of body regions with new HO per participant at Month 36 were: 2-<8 years, 3.9 (2.5); 8-<15 years, 2.8 (1.7); 15-<25 years, 2.4 (2.1). Across median follow-up durations of 34.0, 30.1 and 24.0 months for those aged 2-<8, 8-<15 and 15-<25 years, mean (SD) number of flare-ups reported through clinic visits/telephone contacts per participant were 3.2 (3.2), 2.1 (2.1) and 1.7 (1.8), respectively. Mean

(SD) number of flare-ups per participant-month were 0.1 (0.1), 0.1 (0.2) and 0.1 (0.1), respectively. Mean CfB to Month 36 in CAJIS and FOP-PFQ were small across age groups (CAJIS: 0.9-1.6 [n=34]; FOP-PFQ: 2.4%-10.3% [n=29]).

Conclusions: Total WBCT HO volume increased over 36 months confirming the progressive nature of FOP. Participants aged 2-<8 years reported the highest mean number of flare-ups and had more body regions with new HO versus participants 8-<25 years, despite lower new HO volumes. Measurement of new HO by WBCT is a viable method to monitor disease progression. CAJIS and FOP-PFQ were not sufficiently sensitive to assess progression over 36 months.

LB4

The impact of acetabular inclination on acetabular and femoral head radii

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Abstract

Background: Acetabular morphology and orientation differs from ethnic group to another . Thus, investigating the normal range of the parameters that are used to assess both was a matter of essence. Nevertheless, the main aim of this study was clarification the relationship between acetabular inclination (AI) and acetabular and femoral head arcs' radii (AAR and FHAR).

Methods: A cross-sectional retrospective study that had been done in a tertiary center where Computed tomography abdomen scouts' radiographs of non-orthopedics patients were included. They had no history of pelvic or hips' related symptoms or fractures in femur or pelvis.

Results: A total of 84 patients was included with 52% of them were females. The mean of age was 30.38± 5.48. Also, Means of AI were 38.02±3.89 and 40.15±4.40 (P 0.02, significant gender difference) for males and females, respectively. Nonetheless, HNSA means were 129.90±5.55 and 130.72±6.62 for males and females, respectively. However, AAR and FHAR means for males and females were 2.13±0.31, 1.99±0.31, P 0.04 and 1.97±0.31,1.81±0.27, P 0.019, respectively. In addition, negative significant correlations were detected between AI against AAR, FHAR, HNSA and body mass index (BMI) (r 0.529, P ≤0.0001, r 0.445, P ≤0.0001, r 0.238, P 0.029, r 0.329, P ≤0.007, respectively). On the other hand, high BMI was associated with AAR and FHAR (r 0.577, P 0.0001 and r 0.266, p 0.031, respectively).

Conclusion: This study shows that high AI is correlated with lower AAR, FHAR. Each ethnic group has its own normal values that must be studied to tailor the path for future implications in clinical setting.

LB5

Natural acetabular development in childhood among Saudi population

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Abstract

Background: Acetabular natural development differs from ethnic group to another. Thus, investigating the natural history of the parameters that are used to assess both was a matter of essence. Nevertheless, clarification the picture of normal value in our society was the main aim of this study. However, Acetabular head index (AHI) and center edge angle (CEA) were the most sensitive indicative parameters for acetabular dysplasia. Hence, they were the main variables used in evaluation of acetabular development.

Methods: A cross-sectional retrospective study that had been done in a tertiary center where Computed tomography abdomen scouts' radiographs of non-orthopedics patients were included. They had no history of pelvic or hips' related symptoms or fractures in femur or pelvis.

