ORAL COMMUNICATIONS

The 4th Joint Meeting of ECTS and IBMS
Rotterdam, The Netherlands
25–28 April 2015

OC1.1 Wnt16 Promotes Osteoblastogenesis and is Negatively Regulated by Glucocorticoids In Vitro and In Vivo
Susanne Hildebrandt¹, Sylvia Thiele¹, Ulrike Baschant¹, Juliane Salbach-Hirsch¹, Jan Tuckermann², Lorenz C. Hofbauer¹,³, Martina Rauner¹
¹Division of Endocrinology, Diabetes, and Bone Diseases; Department of Medicine III, Technische Universität Dresden, Dresden, Germany, ²Institute of General Zoology and Endocrinology, University of Ulm, Ulm, Germany, ³Center for Regenerative Therapies Dresden, Dresden, Germany

Glucocorticoids (GCs) are effective drugs to treat inflammatory diseases, but exert detrimental effects on bone when used over longer periods of time. One of the main mechanisms of GC-induced bone loss is the suppression of osteoblast activity. Osteoblast-derived Wnt16 has recently been shown to determine cortical bone mass by regulating osteoclast function. However, its role in osteoblastogenesis and its regulation by GCs remain unknown. Here, we assessed the role of Wnt16 during osteoblast differentiation and tested whether GCs regulate Wnt16 expression. Wnt16 was highly expressed in primary murine bone marrow stromal cells (BMSCs), promoted osteoblastogenesis and activated canonical Wnt signalling in MC3T3-E1 cells. GC treatment using dexamethasone (DEX) decreased Wnt16 mRNA expression levels by 50% in BMSCs. Wnt16 suppression was dose- and time-dependent, reaching a maximum after 48 h at a concentration of 1 µM. Consistently, treatment of mice with GC-containing slow-release pellets for two weeks reduced vertebral bone mineral density by 13% and Wnt16 mRNA levels by 35% in bone tissue. The suppression of Wnt16 by GCs was strictly GC receptor (GR)-dependent. Co-treatment of BMSCs with DEX and the GR antagonist RU-486 completely abrogated the GC-mediated suppression of Wnt16. Likewise, DEX failed to suppress Wnt16 expression in BMSCs derived from GR knockout mice. Additionally, Wnt16 mRNA levels were unaltered after GC treatment in bone tissue of GR<sup>dim</sup> mice, which lack the ability of GR dimerisation and therefore binding of the GR to DNA, suggesting that GCs suppress Wnt16 via direct DNA-binding mechanisms. In line with this, DEX treatment reduced Wnt16 promoter activity in MC3T3-E1 cells. Thus, this study underlines the pro-osteogenetic effect of Wnt16 and identifies Wnt16 as a novel GC target. As the suppression of Wnt16 could define a mechanism of reduced osteoblast activity, Wnt16 may represent a novel target for therapeutic intervention in GC-induced bone loss.

Disclosure: The authors declared no competing interests.

OC1.2 Osteoblast N-Cadherin Restrains Wnt/β-Catenin Signalling and the Osteo-Anabolic Effect of Dkk1 Inhibition
Valerie Salazar¹,², Cynthia Brecks¹, Leila Revollo¹,², Susan Grimston¹, Marcus Watkins¹, Gabriel Mbalaviele¹, Roberto Civitelli¹
¹Washington University in St. Louis, St. Louis, MO, USA, ²Harvard University, Boston, MA, USA

We and others have shown that N-cadherin (Ncad) physically interacts with low density lipoprotein receptor-related protein-5 or 6 (Lrp5/6) and Axin, resulting in negative regulation of canonical Wnt/β-catenin signalling in osteoblasts. We tested whether removal of the N-cadherin gene (Cdh2) alters bone mass accrual in response to Lrp5/6 signalling. We administered a Dickkopf-1 (Dkk1) neutralizing antibody (αDkk1) to activate Lrp5/6 in mice with conditional Cdhh2 ablation driven by the 2.3 Col1A1 promoter (Col1-cKO). At the dose of 5 mg/kg body weight i.p., 3 times/week for 4 weeks, αDkk1 was ineffective in WT mice, but produced a 2-fold increase of BV/TV (0.496±0.085 p<0.01 vs. baseline) in Col-Cdhh2 cKO mice. A higher dose (20 mg/kg) was equally effective in both genotypes. At the molecular level, a single dose of αDkk1 produced accelerated accumulation of β-catenin in bone of Col1-cKO relative to WT mice, indicating direct Wnt/β-catenin signalling activation in bone by αDkk1 and enhanced responsiveness in the absence of Ncad. To corroborate this finding, we introduced one Dkk1-resistant Lrp5<sup>A214V</sup> allele associated with high bone mass (HBM), in conditionally Cdhh2 ablated mice driven by Osx1-Cre (Osx1-cKO). Although bone mass (by μCT) was lower in Osx1-cKO than in WT, the compound Lrp5<sup>A214V</sup>;Cdhh2 cKO mutants were osteoclasteric and indistinguishable from Lrp5<sup>A214V</sup> HBM mice. Despite lower total β-catenin abundance in Osx1-cKO bone marrow stromal cells, steady state cytosolic β-catenin was not decreased. Upon Wnt3a stimulation, N-terminally un-phosphorylated β-catenin was more abundant in Osx1-cKO than in WT cells, suggesting higher levels of active β-catenin in the absence of N-cadherin in response to Wnt3a. In summary, mice lacking Cdhh2 in osteolineage cells are hyper-responsive to Wnt signalling activation and to its osteo-anabolic effect. These results provide in vivo proof of the concept that Ncad restrains anabolic Lrp5/6 signalling in bone forming cells.

Disclosure: The authors declared no competing interests. This work has been supported by NIH grant AR055913 (RC).
Multiple factors contribute to bone loss in inflammatory diseases such as rheumatoid arthritis (RA), but circulating inflammatory factors and immobilisation play a crucial role. Mechanical loading prevents bone loss in the general population, but the effects of mechanical loading in patients with RA are less clear. Therefore, we aimed to investigate whether mechanical stimuli can reverse the modulatory effects of circulating inflammatory factors present in RA-serum on osteocyte-to-osteoclast signalling. We also investigated whether inflammatory factors present in RA-serum alter the response of osteocytes to mechanical stimuli. Human primary osteocytes from trabecular bone pieces were treated with 10% serum osteocytes to mechanical stimuli. Human primary osteocytes in inflammatory factors present in RA-serum alter the response of osteocyte-to-osteoclast signalling. We also investigated whether inflammatory factors present in RA-serum alter the response of osteocytes to mechanical stimuli. Human primary osteocytes from trabecular bone pieces were treated with 10% serum from active RA patients or healthy controls for 7 days. Then cells were subjected to 1 h mechanical loading by pulsating fluid flow (PFF; 0.7±0.7 Pa, 5 Hz) or static control culture, and cells were subjected to 1 h mechanical loading by pulsating from active RA patients or healthy controls for 7 days. Then cells were subjected to 1 h mechanical loading by pulsating fluid flow (PFF; 0.7±0.7 Pa, 5 Hz) or static control culture, and medium NO and PGE2 concentrations were determined. Cells were post-incubated without PFF for 1h, and cytokine gene expression was quantified by qPCR. Osteoclast precursors were differentiated into mature osteoclasts on osteoblasts, and osteoclast number and bone resorption marker CTX over the osteoclast marker TRACP 5b (+1.8-fold, p=0.002), and decreased trabecular BV/TV (-19%, p=0.04) and Tb.N (-16%, p=0.05) vs scrambled-siRNA treated ADO2 mice. After 4 weeks, trabecular BV/TV (-21%, p=0.03) and trabecular variables (Tb.N -19%, Tb.Sp +1.2-fold, p=0.03) returned to WT level, with a full rescue of the bone phenotype. In the rescued ADO2 mice, serum CTX/TRACP, osteoclast number and bone loss were normalised (+1.2-fold, -32%, +2.1-fold, p=0.03, p=0.02, respectively, vs control ADO2 mice), while osteoblast and dynamic parameters were unremarkable. Treatment was well tolerated, with no adverse events, and with normalisation of liver aspartate aminotransferase. To the best of our knowledge, this is the first experimental curative treatment of ADO2, which rescued osteoclast function and returned the bone phenotype to normal by a systemic RNA interference strategy. The invention is protected by the patent application RM2014A000272, which could provide the means to develop this siRNA strategy for therapy in humans.

Disclosure: The authors declared no competing interests.

OC1.5 Dominant Mouse Model with Uncleavable Type I Collagen C-propeptide Processing Site has Extremely Brittle Bones
Aileen M. Barnes1, Joseph E. Perosky2, M. Helen Rajpar1, Kenneth M. Kozloff2, Joan C. Marini1
1NICHD/NIH, Bethesda, MD, USA, 2University of Michigan, Ann Arbor, MI, USA

Classical osteogenesis imperfecta (OI) is caused by type I collagen mutations. Mutations in the C-propeptide cleavage site of both COL1A1 and COL1A2 cause high bone mass OI, characterised by bone hypermineralisation. To elucidate the role of type I procollagen C-propeptide processing in bone formation, we generated a mouse with a heterozygous C-propeptide cleavage site defect (high bone mass, HBM), substituting both COL1A1 cleavage site residues to prevent BMP1 cleavage. Western blots on long bone extracts revealed unprocessed pro- and pC-collagen and cleaved C-propeptide in HBM bone. At 2 months, male HBM mice are significantly smaller in weight (77%) and length (92%) and have shorter femurs (92%). All 2

Disclosure: The authors declared no competing interests.