Results: A total of 81 patients was included with 51% of them were males. The mean of age was 10.38± 3.96. Wiberg CEA was used, means of CEA were 33.71±6.53 and 36.50±7.39 for males and females, respectively. Nonetheless, AHI means were 83.81±6.10 and 84.66±4.17 for males and females, respectively. On the other hand, CEA was increasing by a factor 0.26 for each year (3-18, range). In addition, positive significant correlation was detected between CEA and age as found by linear regression r 2 0.460 (f(df1,79) =21.232, P ≤0.0001). Also, Body mass index (BMI) was positively correlated with CEA r 0.410, P 0.004).

Conclusion: This study shows that obesity and aging are linked to increased CEA. Each ethnic group has its own normal values that must be studied to avoid premature diagnosis.

LB6

Morel-Lavallée lesion induced by Dynamic hip screw (DHS) cut out: a Case Report and literature review

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Abstract

Background: A Morel-Lavallee lesion (MLL) is a benign cystic lesion that occurs due to injury to the soft-tissue envelope's perforating vascular and lymphatic systems, resulting in a distinctive hemolymphatic fluid accumulation between the tissue layers. The MLL has the potential to make a significant impact on the treatment of orthopaedic injuries.

Case presentation: a 79-year-old male patient community ambulatory with assisting aid (cane) known case of Diabetes mellitus, hypertension, bronchial asthma and ischemic heart disease. He was brought to the Emergency, complaining of right hip discomfort and burning sensation for the last 5 days with no history of recent trauma at all. Patient had history of right trochanteric femur fracture 3 years ago, treated with DHS in a privet service. Clinical and Radiological assessment showed that the patient mostly has acute MLL due to lag screw cut out. We offered the patient the surgical intervention, but he refused despite explaining the risks of complications if not treated and preferred to receive the conservative treatment. Compression therapy management explained to him including biker's shorts (instructed to be worn full-time a day) and regular follow up in clinic. Symptom's improvement was reported by the patient in the subsequent visits.

Discussion: In the polytrauma patient, a delayed diagnosis of these lesions is conceivable due to the presence of more visible injuries. It's located over the greater trochanter more commonly, but sometimes in other areas such as the lower lumbar region, the thigh, or the calf. Incorrect or delayed diagnosis and care can have unfavorable outcomes such as infection, pseudocyst development, and cosmetologically deformity. Magnetic resonance imaging (MRI) and ultrasound will aid in MLL diagnosis. However, the effectiveness of MLL therapy remains debatable.

Conclusion: we strongly believe that the MLL caused due to tangential shear forces applied to the soft tissue leads to accumulation of the blood and/or lymph between the subcutaneous and overlying fascia and it often misdiagnosed due to other distracting injuries. Nontheless, in our case we reported MLL occur due to internal pressure on the fascia caused by cut out of DHS lag screw.

LB7

¤Contrast-enhanced ultrasound imaging of displaced humeral fractures: A pragmatic single-centre pilot study

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Abstract

Ultrasound contrast agents (USCA) are used clinically to enhance contrast in tissues to aid non-invasive diagnosis in oncology and cardiovascular medicine. Often comprising formulations of microbubbles, they perfuse blood vessels and enhance the contrast of otherwise poorly distinguished tissues. However, there is limited information on whether USCAs can be used as a means of enhancing contrast in acute bone fracture. In this study we hypothesised that Sonovue, a commercially available contrast agent, would be detectable in murine and human bone fractures. In a pragmatic, non-randomised, observation pilot study, 10 patients with a displaced fracture of the humerus were recruited for study for examination 7 to 14 days post fracture under NREC ethical approval. Sonovue was administered peripherally and a 120s US B-mode video was obtained at the region of the widest visible fracture gap using a Logiq E10 instrument. 40s post injection a marked increase in contrast was visible in the fracture gap region (increase from -66 to -54dB) and in the periosteal regions. Similar results were obtained in murine models. These data indicated that bone fractures are well-perfused and amenable to CEUS imaging or remote activation of microbubbles for other applications, including therapy.

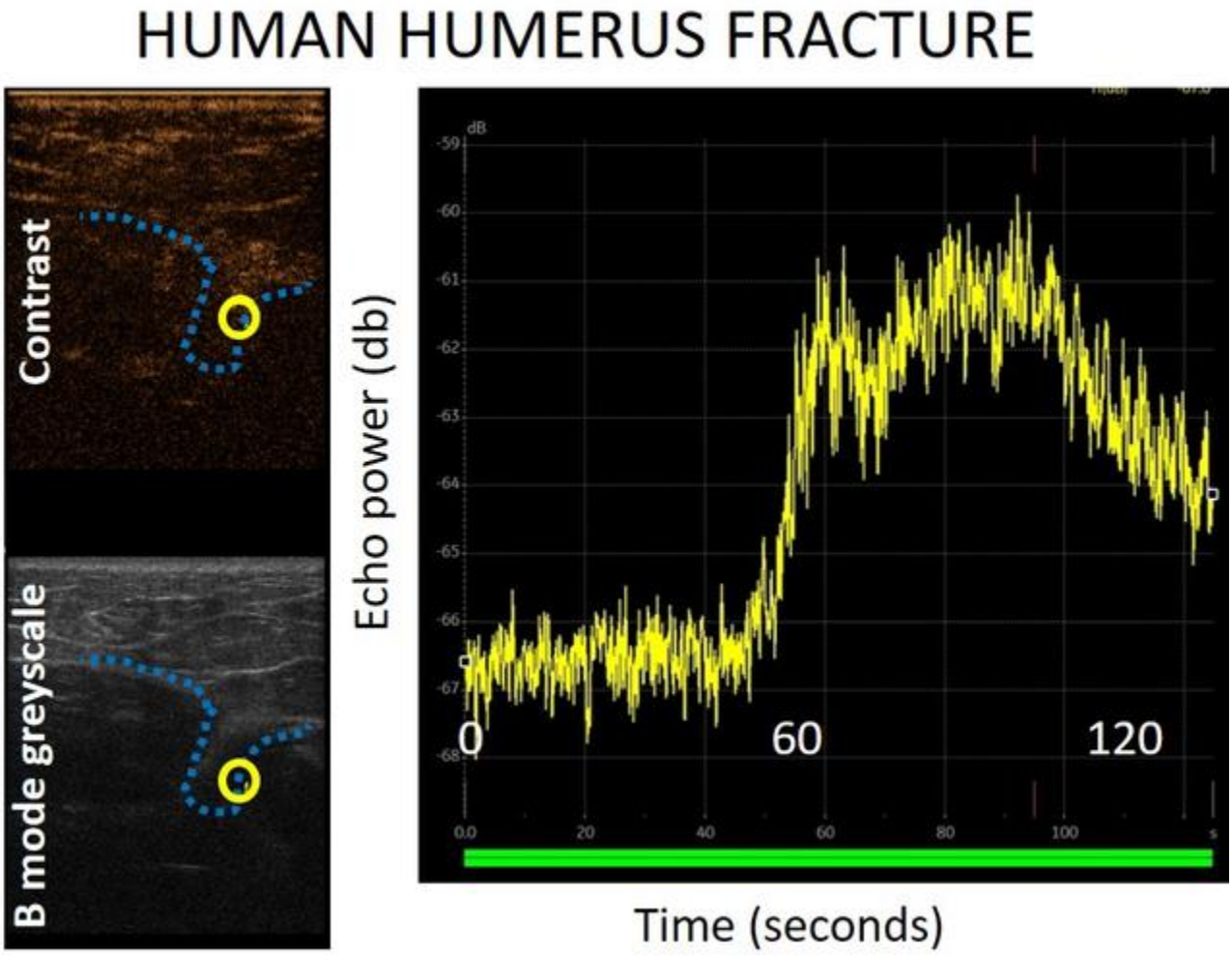

Figure 1. Time intensity curve analysis of contrast enhancement in the region (yellow circle on LH images) of a displaced humeral fracture 14 days post-injury.