OC1.4 RNA interfering Strategy to Cure Autosomal Dominant Osteopetrosis Type 2 (ADO2)
Mattia Capulli1, Antonio Maurizi1, Luca Ventura2, Nadia Rucci1, Anna Teti1
1University of L’Aquila, Dept. of Biotechnological and Applied Clinical Sciences, L’Aquila, Italy, 2University of L’Aquila, Department of Pathology, San Salvatore Hospital, L’Aquila, Italy

Genetic autosomal dominant diseases are generally due to heterozygous missense mutations that could be eradicated by RNA interference. We hypothesised that this approach could cure ADO2 and tested this treatment in ADO2 mice carrying a G213R amino acid substitution in the CLC-7 protein, encoded by the Clcn7 gene. Using a systematic mutation-driven strategy, we designed and tested in-vitro various small interfering (si)RNAs against this mutation and found a Clcn7G213R-siRNA that silenced specifically the mutant transcript in transfected HEK293 cells (-85%, p=0.02), without affecting the WT gene and rescuing bone resorption in ADO2 osteoclasts (+2.6-fold, p=0.003). This siRNA was made “sticky” by 3’dAdT overhangs, conjugated with the delivery system ‘in-vivo-JetPEI™’ and injected i.p. in ADO2 mice. Time- and dose-dependent experiments evidenced 4 mg/Kg every 48 h to be the most effective treatment, decreasing the mutant mRNA in tibias (-80%, p=0.01). Two-weeks treatment of ADO2 mice, down-regulated Clcn7G213R mRNA expression in bone and other organs, increased the serum bone resorption marker CTX over the osteoclast marker TRACP 5b (+1.8-fold, p=0.002), and decreased trabecular BV/TV (-19%, p=0.04) and Tb.N (-16%, p=0.05) vs scrambled-siRNA treated ADO2 mice. After 4 weeks, trabecular BV/TV (-21%, p=0.03) and trabecular variables (Tb.N -19%, Tb.Sp +1.2-fold, p=0.03) returned to WT level, with a full rescue of the bone phenotype. In the rescued ADO2 mice, serum CTX/TRACP, osteoclast number and bone loss were normalised (+1.2-fold, -32%, +2.1-fold, p=0.03, p=0.02, respectively, vs control ADO2 mice), while osteoblast and dynamic parameters were unremarkable. Treatment was well tolerated, with no adverse events, and with normalisation of liver aspartate aminotransferase. To the best of our knowledge, this is the first experimental curative treatment of ADO2, which rescued osteoclast function and returned the bone phenotype to normal by a systemic RNA interference strategy. The invention is protected by the patent application RM2014A000272, which could provide the means to develop this siRNA strategy for therapy in humans.

Disclosure: The authors declared no competing interests.

OC1.3 Mechanical Loading Reduces Inflammation-Induced Human Osteocyte-to-Osteoclast Signalling
Janak L. Pathak1,2, Nathalie Bravenboer1, Frank P. Luyten2, Patrick Verschuere2, Willem F. Lems1, Jenneke Klein-Nulend1, Astrid D. Bakker1
MOVE Research Institute Amsterdam, Amsterdam, The Netherlands, 2Skeletal Biology and Engineering Research Center, KU Leuven, Leuven, Belgium

The authors declared no competing interests.
month HBM mice have pelvic deformities; 40% have kyphosis. Femoral aBMD in HBM mice is decreased 25% (p<0.001), but vertebral BMD is normal. On μCT, HBM femora have thinner cortices with decreased cortical area. Four-point bending revealed significantly decreased HBM femoral stiffness, yield load, and ultimate load. HBM femora are also extremely brittle; post-yield displacement is only ~10% of WT (0.23 vs 0.03, p<0.001). Collagen from HBM calvarial osteoblasts had normal biochemistry with normal trimer incorporation, however, HBM osteoblasts deposited only about 50% of WT matrix. Sost transcripts in HBM femora are decreased ~40% and suggests C-propeptide processing may also influence cellular mal biochemistry with normal trimer incorporation, however, osteoblasts deposited only about 50% of WT matrix. Sost transcripts in HBM femora are decreased ~40% and suggests C-propeptide processing may also influence cellular differentiation. Dermal fibril diameters were smaller and more homogeneous in HBM than WT, with loss of large fibrils. The HBM mouse phenotype is similar to that of the Bmp1<sup>−/−</sup>/Tll1<sup>−/−</sup> mouse which also has small size, thin cortices, reduced maximum load and a dramatic decrease in post-yield displacement. The HBM mouse demonstrates that the essential elements of the broader enzyme deficiency are reproduced by a substrate defect in type I C-propeptide cleavage. These data show the importance of the type I procollagen C-propeptide to both collagenous and mineral properties of bone.

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

**OC1.6**

**Combination Sclerostin Antibody and Zoledronic Acid Treatment Outperforms Either Treatment Alone in a Mouse Model of Osteogenesis Imperfecta**

David Little<sup>1</sup>, Lauren Peacock<sup>1</sup>, Kathy Mikulec<sup>1</sup>, Michaela Kneissel<sup>2</sup>, Ina Kramer<sup>1</sup>, Tegan Cheng<sup>1</sup>, Aaron Schindeler<sup>1</sup>, Craig Munns<sup>1</sup>

<sup>1</sup>The Children’s Hospital at Westmead, NSW, Australia, 2Novartis Pharma AG, Basel, Switzerland

**Background:** Osteogenesis Imperfecta (OI) is a genetic disorder featuring bone fragility and decreased bone mass. Bisphosphonates in children with OI reduce bone catabolism and rely on modelling to form new bone. An anabolic treatment, Anti-Sclerostin Antibody (Anti-SOST Ab), is being investigated in clinical trials. We hypothesised that combined treatment may produce superior outcomes.

**Methods:** Female Col1a2 G610C mice and their wild type (WT) littermates were treated from week 5 to week 9 of life to either saline (control), zoledronic acid (ZA) 0.025 mg/kg IV weekly (Anti-SOST), or a combination of both (ZA Anti-SOST). Outcomes included weekly DEXA for areal bone mineral density (BMD) (GE Lunar PIXImus WI, USA), μCT (SkyScan 1174 Kontich, Belgium), mechanical testing of Tibia in 4 point bending (Instron 5944, Massachusetts, USA). Data were analysed with one-way ANOVA (SPSS v11).

**Results:** Increases in tibial BMD were seen over time in all groups. Anti-SOST treatment alone had no effect on tibial BMD, while ZA (16%) and ZA Anti-SOST (27%) treatments produced significant increases from weeks 1-4 (P<0.05). μCT analysis showed increases in Tissue Mineral Density and Cortical Thickness for combined treatment over respective controls. Tibial 4-point bending showed only combined ZA Anti-SOST yielded a significant increase in strength and energy to failure in OI mice, restoring bone strength to the values of untreated WT mice. In the spine, all treatments increased compression strength over controls, Anti-SOST 30%, ZA 43% and ZA Anti-SOST 91% (P<0.05).

**Conclusion:** Anti-SOST Ab alone had effects on trabecular but not cortical sites in this study in Col1a2 G610C mice. Roschger et al. reported minimal effect in the Col1a1(Jrt)/+ mouse model treated with Anti SOST Ab, whereas large effects were noted with just 2 weeks treatment in 8 week-old Btii/+ mice, leading to increase in bone size and strength. A combination of zoledronic acid and anti-sclerostin antibody is superior over either treatment alone in the Col1a2 G610C model of OI. Further studies are required in alternate mouse models of OI to confirm efficacy across different models, and thus to predict possible efficacy across the heterogeneous population of OI patients.

**Disclosure:** Little DG Research Contracts Novartis Pharma; Kneissel M, Kramer I Novartis Pharma employees; Anti-SOST Antibody supplied by Novartis Pharma.

**OC2.1**

**Melatonin Improves Bone Mineral Density (BMD) at the Femoral Neck in Post-Menopausal Women with Osteopenia: a Randomised Controlled Trial**

Anne-Kristine Amstrup, Tanja Slikjaer, Lene Heickendorff, Leif Mosekilde, Lars Rejnmark

Aarhus University Hospital, Aarhus, Denmark

**Background:** Melatonin is known for its regulation of circadian rhythm, however, over recent years, studies have shown that melatonin also has a positive effect on bone. With age, the melatonin levels decrease leading to further imbalanced bone remodelling. We aimed to investigate whether treatment with melatonin may improve bone parameters.

**Method:** In a double-blind placebo-controlled investigator initiated study, we randomised 81 healthy post-menopausal women with osteopenia to one-year of treatment with melatonin in a nightly dose of 1 mg (N=20), or 3 mg (N=20), or similar placebo (N=41). At baseline and after 12 months of treatment, DXA measurements of body composition, and BMD at the spine and hip were collected. Biochemical markers of calcium homeostasis were measured throughout the trial.

**Results:** Mean age was 63 (range 56-73) years. Compared with placebo, BMD at the femoral neck increased by 1.4% (95%CI: -2.7; -0.0, p<0.05) in response to melatonin. A dose-response relationship was present (p<0.01) as BMD at the femoral neck increased by 2.3% (95%CI: 0.7; 4.0, p<0.01) in the high dose (3mg/d) melatonin group compared with placebo. Compared with 1 mg/d of melatonin, BMD in the 3 mg/d group increased by 1.9% (95%CI: 0.0; 3.7, p<0.05). Treatment did not affect BMD at other skeletal sites or levels of bone turnover markers, however, there was a significant decrease in 24 h urinary calcium in the melatonin group (-3.7%, IQR:-2.9;57.0) compared with placebo (8.5%, IQR:-11.5;19.4, p=0.02). Moreover, compared with placebo, melatonin decreased fat mass significantly by 6.8% (95%CI: -3.7%; 0.0, p<0.05). While lean body mass increased by 2.2% (95%CI: -4.8; 0.0, p=0.08).

**Conclusion:** One year of treatment with melatonin improved BMD dose-dependently at femoral neck and showed beneficial effects on body composition in terms of a reduced fat mass and borderline increased lean tissue. Further studies are needed to assess mechanisms of action and whether nighttime melatonin may protect against fractures.

**Disclosure:** The authors declared no competing interests.
OC2.2
Spontaneous Femoral Varization as a Risk Factor for Atypical Femoral Fractures
Carmen García Ibarbia, Mª Angeles Red Gallego, Ana Alfonso Fernández, Jose Antonio Riancho, Mª Isabel Pérez Núñez
Hospital Universitario Marqués de Valdecilla, Santander, Cantabria, Spain

Background: Several reports have linked bisphosphonates (BPs) with atypical femoral fractures (AFFs), but there is still debate regarding the real influence of these drugs on the development of such fractures. We speculated that AFFs could be related to lower limb geometry, specifically to spontaneous femoral varization, which would result in increased stress on the femoral cortices.

Methods: In order to test this hypothesis, we conducted a case-control study examining the geometric characteristics of the femur in patients who had suffered an AFF during treatment with BPs and in control patients taking BPs for a long time and not experiencing AFFs. A standing X-ray of the lower extremities was obtained. The following parameters were measured: curvature of the femur, distance from the femur to the load line, femoro-tibial angle and load angle. Eight women on BPs suffered 11 AFF (8 complete fractures and 3 incomplete fractures). Three patients had AFFs of both femora, the control group included 21 women with postmenopausal osteoporosis.

Results: The geometric features of patients with AFFs were very different from those of the control group, with a marked tendency to increased curvature of the femur in the patient’s group (Table 1).

Conclusion: Our results suggest that patients with disturbed lower limb geometry are at higher risk of AFFs. Therefore, it may be worthwhile to obtain a standing X-ray of the lower legs in patients on long-term BPs in order to identify those individuals more susceptible to AFF.

Disclosure: The authors declared no competing interests.

Table 1 [OC2.2]: Geometric features

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CASES</th>
<th>CONTROLS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>curvature of the femur (grade)</td>
<td>9</td>
<td>2-20</td>
<td>-4.4</td>
</tr>
<tr>
<td>distance from the femur to the load line (mm)</td>
<td>37.9</td>
<td>14-53</td>
<td>6.3</td>
</tr>
<tr>
<td>femoro-tibial angle (grades)</td>
<td>4</td>
<td>1-9</td>
<td>-3.6</td>
</tr>
<tr>
<td>load angle (grades)</td>
<td>7.4</td>
<td>5-12</td>
<td>-7.6</td>
</tr>
</tbody>
</table>

OC2.3
Osteoprotegerin Autoantibodies are Independently Associated with Reduced Bone Density in Coeliac Disease
Philip Riches1, Tamara Gilchrist1, Micaela Rios-Petrakis1, Barbara Hauser1, Nick Kennedy1,2, Helen Gillett2, Peter Gillett2, Clive Goddard2, Alan Shand2, Jack Satsangi1,2, Stuart Ralston1
1Institute of Genetics and Molecular Medicine, Edinburgh, UK, 2NHS Lothian Gastroenterology Department, Edinburgh, UK

Background: Autoantibodies neutralising the effect of the bone regulatory cytokine osteoprotegerin (OPG) have been described in a patient with severe osteoporosis and coeliac disease. This study aimed to determine the prevalence of autoantibodies to OPG in patients with coeliac disease, and correlate their presence with bone mineral density.

Methods: A direct enzyme linked immunosorbent assay using recombinant OPG as a capture antigen was developed and used to screen serum from 282 patients with coeliac disease for autoantibodies to OPG. Bone mineral density data was available in 254 patients. A threshold for the presence of OPG antibody was defined as the mean plus three standard deviations of values obtained from 102 healthy controls.

Results: OPG autoantibodies were found in 14/282 (5%) patients with coeliac disease. Bone mineral density results are summarised in Table 1. The presence of OPG antibodies was associated with lower spine bone mineral density T and Z-scores on both univariate analysis, and multivariate analysis including age, sex, height and weight as covariates (p<0.05). This association was also seen when analysing the titre of OPG antibody as a continuous trait. A non-significant reduction in mean bone mineral density hip scores was seen in patients with OPG antibodies. (See table 1.)

Conclusion: Raised levels of OPG autoantibodies are found in 5% of patients with coeliac disease and are independently associated with reduced spine bone mineral density. Further work is required to establish the clinical utility of testing for OPG antibodies.

Disclosure: PLR and SHR are co-applicants on a patent application protecting the detection and/or treatment of diseases associated with autoantibodies to osteoprotegerin. This work was supported by the ECTS Amgen Bone Biology Fellowship (2010) and Coeliac UK/CORE charity (2013).

Table 1 [OC2.3]: Bone mineral density of coeliac patients defined by OPG antibody status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>OPG antibody present</th>
<th>OPG antibody absent</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spine BMD T-score</td>
<td>-2.00 (±1.2)</td>
<td>-1.05 (±1.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Spine BMD Z-score</td>
<td>-1.12 (±1.39)</td>
<td>-0.10 (±1.2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hip BMD T-score</td>
<td>-1.36 (±0.99)</td>
<td>-1.01 (±1.10)</td>
<td>0.29</td>
</tr>
<tr>
<td>Hip BMD Z-score</td>
<td>-0.38 (±0.84)</td>
<td>-0.03 (±0.97)</td>
<td>0.24</td>
</tr>
</tbody>
</table>
OC2.4 Mitochondrial DNA Point Mutation is Associated with Lower Bone Mineral Density and Altered Bone Structure in a Matched Case-Control Study

Jakob H. Langdahl1,2, Stinus J. Hansen3, Knud B. Yderstræde4, Per H. Andersen2, John Vissing5, Morten Dune6, Anja L. Frederiksen7, Morten F. Nielsen3

1Clinical Genetic Research Unit / Clinical Institute University of Southern Denmark, Odense, Denmark, 2Department of Endocrinology / Hospital of Southwest Jutland, Esbjerg, Denmark, 3Endocrine Research Unit / Clinical Institute University of Southern Denmark, Odense, Denmark, 4Department of Endocrinology / Odense University Hospital, Odense, Denmark, 5Department of Neurology / Rigshospitalet, Copenhagen, Denmark, 6Department of Clinical Genetics / Rigshospitalet, Copenhagen, Denmark, 7Department of Clinical Genetics / Odense University Hospital, Odense, Denmark

Background: Mitochondrial dysfunction is associated with several clinical outcomes including diabetes, myopathy, hearing loss and is implicated in the human ageing process. Mitochondrial mutations cause osteoporosis in mouse models. The effect of mitochondrial dysfunction on bone has not been studied in humans.

Methods: We recruited 38 patients (24 female, 14 male) with the mtDNA3243A>G mutation aged 45.8 ±14.9 years. Twenty-three of the cases had diabetes mellitus. Cases were matched with respect to sex, age, height and menopausal status with healthy controls. All participants underwent DXA and HR-pQCT scans. Finite element analysis was used to assess bone strength.

Results: Cases and controls were matched with regard to age, sex and height, but cases had a lower body weight (63.3 vs. 75.7 kg) and higher calcium and vitamin D supplements. Based on DXA, cases had a lower total hip aBMD (0.82 vs. 0.95 g/cm², p<0.01), femoral neck aBMD (0.65 vs. 0.80 g/cm², p<0.01) and spine aBMD (0.91 vs. 0.98 g/cm², p=0.02). Compared to controls, cases had smaller cortical area (radius: 56.0 vs. 64.2 mm², p<0.01, tibia: 98.4 vs. 134.6 mm², p<0.01), thinner cortices (radius: 0.80 vs. 0.92 mm, p<0.01, tibia: 1.06 vs. 1.29 mm, p<0.01) and lower total bone vBMD (radius: 312.6 vs. 370.8 mg/cm³, p<0.01, tibia: 275.8 vs. 316.2 mg/cm³, p<0.01). In cases, cortical density was lower at the radius (888.8 vs. 913.9 mg/cm³, p=0.02) and trabecular density was lower in tibia (154.1 vs. 176.8 mg/cm³, p=0.02). In tibia, but not radius, estimated bone stiffness (165.8 vs. 209.4 kN/mm, p<0.01) and failure load (8.5 vs. 10.7 kN, p<0.01) was lower in cases. Hip BMD remained lower in cases after adjusting for weight.

Conclusion: Bone mass, microarchitecture and strength were compromised in patients with mitochondrial dysfunction. Further studies are needed to describe the effects of mitochondrial dysfunction on bone remodelling.

Disclosure: The authors declared no competing interests.

OC2.5 Bone Marrow Lesions Detected by Different Magnetic Resonance Sequences as Potential Biomarkers for Knee Osteoarthritis: Comprehensive Tissue Level Analysis

Julia Kuliwaba1,2, Dzenita Muratovic1,2, Flavia Cicuttini3, Anita Wiuka3, Yuanyuan Wang3, Sophia Otto1, David Taylor4, Sue Collings4, Julia Humphries1, Yea-Rin Lee1, Graham Mercer5, David Findlay2

1SA Pathology, Adelaide, Australia, 2The University of Adelaide, Adelaide, Australia, 3Monash University, Melbourne, Australia, 4Royal Adelaide Hospital, Adelaide, Australia, 5Repatriation General Hospital, Adelaide, Australia

Background: MRI-detected bone marrow lesions (BMLs) are associated with symptom severity and structural degeneration in knee osteoarthritis (OA). What BMLs represent at the tissue level is poorly described. The study aim was to characterise the cartilage-subchondral bone features corresponding to BMLs detected using two different MRI sequences for a knee OA cohort.

Methods: Whole tibial plates were retrieved from 54 patients (27-female, 27-male), aged 51-86 years, undergoing total knee replacement surgery for OA. To identify BMLs ex vivo, 3T-MRI scans were performed using T1 and PDFS-weighted sequences. MRI images were used for cartilage volume measurement. Micro-CT was used to assess microstructure of subchondral bone plate (SBP) and trabecular bone (STB). Cartilage and subchondral bone were assessed by OARSI and histopathology. Bone turnover indices were quantitated.

Results: BMLs were detected in 78% of patients (remainder formed No-BML group). Of all BMLs, BML-1 group (BML detected by PDFS only) represented 62%; BML-2 group (BML detected by PDFS and T1) represented 38%. BML-2 had reduced cartilage volume (p=0.007) with increased OARSI degenerative changes (p=0.009) compared to No-BML. BML-2 SBP was thicker and had lower porosity compared with No-BML (p<0.0001). BML-2 STB had higher bone volume (p=0.003), thicker (p=0.002) and more plate-like trabeculae (p=0.0004). SBP and STB osteoid volume and thickness were increased for BML-2 compared to No-BML (p<0.0001). More bone marrow oedema, necrosis and fibrosis was present in BML-2 compared to BML-1 and No-BML (p=0.03).

Conclusion: Knee OA BMLs are associated with loss and degeneration of the overlying cartilage, together with more sclerotic bone morphology. These relationships are more significant for BML-2, suggesting that BML-2 type lesions represent a later stage of OA disease. BMLs detected with specific MRI sequences may act as potential MRI biomarkers for identification of individuals at high risk of progressive OA and inform development and monitoring of new therapies.

Disclosure: The authors declared no competing interests. This work was supported by the National Health and Medical Research Council of Australia (APP1042482).
OC2.6
Gender-Specific Effects of Bisphosphonates on Mortality among Austrian Hip Fracture Patients Aged ≥50 Years
Wolfgang Brozek1, Berthold Reichardt2, Jochen Zwerina1, Hans Peter Dima3, Klaus Klaushofer1, Elisabeth Zwettler1
1Ludwig Boltzmann Institute of Osteology, Hanusch Hospital of the WGKK and AUVA Trauma Center, 1st Medical Department at Hanusch Hospital, Vienna, Austria, 2Sickness Fund Burgenland, Burgenländische Gebietskrankenkasse, Eisenstadt, Austria, 3Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Graz, Austria

We retrospectively analysed effects of bisphosphonates (BPs) on mortality in Austrian hip fracture patients. For 31,668 patients ≥50 years sustaining a hip fracture in Austria between July 2008 and December 2010, information on survival with follow-up until June 2011 and on prescription of BPs between July 2007 and June 2011 was available. Using Cox and logistic regression analysis, cumulative all-cause mortality among patients who started treatment before or after fracture was compared with that among age- and sex-matched hip fracture patients without anti-osteoporotic medication. The minimum prescription interval was set at half a year, and matched subjects had to be alive during the prescription interval of his/her assigned treated subject. Compared with female patients receiving no anti-osteoporotic prescription, women who initiated BPs before first fracture (n=8,868) displayed unaltered short-term mortality (hazard ratio [HR] at 90 days after fracture: 0.91 [95%-CI: 0.80-1.04, p=0.30]) but decreased long-term mortality (odds ratios [ORs] at one year and three years’ post-fracture, respectively: 0.70 [0.62-0.79, p<0.0001], 0.68 [0.61-0.76, p<0.0001]). Women starting BPs after first fracture (n=3,216) exhibited relative HRs of 0.29 (0.16-0.55, p<0.001) and 0.39 (0.29-0.52, p<0.0001) one year and three years’ post-fracture, respectively. For males using BPs already before fracture (n=837), no statistically significant reduction in mortality emerged, however, lowered mortality at one year post-fracture was observed for men treated only after fracture (n=633) (HR 0.12 [0.02-0.88, p<0.05]). Among hip fracture patients using BPs, mortality was reduced predominantly in females. The smaller effect of BPs on pre-fracture users’ relative to post-fracture users’ survival might reflect a selection bias inherent to this observational study with more co-morbidity among BP users than non-users. However, the high extent of mortality reduction found in post-fracture BP users portends a causal relationship with anti-resorptive treatment with BPs.

Disclosure: The authors declared no competing interests.

OC3.2
Neuro-Protein CRMP4 Inhibits Bone Formation by Regulating BMP Signalling and Rhoa-FAK Network
Basem M Abdallah1, Florence Figeac1, Kenneth Larsen1, Nicholas Ditzel1, Adiba Isa1, Toshio Ohshima2, Moustapha Kassem1,3
1Odense University Hospital, Odense, Fyn, Denmark, 2Waseda University, Tokyo, Japan, 3University of Copenhagen, Copenhagen, Denmark

We employed a global gene expression profiling using DNA microarrays to characterise non-canonical osteogenic factors regulating the differentiation of bone marrow skeletal stem cells (marrow stromal stem cells, BMSCs) into osteoblastic cells. We identified CRMP4 (collapsing response mediator protein-4) that was the only member of CRMP1-5 family to be expressed by BMSCs. We found CRMP4, a cytosolic phosphoprotein that mediates Semaphorin-3A effects in neuronal differentiation to be expressed by proliferating chondrocytes and osteoblastic cells and its expression was detected in bone lining osteoblasts in postnatal and adult mouse bones. In vitro gain and loss of CRMP4 function in bone marrow stromal cell line ST2 revealed the inhibitory effect of CRMP4 on osteoblast differentiation. Consistently, mice lacking Crmp4 expression displayed significant increased bone mass by 40% compared with wild type controls due to increased trabecular and cortical bone

Disclosure: The authors declared no competing interests.
OC3.3

Transgenic Over-Expression of Vitamin D Receptor in Mature Osteoblasts Enhances Catabolic Activities under Dietary Calcium and Phosphorus Restriction

Rahma Triliana1,2, Rebecca Sawyer3, Howard Morris2,3, Paul Anderson2,3

1Faculty of Medicine, Islamic University of Malang, Malang, East Java, Indonesia, 2School of Medicine, The University of Adelaide, Adelaide, South Australia, Australia, 3Centre for Orthopaedics and Trauma Research, University of Adelaide, SA, Australia, Adelaide, Australia

Osteoblast-specific over-expression of vitamin D receptor (VDR) in a transgenic mouse on a FVB/N genetic background (OSVDR) increases bone volume due to both reduced RANKL-mediated osteoclastic bone resorption and enhanced bone formation. These observations are in contrast to reports of 1,25-dihydroxyvitamin D (1,25D) enhancing osteoclastic bone resorption and inhibiting bone mineralisation. To address this conundrum, 3w female mice with Osteoblast-specific Over-expression of Vitamin D Receptor (VDR) (ObVDR-Tg), Osteoblast-specific VDRKO (ObVDR-KO) and littermate control mice (WT, VDRfl/fl) all on a C57/B16 genetic background were fed calcium/phosphorus restricted diet (0.03%Ca, 0.08%Phos; LowCa/P) for 17 weeks and compared with a normal diet (1%Ca, 0.625%Phos; NormCa/P). ObVDR-Tg mice fed the NormCa/P diet demonstrated increased trabecular (64% P<0.01) and cortical bone volumes (8%, P= 0.056) when compared with WT mice with increased peristeal circumference (P<0.05). All mice fed the LowCa/P diet resulted in marked osteopenia with almost total absence of metaphyseal trabecular bone. However, LowCa/P fed ObVDR-Tg mice maintained the increased peristeal circumference, whereas LowCa/P fed ObVDR-KO mice decreased peristeal circumference. Furthermore, LowCa/P fed ObVDR-Tg increased the endosteal circumference, whereas LowCa/P fed ObVDR-KO decreased the endosteal circumference. Interestingly, LowCa/P fed Ob-VDR-Tg mice exhibited marked intra-cortical porosity and a 22% reduction in cortical osteocyte density. While, serum calcium and phosphorus levels were unaltered in LowCa/P fed ObVDR-Tg mice, serum FGF23 levels were 2-fold lower and serum 1,25D levels were 2-fold higher when compared with WT mice. In addition, RANKL mRNA levels and RANKL:OPG ratio were markedly raised in LowCa/P fed ObVDR-Tg mice. Thus, while overexpression of VDR in osteoblasts can mediate anabolic activities, under conditions of limited dietary calcium and phosphorus, profound bone catabolism prevails possibly due to a lack of appropriate FGF23 feed-back on renal 1,25D synthesis and enhanced RANKL-mediated catabolism.

Disclosure: The authors declared no competing interests. This work was supported by a Project Grant from the National Health and Medical Research Council, Australia (APP1003433) and Career Development Fellowship for P.A. (APP1034698). PhD scholarship for Rahma Triliana is provided by the Directorate General of Higher Education (DGHE/DIKTI), the Government of Indonesia.

OC3.4

Absence of VDR in Mature Osteoclasts Results in Enhanced Resorptive Activity

Yolandi Starczak1, Daniel Reineke2, Rachel Davey2, Howard Morris3, Gerald Atkins3, Paul Anderson1

1Musculoskeletal Biology Research, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia, 2Department of Medicine, Austin Health, University of Melbourne, Melbourne, Australia, 3Centre for Orthopaedics and Trauma Research, University of Adelaide, SA, Australia, Adelaide, Australia

Mature osteoclasts express the vitamin D receptor (VDR) and while we have shown that these cells respond to active vitamin D (1,25(OH)2D3), the role for direct activity of vitamin D in regulating osteoclast function is not well understood. To assess the role for VDR-mediated activity in osteoclasts, osteoclast-specific vitamin D receptor knockout mice (OclVDR-/-) were generated by mating Cathepsin KCre with floxed VDR mice (VDRfl/fl). Male and female OclVDR-/- and VDRfl/fl littermates were assessed at 6 and 12 weeks of age under normal dietary conditions. In addition, isolated spleenocytes from global OclVDRKO mice or their wild-type (WT) littermates were assessed for osteoclast formation, resorption activity and gene expression under osteoclast-forming conditions. 6w old OclVDR-/- mice demonstrated increased osteoclast surface (Oc.S/Bs) in L1 vertebra in both female (+20%, P<0.05) and male (+67%, P<0.05) mice when compared to VDRfl/fl mice. In OclVDR-/- mice, V-ATPase (V0 subunit) mRNA was increased (P<0.05) and Calcitonin Receptor (CTR) mRNA markedly decreased (P<0.05). Despite this, biomarkers such as serum X-laps and TRAP5b were not significantly different between OclVDR-/- and VDRfl/fl mice. Furthermore, only males demonstrated a trend for decreased vertebral BV/TV% due to increased trabecular spacing (Tb.Sp) (P=0.05). Interestingly, RANKL mRNA levels were significantly decreased suggesting reduced signalling for osteoclastogenesis. VDRKO spleenocytes cultured under osteoclastogenic conditions resulted in 2-fold fewer TRAP-positive multinucleated cells (P<0.05) compared with WT cells. However, the resorption area on Osteologic™ slides was 3-fold greater per VDRKO osteoclast (P<0.05). VDRKO osteoclast CTR mRNA levels associated with reduced Bax/Bcl mRNA ratio were markedly decreased compared with WT suggesting resistance to apoptosis. Thus, while vitamin D
Active vitamin D (1,25D), bound to the vitamin D receptor (VDR), can directly regulate osteoblast activity modulating bone resorption via induction of RANKL. However, it is somewhat unclear as to which cells of the osteoblast lineage are predominantly responsible for this activity. We have generated mature Osteoblast-VDR Knock Out (mOb-VDRKO) mice using an osteocalcin promoter-Cre to demonstrate the role of VDR-mediated bone resorption in mature osteoblasts during growth and under dietary calcium/phosphorus restriction. 6 week old female mOb-VDRKO mice displayed a pronounced reduction in RANKL mRNA expression, metaphyseal osteoclast surface (OcSur/BS) and serum X-laps. As a consequence, trabecular bone volume (BV/TV%) was increased in the femur (19%, p<0.05) and vertebra (21%, p<0.05) in comparison to littermate controls. The increase in trabecular bone in female mOb-VDRKO persisted at 12w of age but was absent by 26w of age. By comparison, 6 week old female Osteocyte-specific-VDRKO mice (deletion driven by Dmp1-Cre), exhibited no structural differences in femoral trabecular BV/TV%, and unchanged OcSur/BS. However, vertebral BV/TV% was modestly increased (8%, p<0.05) in Oy-VDRKO mice. When 3 week old female mOb-VDRKO mice were subjected to a low calcium (0.03%) and phosphorus diet (0.08%) (LowCa/P) for 3 weeks, serum PTH levels and X-laps levels were approximately 2-fold greater than LowCa/P fed control mice, resulting in the abrogation of the bone phenotype to levels comparable to control mice. When the LowCa/P was continued to 20 weeks of age, higher serum PTH and X-laps levels persisted in mOb-VDRKO mice resulting in deleterious effects on bone including significant intra-cortical porosity. Collectively, these data suggest that mature osteoblasts play a greater role in VDR-mediated bone resorption than osteocytes in young mice. Furthermore, the absence of VDR in mature osteoblasts during calcium/phosphorus restriction results in inappropriately high PTH-mediated bone resorption, possibly through lack of appropriate VDR-mediated bone resorption.

Disclosure: The authors declared no competing interests. This work was supported by National Health and Medical Research Council (NHMRC) APP1029926, 2012-2014.

OC3.5

Mature Osteoblasts Regulate Vitamin D-Mediated Bone Resorption during Growth and Dietary Calcium/Phosphorus Restriction

Jackson Ryan1, Rahma Tirliana4, Rachel Davey3, Gerald Atkins2,4, Howard Morris1,4, Paul Anderson1
1School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia, 2Centre for Orthopædics and Trauma Research, University of Adelaide, Adelaide, SA, Australia, 3School of Medicine, Austin Health, University of Melbourne, Melbourne, VIC, Australia, 4School of Medicine, University of Adelaide, Adelaide, SA, Australia

Background: The adverse effects of weight loss on bone mineral density in postmenopausal women are well documented, and increased risk of distal forearm and hip fractures has been reported in studies with average follow-up periods of around 6 years after weight loss. The aim of this study was to investigate the effects of unintentional weight loss in postmenopausal women on the incidence of clinical fractures at multiple sites in the following year.

Methods: GLOW is an observational longitudinal study of non-institutionalised women aged ≥55 years recruited from 723 primary physician practices in 10 countries. Self-administered questionnaires were mailed and data collected included demographics, medical history, fracture occurrence, medications and weight loss of 10 lb (4.5 kg) or more over the preceding year. Cox models treating weight loss as a time-varying covariate were used to predict fracture in the following survey year, adjusting for factors such as age, prior fracture, co-morbidities, and falls that we have previously shown to be associated with the specific fracture.

Results: Unintentional weight loss of ≥10 lb during the previous 12 months was reported in Year 2 by 3405 (8.0%) of 42,756 and in Year 3 by 3322 (7.7%) of 43,004 women. After adjustment for clinically relevant variables, a significantly increased risk was seen for hip (HR 1.83, 95% CI 1.25–2.69, p<0.01) and spine fracture (HR 1.46, 95% CI 1.02–2.09, p=0.04) in the year following the unintentional weight loss.

Conclusions: Unintentional weight loss in postmenopausal women is associated with increased risk of hip and spine fracture within the year following weight loss. The rapid time course of this increase in risk has not previously been reported and emphasises the need for prompt fracture risk assessment and appropriate management in women with unintentional bone loss.

Disclosure: The Alliance for Better Bone Health. Financial support for the GLOW study is provided by Warner Chilcott Company, LLC and sanofi-aventis to the Center for Outcomes Research, University of Massachusetts Medical School. JEC acknowledges support from the Cambridge Biomedical Research Centre and the National Institute for Health Research (NIHR).

Reference
Hypophosphatasia (HPP) is the rare inherited metabolic bone sequelae in children, including muscle weakness and common phosphatase (TNSALP). HPP can cause a spectrum ofvariables. Fractures were more common in cannabis users no difference in BMD values between cannabis users and controls (data not shown). Heavy cannabis users were more likely to use other illicit drugs (65.8% vs. 2.9% for controls; p<0.001). There was no difference in BMD values between cannabis users and controls after adjustment for age, BMI, gender and other relevant variables. Fractures were more common in cannabis users (58% vs. 46%; p=0.06), and multiple fractures were significantly more common (10.6% vs. 1.9%, p=0.008). Heavy cannabis use is associated with reduced fat mass and an increased risk of fracture, but is not associated with BMD. The differences between mice and men may be due to the complex nature of cannabis, which contains not only THC, a CB1 agonist, but multiple other cannabinoids.

Disclosure: The authors declared no competing interests. This work was supported by Arthritis Research UK.

OC4.3
Significantly Improved Muscle Strength, Running Speed, and Agility in Children with Hypophosphatasia Treated with Asfotase Alfa
Dawn Phillips1, Kimberly Hamilton2, Scott Moseley3, Tatjana Odrljin3, Kenji P Fujita3, Amy Reeves4, Amy Yakimoski2, Katherine L Madson4, Cheryl Rockman-Greenberg2, Michael P Whyte4
1University of North Carolina Division of Physical Therapy, Chapel Hill, NC, USA, 2University of Manitoba, Winnipeg, Manitoba, Canada, 3Alexion Pharmaceuticals, Cheshire, CT, USA, 4Shriners Hospital for Children, St. Louis, MO, USA

Hypophosphatasia (HPP) is the rare inherited metabolic bone disorder resulting from deficiency in tissue-nonspecific alkaline phosphatase (TNSALP). HPP can cause a spectrum of sequelae in children, including muscle weakness and compromised physical function. 5-12 year-old children treated ≥3 years with asfotase alfa, a recombinant bone-targeted human TNSALP, had improved skeletal mineralisation, growth, and physical function. Here, we report muscle strength and the individual subtests of the Bruininks Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2) in these children. This ongoing Phase II open-label extension study (6 mg/kg/wk subcutaneous asfotase alfa) assessed bilateral hip and knee extension and flexion, hip abduction, and grip strength by hand-held dynamometry (HHD), reported as percent predicted (%P; right side) for matched, healthy peers. Physical function was evaluated using the BOT-2 Strength subtest (e.g. long jumps, push-ups, etc.), and Running Speed/Agility subtest (e.g. shuttle runs, one-legged hop, etc.). 11/12 patients in the extension study participated in functional testing with last assessment (LA) at 3 years (n=7) or 3.5 years (n=4). Right-side strength (%P) ranged from median (min-max) 32 (9-53; hip extensor) to 60 (21-148; grip) at baseline. Strength in all right-side muscle groups improved at 3 months (P<0.05) except grip, and continued to improve to LA (median 59-98 %P; hip and knee extensor, respectively) (P<0.05). Left side results were similar. BOT-2 Strength scaled score (mean±SD) for healthy peers: 15(6) improved from median (min-max) 4 (1-13) at baseline to 15 (10-24) at LA (P<0.0001). Median Running Speed/Agility scaled score improved from 3 (1-9) at baseline to 12 (7-19) at LA (P<0.0001). Performance on all BOT-2 subscales improved significantly. These children with HPP had substantial muscle weakness and impaired function at baseline. With asfotase alfa treatment, rapid and continued improvements in strength contributed to significant gains in physical function, which impact ability to perform activities of daily living.

Disclosure: SM, TO, and KF are employees of Alexion Pharmaceuticals, Inc. DP receives consulting fees from Alexion. KM, CRG, and MW have received honoraria, travel support, and research grant support from Alexion. Editorial support was provided by Fishawack Communications, GmbH, Basel, Switzerland, and was funded by Alexion Pharmaceuticals, Inc., Cheshire, CT, USA.

OC4.4
Odanacatib Anti-Fracture Efficacy And Safety in Postmenopausal Women with Osteoporosis: Results from the Phase III Long-Term Odanacatib Fracture Trial (LOFT)
Michael R McClung1, Bente Langdahl2, Socrates Papapoulos3, Kenneth Saag4, Henry Bone5, Andrea Rybak-Feiglin6, Dosinda Cohn6, Carolyn A DaSilva6, Rachid Massaad7, Arthur C Santora8, Boyd B Scott6, Keith D Kaufman6, Nadia Verbruggen7, Albert Leung8, Antonio Lombardi6
1Oregon Osteoporosis Center, Portland, OR, USA, 2Aarhus University Hospital, Aarhus, Denmark, 3Leiden University Medical Center, Leiden, The Netherlands, 4University of Alabama at Birmingham, Birmingham, AL, USA, 5Michigan Bone & Mineral Clinic, Detroit, MI, USA, 6Merck & Co., Inc., Whitehouse Station, NJ, USA, 7MSD Europe Inc., Brussels, Belgium, 8Formerly Merck & Co., Inc., Whitehouse Station, NJ, USA

LOFT (NCT00529373) is a randomised, double-blind, placebo-controlled, event-driven trial of odanacatib (ODN), an oral selective inhibitor of cathepsin K. Postmenopausal women ≥65 years with bone mineral density (BMD) T-score ≤-2.5 at
Rebecca Moon1,2, Sarah Crozier1, Sian Robinson1, Hazel Inskip1, Keith Godfrey1,3, Cyrus Cooper1,4, Nicholas Harvey1,3
1MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, Hampshire, UK, 2Paediatric Endocrinology, University Hospital Southampton NHS Foundation Trust, Southampton, Hampshire, UK, 3NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, Hampshire, UK, 4NIHR Musculoskeletal Biomedical Research Unit, University of Oxford, Oxford, Oxfordshire, UK

Background: When assessed in pregnancy studies, 25(OH)D is usually measured only once. However, it is unknown whether the ranking of an individual’s 25(OH)D is maintained across pregnancy, which crosses several seasons. We therefore assessed the tracking of 25(OH)D from early to late pregnancy in a prospective mother-offspring study, the Southampton Women’s Survey.

Methods: At 14 and 34 weeks gestation, serum 25(OH)D was measured, and diet and lifestyle questionnaires completed. We modelled seasonal variation in 25(OH)D separately for each time point using Fourier transformations, and then calculated the difference between actual 25(OH)D and the modelled value corresponding to the sampling date for each individual (denoted 25(OH)Ddev). We used Spearman’s rank correlation to test tracking of 25(OH)Ddev from 14 to 34 weeks gestation. Multivariate linear regression was used to determine factors associated with alterations in an individual’s 25(OH)Ddev ranking.

Results: 25(OH)D was available in 2060 and 2322 women at 14 and 34 weeks, respectively, with 1756 women included at both gestations. 25(OH)Ddev tracked moderately from 14 to 34 weeks (r=0.57, p<0.0001), although some women had marked changes in 25(OH)Ddev across pregnancy (median: -0.8; range: -150.1 to 129.6nmol/l), 25(OH)D tended to fall with greater pregnancy weight gain (25(OH)Ddev β=-0.4nmol/l per kg, p=0.02), and to rise with greater strenuous activity in late pregnancy (β=1.0nmol/l per hour/week, p=0.03). Vitamin D supplementation was the strongest influence on tracking: compared with women who never used supplements, discontinuing supplementation after 14 weeks was associated with negative change in 25(OH)Ddev (β=-7.2nmol/l, p<0.001), whereas commencing (β=12.2nmol/l, p<0.001) or continuing (β=8.0nmol/l, p<0.001) supplementation were positively associated.

Conclusion: Stability of an individual’s gestational 25(OH)D relative to the population is modest, and affected by weight changes, activity levels and vitamin D supplementation. These findings may explain some of the observed heterogeneity in studies relating maternal vitamin D status to offspring health.

Disclosure: KMG has acted as a consultant to Abbott Nutrition and Nestle Nutrition. He is part of an academic consortium that has received research funding from Abbott Nutrition, Nestec and Danone. This work was supported by the Medical Research Council, British Heart Foundation, Arthritis Research UK, National Osteoporosis Society, International Osteoporosis Foundation, Cohen Trust, NIHR Southampton Biomedical Research Centre, and NIHR Musculoskeletal Biomedical Research Unit, University of Oxford and the Dunhill Medical Trust.

OC4.5 Tracking of 25-Hydroxyvitamin D Status in Pregnant Women
Rebecca Moon1,2, Sarah Crozier1, Sian Robinson1, Hazel Inskip1, Keith Godfrey1,3, Cyrus Cooper1,4, Nicholas Harvey1,3
1MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton, Hampshire, UK, 2Paediatric Endocrinology, University Hospital Southampton NHS Foundation Trust, Southampton, Hampshire, UK, 3NIHR Southampton Biomedical Research Centre, University of Southampton and University Hospital Southampton NHS Foundation Trust, Southampton, Hampshire, UK, 4NIHR Musculoskeletal Biomedical Research Unit, University of Oxford, Oxford, Oxfordshire, UK

Background: When assessed in pregnancy studies, 25(OH)D is usually measured only once. However, it is unknown whether the ranking of an individual’s 25(OH)D is maintained across pregnancy, which crosses several seasons. We therefore assessed the tracking of 25(OH)D from early to late pregnancy in a prospective mother-offspring study, the Southampton Women’s Survey.

Methods: At 14 and 34 weeks gestation, serum 25(OH)D was measured, and diet and lifestyle questionnaires completed. We modelled seasonal variation in 25(OH)D separately for each time point using Fourier transformations, and then calculated the difference between actual 25(OH)D and the modelled value corresponding to the sampling date for each individual (denoted 25(OH)Ddev). We used Spearman’s rank correlation to test tracking of 25(OH)Ddev from 14 to 34 weeks gestation. Multivariate linear regression was used to determine factors associated with alterations in an individual’s 25(OH)Ddev ranking.

Results: 25(OH)D was available in 2060 and 2322 women at 14 and 34 weeks, respectively, with 1756 women included at both gestations. 25(OH)Ddev tracked moderately from 14 to 34 weeks (r=0.57, p<0.0001), although some women had marked changes in 25(OH)Ddev across pregnancy (median: -0.8; range: -150.1 to 129.6nmol/l), 25(OH)D tended to fall with greater pregnancy weight gain (25(OH)Ddev β=-0.4nmol/l per kg, p=0.02), and to rise with greater strenuous activity in late pregnancy (β=1.0nmol/l per hour/week, p=0.03). Vitamin D supplementation was the strongest influence on tracking: compared with women who never used supplements, discontinuing supplementation after 14 weeks was associated with negative change in 25(OH)Ddev (β=-7.2nmol/l, p<0.001), whereas commencing (β=12.2nmol/l, p<0.001) or continuing (β=8.0nmol/l, p<0.001) supplementation were positively associated.

Conclusion: Stability of an individual’s gestational 25(OH)D relative to the population is modest, and affected by weight changes, activity levels and vitamin D supplementation. These findings may explain some of the observed heterogeneity in studies relating maternal vitamin D status to offspring health.

Disclosure: Authors conflicts of interest for Merck include: consulting fees (MMR, BL, SP, KS, HB); grants (MMR, BL, KS, HB); royalties (MMR); participation in speakers bureaus (MMR, BL); employee (AR-F, DC, CAD, RM, AS, BBS, KDK, NV, ALo); former employee (ALE). This study was sponsored by Merck & Co., Inc.
inhibitors (DPP4-is). Currently, there is no data available from electronic healthcare databases. The objective of this study was to evaluate the association between incretin agents and risk of fracture.

**Methods:** We used data from the UK Clinical Practice Research Datalink (CPRD), the world’s largest primary care database, representative for the total UK population (2007-2012, n=13 million) and from the full country of Denmark (2007-2011, n=5.5 million). We used a cohort design and Cox regression analysis with CPRD data and a case-control study with conditional logistical regression in Denmark (which comprised all patients with a first fracture matched to controls). We compared current incretin use with non-use. A meta-analysis extracted hazard- (HRs) and odds ratios and their corresponding 95% confidence intervals (CIs) using generic inverse variance methods assuming a random effects model.

**Results:** Use of incretin agents was not associated with fracture risk in both countries (adj. pooled risk ratio DPP4-I and GLP1-ra: 1.01; 95% CI 0.92 – 1.12, 1.03; 95% CI 0.87 – 1.22, respectively). Increasing cumulative dose did not further decrease risk of fracture yielding adj. HRs of 1.07; 95% CI 0.90 – 1.27 (0-18.2 mg) adj. HR 0.84; 95% CI 0.67 – 1.06 (18.3-36.5 mg) adj. HR 1.05; 95% CI 0.81 – 1.37 (36.6-54.7 mg), adj. HR 0.97; 95% CI 0.78 – 1.20 (> 54.7 mg).

**Discussion:** Use of incretin agents was not associated with fracture risk in both countries, and higher cumulative dosages did not result in an inverse association. Our results do not support the conduct of further clinical research to study beneficial effects of incretin agents on fracture risk.

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

**OC5.1**

**Inhibition of the Interleukin-6-Induced STAT3 Signalling Pathway is Chondroprotective**

Augustin Latourte1,2, Chahrazad Cherifi1, Hang Korng Ea1,2, Wafa Bouaziz1, Thomas Funck-Brentano1,2, Martine Cohen-Solal1,2, Eric Hay3, Pascal Richette1,2

1INSERM UMR-1132, Hôpital Lariboisière, Paris, France,
2Fédération de Rhumatologie, Centre Vigo Petersen - Hôpital Lariboisière, Paris, France

**Background:** High levels of interleukin-6 (IL-6) have been found in the synovial fluid of patients with osteoarthritis (OA), suggesting that IL-6 may be involved in the pathogenesis of OA. The objectives were to investigate the effects of IL-6 in chondrocytes and to determine its main signalling pathways; and to study the impact of IL-6 inhibition in an experimental mouse model of OA.

**Methods:** The effects of IL-6 (10-50-100 ng/mL) were determined in vitro (primary culture of mouse chondrocytes) and ex vivo (mouse femoral articular cartilage). Proteoglycan content (Alcian blue and Safranin O staining, DMM blue assay), expression of catabolic factors (qPCR, Western Blot, immunostaining), NO and PGE2 production and apoptosis (TUNEL assay) were evaluated. IL-6-induced signalling pathways were determined by western blot. The impact of STAT3 blockade was investigated using a specific inhibitor – Stattic – ex vivo and in a mice model of OA induced by destabilisation of the medial meniscus (DMM).

**Results:** In vitro and ex vivo, IL-6 dose-dependently induced a dramatic loss of proteoglycan content through an increase in the expression of MMP3, MMP13, ADAMTS4 and ADAMTS5. By contrast, IL-6 had no effect on col2, aggrecan, col10 or VEGF. IL-6 induced chondrocytes apoptosis without increasing NO or PGE2 production. Inhibition of STAT3 by Stattic counteracted the catabolic and pro-apoptotic effects of IL-6 ex vivo. Finally, we orally administrated either Stattic (25 mg/kg/2d) or a saline for 6 weeks in C57/B16 mice (n=18) subjected to DMM. The severity of the OA lesions as assessed with the OARS histological score was significantly lower in the Stattic group: 2.65 ± 1.44 vs. 4.5 ± 0.93 (p=0.004).

**Conclusion:** Our findings indicate that IL-6 has numerous catabolic effects in cartilage, mainly mediated by STAT3. STAT3 blockade protects against DMM-induced OA in mice, suggesting that IL-6 might be a promising therapeutic target in OA.

**Disclosure:** The authors declared no competing interests. This work was supported by the Sociétè Française de Rhumatologie.

**OC5.2**

**Genetic Variants in the SUPT3H-RUNX2 Locus Confer Susceptibility for Bone and Cartilage Related Disorders via Long-Range Regulation Of RUNX2**

Cindy Boer1, Roberto Narsici1, Yolande Ramos2, Wouter den Hollander2, Nils Borner2, Martha Castano Betancourt1, André Uitterlinden1, Gerjo van Osch1, Ingrid Meulenbelt2, Joyce van Meurs1

1Erasmus Medical Center, Rotterdam, The Netherlands,
2Leiden University Medical Center, Leiden, The Netherlands

Genome-wide association studies (GWAS) have identified in total 6 independent SNPs within the 5’ region of the RUNX2 gene to be robustly associated with 5 different cartilage and bone related phenotypes. We aim to elucidate the effect of the identified SNPs on the regulation and expression of RUNX2 and how these confer susceptibility to cartilage and bone related disorders, such as osteoarthritis and osteoporosis. Independent GWAS signals and SNPs in LD with the GWAS loci were identified with GCTA conditional joint analysis, the SNAP tool, and HaploReg (V2.2, Broad Institute). GWAS SNPs and SNPs in high LD were analysed for enrichment in genomic regulatory regions, and co-location with DNA binding proteins using data from the ENCODE-Project, Roadmap epigenetics project, and the FANTOM5 database. In human cartilage explants we measured RUNX2 expression by RNA sequencing, CTCF-DNA binding by ChIP-qPCR and preformed eQTL analysis to determine the effect of the SNPs on gene expression. We found 6 genetically independent GWAS signals to co-localise to regions with enrichment of active enhancer markers, H3K4me1, H3K27ac, DNase1 hypersensitivity enrichment and bi-directional CAGE reads, in osteoblast and chondrogenic cells. The BMD associated SNP located ~700 kb away from the RUNX2 promoter, had a significant effect (p<0.05) on RUNX2 gene expression in human cartilage. In addition, we observed that when we stimulated RUNX2 expression in human chondrocytes by TGFβ stimulation, there is an increase in binding of the chromatin-loop mediating protein, CTCF, near the RUNX2 promoter. We have found that variants in the SUPT3H-RUNX2 locus associated to cartilage and bone phenotypes are located in gene regulatory regions, and affect RUNX2 gene expression. We hypothesise that the SNPs are localised in long-range enhancers which, mediated by CTCF
chromatin-loop to the RUNX2 promoters, regulate RUNX2 gene expression in bone and cartilage development.

Disclosure: The authors declared no competing interests. This work was supported by the Netherlands organisation for scientific research (NWO) VIDI-scheme.

OC5.3
MUC1 in Osteoblasts Balances Osteogenesis and Angiogenesis under Hypoxia
Jyotirmaya Behera1,2, Andrea Brum1, Marijke Schreuders-Koedam1, Cindy van der Leije1, Suvro Chatterjee2, Johannes van Leeuwen1, Bram van der Eerden1

1Erasmus MC, Rotterdam, The Netherlands, 2AU-KBC Research Centre, Anna University, Chennai, India

It is critical that bone formation and angiogenesis are tightly coordinated during bone development and fracture healing. Oxygen tension impacts both processes. Previously we demonstrated that hypoxia limits osteoblast differentiation/mineralisation and strongly induces mucin1 (MUC1) expression in human osteoblasts. Expression of MUC1 is positively associated with hypoxia-driven angiogenesis. Thereby MUC1 is a likely candidate to control both osteogenesis and angiogenesis. We investigated MUC1 function in osteoblasts and its role in the interaction between bone formation and angiogenesis. Hypoxia (2% O2)-induced inhibition of osteoblast differentiation (Alkaline phosphatase activity -64%) and mineralisation (-89%) was prevented by blocking MUC1 function using either a specific inhibitor (GO-201) or 2 shRNAs. This was supported by studies using osteoblasts cultured from bone marrow of Muc1 knockout mice. Conditioned medium of osteoblasts cultured under hypoxia (HCM) stimulated endothelial migration (+80%) and angiogenesis (+150%), which was prevented by blocking MUC1 in osteoblasts using GO-201 or shRNA. Mass spectrometry analysis identified among others vascular endothelial growth factor (VEGF)-A and mVGEF migration inhibitory factor (MIF) to be present in control HCM but not in HCM of osteoblasts treated with GO-201 and shRNA. VEGF neutralising antibody or MIF inhibitor 4-IPP prevented HCM-induced endothelial morphogenesis. HCM induced nitric oxide (NO) production (1.8 fold increase) in human endothelial cells and inhibition of NO production blocked the angiogenic effect of HCM. Finally, it was shown that nuclear translocation of the MUC1 cytoplasmic tail in osteoblasts is essential for the effects observed. In conclusion, we demonstrate that MUC1 in osteoblasts is at the crossroad of oxygen control of osteoblast differentiation/mineralisation and angiogenesis. The level and nuclear translocation of MUC1 in osteoblasts determines whether under hypoxia either bone formation or angiogenesis prevails. Thereby, these data contribute to the molecular understanding of the balance between osteogenesis and angiogenesis in bone development and fracture repair.

Disclosure: The authors declared no competing interests. This work was supported by the European Union (PIRSES-GA-2011-295181).

OC5.4
Osteoblast-Secreted Extracellular Vesicles Stimulate the Expansion of CD34+ Human Umbilical Cord Blood Cells
Jess Morhayim1, Jeroen van de Peppel1, Eric Braakman1, Bram van der Eerden1, Mariette ter Borg1, Marijke Schreuders-Koedam1, Andre van Wijnen2, Jan Cornelissen1, Johannes P. van Leeuwen1

1Erasmus MC, Rotterdam, The Netherlands, 2Mayo Clinic, Rochester, MN, USA

Umbilical cord blood (UCB) is increasingly used in haematopoietic stem cell (HSC) transplantations; however, the low cell numbers are still remaining as a limiting factor for proper engraftment. Osteoblasts play important roles in regulating HSC self-renewal and differentiation. Recently, extracellular vesicles (EVs) have been implicated in stem cell fate regulation via horizontal transfer of proteins and nucleic acids between cells. In this study, we focused on the characterisation of osteoblast EVs and investigated their potential in ex vivo expansion of CD34+ UCB cells for clinical use. We used human pre-osteoblasts (SV-HFO cells) to isolate EVs, and characterised EVs by electron microscopy, proteomics, and RNA sequencing, and investigated their functional effect on human CD34+ UCB cells by qPCR and flow cytometry. Characterisation analyses demonstrated that osteoblast EVs are heterogeneous in size, contain novel osteoblast EV proteins primarily linked to ribosomal activity and RNA processing, and are enriched with small RNAs. Treatment of CD34+ UCB cells with osteoblast EVs led to donor-dependent 2-3-fold expansion (p < 0.01) of the CD34+ expressing progenitors in 10 days. MicroRNA profiling demonstrated that osteoblast EVs contain abundant amounts of miR-29a, one of the key regulators of early haematopoiesis. Interestingly, EVs treatment led to the two-fold down-regulation (p < 0.01) of HBP1, a miR-29a target that has been shown to be a cell cycle inhibitor, in CD34+ UCB cells. Consequently, cell cycle analysis showed that EVs stimulated progression from G0/G1 to S/G2 phase (p < 0.05), which may explain the mechanism by which EVs stimulate UCB cell expansion. Finally, EV-expanded CD34+ UCB cells showed good clonogenicity and differentiation potential in vitro and successful engraftment in a NOD/SCID-IL2Rγ (NSG mice) xenograft model in vivo. In this study, we demonstrated that osteoblasts secrete EVs that expand UCB cells ex vivo, and uncovered the first clues that contributed to the understanding of EV function.

Disclosure: The authors declared no competing interests. Erasmus MC Stem Cell and Regenerative Medicine Institute.

OC5.5
Sclerostin Depletion and its Effect on Fracture Healing in the Mouse Model
Mohammad Alzahrani1,2, Reggie Hamdy1, Frank Rauch1

1Division of Orthopaedic Surgery, Shriners Hospital for Children, Montreal Children Hospital, McGill University, Montreal, Quebec, Canada, 2Department of Orthopaedic Surgery, University of Dammm, Dammm, Saudi Arabia

Background: Sclerostin is a secreted glycoprotein that interacts with LRP5 receptor on osteoblasts and inhibits the intracellular Wnt signalling pathway, leading to decreased bone formation. When sclerostin is inactivated bone formation is therefore stimulated. This stimulation has been proven in
fracture studies, which showed that sclerostin deficient mice have larger and stronger calluses with accelerated fracture healing, both in sclerostin knockout and sclerostin antibody injection models. These observations suggest that sclerostin inhibition and depletion show improved and accelerated fracture healing, but the effect of these two mechanisms have not been compared to assess the accurate effect of the Scl-Ab injections. Therefore we designed a study to compare the effect of sclerostin depletion (sclerostin knockout) and inhibition (Scl-Ab injection).

Methods: Ten-week-old male SOST knockout (KO) (N=20) and wild-type (WT) (N=40) mice underwent insertion of a tibial intramedullary pin after which a mid-shaft tibial osteotomy was performed. The mice were divided into three groups: SOST KO (N=20), WT with Scl-Ab injection (N=20) and WT with saline injection (N=20). The Scl-Ab group received an intravenous dose of 100mg/kg weekly starting on day 7. Each group was managed and sacrificed according to the specified protocol (Figure 1). For data analysis, one-way ANOVA (Analysis Of Variance) was performed followed by Tukey’s post hoc test at each time point. P values<0.05 were considered statistically significant.

Results: Both Scl-Ab and KO groups showed significantly increased trabecular BV/TV (bone volume/ total volume) at the fracture site (mid-shaft of the tibia) compared to the saline group at all time points and also showed no significant difference between them at all time points (except at 28 days postoperative) (Figure 2). On biomechanical testing the Scl-Ab and KO groups showed significant increased strength in stiffness at days 14, 28 and 35 compared to the saline group (Figure 3A). Concerning ultimate force and work to failure the KO group showed significant increase in the force required compared to both the Scl-Ab and saline groups at 21,28 and 35 days. While the Scl-Ab group showed increased forced required to fracture the callus compared to the saline group at these time points, but this was only significant for work to failure at 28 days (Figure 3B, D).

Conclusion: Scl-Ab injections showed promising results, which were comparable to the complete depletion of sclerostin, especially at earlier stages of the healing process. In addition, our results indicate that sclerostin antibody exerts its greatest effect in the earlier stages of fracture healing (days 14 and 21), after which the healing process plateaus and thus completing this process at an earlier time point. Further re-
search into accurate dosage and adequate timing of administration is required before these promising results can be implicated as a modality for accelerating fracture healing in humans and management of delayed / nonunion.

Disclosure: The authors declared no competing interests.

OC6.1 Secular Change in Fracture Incidence is Not Associated with Better Post-Fracture Outcomes: a Time-Trend Comparison between Two Birth Cohorts

Dana Bliuc1,2, Tuan Nguyen1,2, John Eisman1,2, Jacqueline Center1,2
1Garvan Institute of Medical Research, Sydney, NSW, Australia, 2UNSW Australia, Sydney, NSW, Australia, 3St Vincent’s Hospital, Sydney, NSW, Australia, 4University of Notre Dame, Sydney, NSW, Australia

During the last decade, hip fracture incidence declined and life expectancy improved. However, it is unclear whether the outcomes following osteoporotic fracture have also changed. The aim of this study was to compare re-fracture risk and excess mortality following osteoporotic fracture between two birth cohorts and over 2 time intervals 1989-1999 and 2000-2010. Study participants comprised women and men 60+ participating into DOES1 (born before 1930) and DOES2 (born after 1930). All fractures excluding head, fingers and toes were recorded between 1989 and 2010. Age-standardised fracture incidence and mortality rates were calculated in two time intervals: 1989-1999 (for DOES1) and 2000-2010 (for DOES2). The difference in excess mortality between the 2 cohorts was assessed using standardised mortality ratios (SMR) calculated for each study cohort using time-specific population mortality rates. The prevalence of osteoporosis declined and the level of treatment increased significantly in DOES 2 compared to DOES 1. Fracture incidence declined by ~10% in both genders, however, not significantly. Interestingly, re-fracture risk was similar for DOES1 and DOES2 [women age-adjusted RR 2.0 (95% CI, 1.6-2.5) in DOES1 and 1.9 (95% CI, 1.7- 2.3) in DOES2 and men, 3.5 (95% CI, 2.7-4.8) in DOES1 and 3.4 (95% CI, 2.7- 4.5) in DOES2]. Crude mortality rates decreased during study follow-up. However, after taking into account the difference in general population life expectancy during the 2 study periods, the excess mortality post-fracture was similar [women, SMR 2.1 (95% CI, 1.7- 2.6) in DOES1 and 1.7 (95% CI, 1.2- 2.4) in DOES2, and men, 1.9 (95% CI, 1.5- 2.5) in DOES1 and 1.9 (95% CI, 1.3- 2.7) in DOES2]. Thus despite a reduction in the prevalence of osteoporosis and improvement in treatment uptake over the last 2 decades, re-fracture risk and fracture-associated excess mortality was similar. The reasons for this deserve urgent exploration.

Disclosure: The authors declared no competing interests. This work was supported by the National Health Medical Research Council Australia (NHMRC project ID: DB 1073430, JRC 1008219, TVN, JRC and JAE 1070187).
Autosomal Recessive Osteopetrosis (ARO) is a rare genetic bone disease with genotypic and phenotypic heterogeneity, sometimes translating into delayed diagnosis and treatment; in particular, intermediate cases often constitute a diagnostic challenge. Mutations in the TCIRG1 gene are responsible for more than 50% of ARO cases, and a wide range of molecular defects have been found. Here we describe the identification of 4 different single nucleotide changes in intron 15 in 5 patients from 4 unrelated families. These novel mutations were in the middle of a 368 nucleotide long intron, far from the canonical splice sites; therefore, they were missed by standard gene amplification and sequencing, focused on exons and exon-intron boundaries, and went ignored by exome sequencing. In 3 out of 5 patients, by cloning and sequencing a number of independent cDNA clones covering exons 14 to 17, we demonstrated a reduced splicing efficiency, which did not completely abrogate the production of the normal transcript. In conclusion, we identified an intronic region in the TCIRG1 gene which seems to be prone to splicing mutations. These molecular defects allow the production of a small amount of protein sufficient to dampen the severe phenotype usually associated to TCIRG1 mutations. Indeed, the patients bearing these variants displayed a different level of severity of the disease, with 3 out of 5 reaching adulthood with a mild presentation. On this basis, we suggest the analysis of the TCIRG1 gene is appropriate not only in the molecular work up of severe patients, but also of intermediate cases. In addition, our results demonstrate that standard protocols for gene testing have to follow these effects in longer term.

Disclosure: The authors declared no competing interests. This work was supported by the Telethon Foundation [GGP12178], PRIN Project [20102M7T8X_003], Giovani Ricercatori from Ministero della Salute [GR-2011-02348266], Ricerca Finalizzata from Ministero della salute [RF-2009-1499,542], the European Community’s Seventh Framework Program [FP7/2007-2013, SYBIL Project], PNR-CNR aging Program 2012-2014, the Leenards Foundation Lausanne and the Swiss National Foundation.
Mortality in Hip Fracture Patients

Background: The relationship between bone metabolism and plasma sodium levels has lately gained increasing interest as hyponatraemia has been linked to both increased risk of osteoporosis and fractures. The aim of this study was to examine the frequency of hypo- and hypernatraemia in patients admitted with a fractured hip and the association with 30-day mortality in these patients.

Methods: A database of all surgically treated hip fracture patients admitted to our hospital between January 1996 and November 2013 was searched for all patients aged 60 years or above. 7755 patients were identified and a search for plasma sodium levels has lately gained increasing interest as hyponatraemia has been linked to both increased risk of osteoporosis and fractures. The aim of this study was to examine the frequency of hypo- and hypernatraemia in patients admitted with a fractured hip and the association with 30-day mortality in these patients.

Results: The patients had a mean age of 82.5 (SD 8.5) years and 76.5% (5845/7644) were female. 19.0% (1455/7644) were hyponatraemic, 1.6% (123/7644) were hypernatraemic and 79.4% (6066/7644) were normonatraemic on admission. There was an increased 30-day mortality rate for patients with hyponatraemia (12.1%, p=0.008 (chi-square)) and hypernatraemia (16.3%, p=0.02 (chi-square)) compared to normonatraemic patients (9.7%). The hazard ratios for 30-day mortality were 1.26 [1.06;1.49] (unadjusted) and 1.35 [1.14;1.60] (adjusted for sex, age and comorbidity) for hyponatraemic patients and 1.74 [1.12;2.72] (unadjusted) and 1.76 [1.13;2.78] (adjusted for sex, age and comorbidity) for hypernatraemic patients.

Conclusion: The study showed that the prevalence of hyponatraemia in hip fracture patients was high. Furthermore, patients with decreased or elevated plasma sodium levels had an increased mortality rate. Disturbances in plasma sodium levels may itself cause increased mortality but could also be a surrogate marker for frailty in these patients.

Disclosure: The authors declared no competing interests.

OCT6.5

The Calcineurin Inhibitor Tacrolimus as a New Therapy in Severe Cherubism

Cherubism is a rare genetic disorder characterised by extensive growth of a bilateral granuloma of the jaws, resulting in facial disfigurement. Cherubism is caused by gain-of-function mutations in the SH3BP2 gene, leading to over-activation of NFATc1-dependent osteoclastogenesis. Recent findings in human and mouse cherubism suggested that calcineurin inhibitors might be drug candidates in cherubism medical treatment. A 4-year-old boy with aggressive cherubism was treated with the calcineurin inhibitor tacrolimus for one year, and clinical, radiological, and molecular data were obtained. Immunohistological analysis was performed to compare pre- and post-operative NFATc1 staining and TRAP activity. Real-time PCR was performed to analyse the relative expression levels of OPG and RANKL. After tacrolimus therapy, the patient showed significant clinical improvement, including stabilisation of jaw size and intra-ossseous osteogenesis. Immunohistological analyses on granuloma showed that tacrolimus caused a significant reduction in the number of TRAP positive osteoclasts and NFATc1 nuclear staining in multinucleated giant-cells. Molecular analysis showed that tacrolimus treatment also resulted in increased OPG expression. We present the first case of effective medical therapy in cherubism. Tacrolimus enhanced bone formation by stimulating osteogenesis and inhibiting osteoclastogenesis.

Disclosure: The authors declared no competing interests. This research was supported by the PHRC TEIOS, NK by AFDS (Association Française Développement de la Stomatologie) and AEC by ANR Osteodiversity (ANR-12-BSV1-0018).
Blood Circulating miRNAs are Indicative of Skeletal Fractures in Postmenopausal Women with and Without Type 2 Diabetes and may be Promising Candidates for General Fracture Risk Prediction

Ursula Heilmeier1, Matthias Hackl3, Susanna Skalicky3, Fabian Schroeder3, Klemens Vierlinger4, Andrew J. Burghardt1, Ann V. Schwartz5, Johannes Grillari2, Thomas M. Link1

1Musculoskeletal Quantitative Imaging Research Group, Department of Radiology & Biomedical Imaging, University of California San Francisco, San Francisco, CA, USA, 2Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria, 3TAmiRNA GmbH, Vienna, Austria, 4Austrian Institute of Technology, Vienna, Austria, 5Department of Epidemiology & Biostatistics, University of California San Francisco, San Francisco, CA, USA

Fracture risk in type-2-diabetes (T2D) and postmenopausal osteoporosis is routinely assessed with FRAX or DXA although these methods show limitations especially in T2Diabetics. Novel, general applicable biomarkers are therefore desirable. MicroRNAs (miRNAs) are secreted into the circulation from cells of various tissues proportional to local disease severity and were recently found to be crucial to bone homeostasis (“osteomiRs”) and T2D aetiology. The objective of this study was to analyse circulating miRNAs in a well-characterised study of postmenopausal and diabetic osteoporosis and to evaluate their utility for general fracture risk assessment. MiRNA-qPCR-arrays and differential-expression-analysis of 153 miRNAs were performed from 74 serum samples drawn from postmenopausal T2D women with (DMFX, n=19) and without fracture history (DM, n=19) as well as from non-diabetic women with (Fx, n=19) and without fracture history (Co, n=17). Group-wise non-parametric statistical comparisons were used with BH-adjustment of p-values for multiple testing. Circulating miRNAs exhibiting significant differences were then used for building multi-parametric models to differentiate fracture patients from controls. Cumulative ROC analyses yielded AUC-values of 0.978 for Fx/Co-comparisons (based on 4 miRNAs) and 0.933 for DMFX/DM-comparisons (based on 4 miRNAs). Interestingly, the 4 highly discriminative miRNAs of each comparison did not overlap. We found that some of them have been previously described as “osteomiRs”, such as miR-155-5p, an initiator of osteoclastogenic differentiation, or miR 96-5p, an osteocyte negative marker. All remaining ones had not been previously characterised (e.g. miR-188-3p and miR-203a) yet. Therefore, additional in vitro tests were performed, to characterise their (anti)-osteogenic activity. Our data provide first evidence that certain circulating miRNA levels are indicative of fragility fractures in postmenopausal women with and without diabetes and may be novel candidates for general fracture risk screenings. Future studies will elucidate if this knowledge can be used to improve current diagnostic techniques to predict fracture risk and therapy response in elderly women.

Disclosure: Susanna Skalicky: employee at TamiRNA; Matthias Hackl: COO at TamiRNA; Johannes Grillari: scientific advisor TamiRNA, CEO of evercyte.GmbH. The study was funded by EU_FP7 Frailomic (JG, SKA), NIH RC1 AR058405 (TML), NIH R01 AR060700 (AJB), AWS Preseedgrant (MH).