

# Oral Presentation Abstracts

**6th International Conference on Osteoporosis and Bone Research: 'Oral Presentation' Abstracts**  
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## OP 01

### **Effects of Teriparatide on Cortical Histomorphometric Variables in Patients on Long-Term Alendronate Therapy**

Jan J Stepan<sup>1</sup>, Harald Dobnig<sup>2</sup>, Astrid Fahrleitner-Pammer<sup>2</sup>, Yanfei L Ma<sup>3</sup>, Qingqiang Zeng<sup>3</sup>, Xiaohai Wan<sup>3</sup>, Fernando Marin<sup>4</sup>, Imre Pavo<sup>4</sup>

<sup>1</sup>Institute of Rheumatology, Charles University Faculty of Medicine, Prague, Czech Republic; <sup>2</sup>Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Graz, Austria; <sup>3</sup>Lilly Research Laboratories, Indianapolis, IN, USA; <sup>4</sup>Lilly Research Center, Windlesham, UK

Teriparatide (TPTD) treatment increases bone turnover and cancellous bone formation in patients previously treated with potent antiresorptives, such as alendronate (ALN, Stepan *et al.*, 2010). However, no histological information exists on the effects of TPTD on cortical bone in the ALN pretreated patients. The aim of this *post-hoc* analysis was to investigate the changes in cortical microstructure and dynamic histomorphometric indices in patients with osteoporosis, with or without prior therapy with ALN, who were treated subcutaneously with TPTD 20 µg per day for 24 months. Sixty-six postmenopausal women with osteoporosis, mean age (s.d.) of 68 (7) years and mean BMD *T*-score of -1.7 (0.9) at total hip and -2.8 (0.8) at lumbar spine, and 62% with prevalent fractures were included in the study. Thirty-eight patients stopped previous ALN treatment (10 mg per day or 70 mg per week for a mean duration of 63.6 months) and switched to TPTD, whereas 28 patients were treatment naive (TN) of osteoporosis pre-treatment. Forty-five paired iliac crest biopsies were collected and analyzed by micro-CT, static and dynamic histomorphometry at baseline and after 24 months. In the ALN pretreated group, the MS/BS (%) values were lower than that in the TN group, at both the periosteal (baseline: 0.61±1.29 vs 1.39±0.96, *P*=0.04; post-TPTD: 1.34±1.05 vs 3.94±2.7, *P*=0.002) and endocortical levels (baseline: 3.16±5.05 vs 6.19±5.07, *P*=0.06; post TPTD: 4.95±4.03 vs 11±9.57, *P*=0.027). In the pooled analysis, percent changes in indices mean of periosteal (MS/BS: 164%; BFR/BS: 282%) and endocortical (MS/BS: 71%; BFR/BS: 107%) dynamic variables, total cortical area (39%), cortical thickness (34%) and porosity area (67%) increased significantly after 24 months. Our results indicate the bone-forming effects of TPTD in the cortical bone in patients previously treated with ALN or the TN patients. The observed increase in

cortical porosity in both groups may be the consequence of increased intracortical remodeling.

## OP 02

### **The association between GGPS1 polymorphisms and response of alendronate treatment in women with postmenopausal osteoporosis**

Lanwen Han<sup>1</sup>, Mei Li<sup>1</sup>, Ling Xu<sup>2</sup>, Eryuan Liao<sup>3</sup>, Zhenlin Zhang<sup>4</sup>, Decai Chen<sup>5</sup>, Tianzun Tao<sup>6</sup>, Wen Wu<sup>7</sup>, Jian Liu<sup>8</sup>, Weibo Xia<sup>1</sup>, Min Nie<sup>1</sup>, Yingying Hu<sup>2</sup>, Xiaoping Xing<sup>1</sup>, Yan Jiang<sup>1</sup>, OU Wang<sup>1</sup>

<sup>1</sup>Department of Endocrinology, Endocrine Key Laboratory of the Ministry of Health of China, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China; <sup>2</sup>Department of Gynaecology and Obstetrics, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China; <sup>3</sup>Institute of Metabolism and Endocrinology, The Second Xiang-Ya Hospital, Central South University, Hunan, China; <sup>4</sup>Metabolic Bone Disease and Genetic Research Unit, Department of Osteoporosis and Bone Diseases, Shanghai Jiao Tong University Affiliated Sixth Peoples Hospital, Shanghai, China; <sup>5</sup>Department of Endocrinology, West China Hospital, Sichuan University, Chengdu, China; <sup>6</sup>Department of Orthopaedics, Second Affiliated Hospital, Harbin Medical University, Harbin, China; <sup>7</sup>Department of Endocrinology, East Ward, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China; <sup>8</sup>Institute of Orthopedic Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an, China

**Objective:** Nitrogen-containing bisphosphonates inhibit osteoclast function through interfering with the mevalonate pathway, and GGPS1 is a key enzyme of this pathway. The objective of this study was to explore the association between GGPS1 polymorphisms and response to alendronate treatment in women with postmenopausal osteoporosis.

**Methods:** A total of 632 postmenopausal osteoporotic or osteopenic women were enrolled and randomly assigned to a low-dose (70 mg per 2 weeks) or a conventional-dose group (70 mg per week). Before and after treatment, serum calcium, phosphate, alkaline phosphatase (ALP), cross-linked C-telopeptide of type I collagen (CTX) levels and BMD were measured. The six single-nucleotide polymorphisms of GGPS1 were determined using TaqMan Pre-designed SNP Genotyping Assays and STR (short tandem repeat), respectively.

**Results:** The genotype frequency of rs3840452 in this population was similar to that of Koreans, whereas the genotype frequency of rs2803851, rs2789367, rs10802624 and rs10925503 were different from that in populations from Japan, Europe

and Africa. The rs10925503 was related to baseline CTX ( $P=0.012$ ), and bone resorption level was higher in patients carrying the TT genotype. In the conventional-dose group, rs10925503 and rs2789366 were significantly correlated to the change in BMD at the trochanter after 6 months treatment ( $P=0.019$ ,  $P=0.048$ ) and the alleles T and C were more sensitive to treatment, respectively. In the low-dose group, rs2789367 was significantly associated with the change of ALP after 3 months treatment ( $P=0.03$ ) and ALP decreased more in the GG genotype. After 6 months treatment in the conventional-dose group, rs10925503 polymorphism affected the response rate of the total hip BMD ( $P=0.015$ ), and the response rate was higher for the allele T. After 1 year treatment in the conventional-dose group, there was a significant difference in the response rate of the total hip BMD between different genotypes of rs3840452, rs2789367 and rs10802624 ( $P=0.034$ ,  $P=0.025$ ,  $P=0.034$ , respectively), and the more sensitive alleles were A, C and A respectively. The rs10925503 polymorphism also affected the response rate for trochanter and total hip BMD ( $P=0.011$ ,  $P=0.044$ , respectively), and the drug effect was better in patients carrying the allele T.

**Conclusion:** The study first reports that GGPS1 polymorphisms may have effects on the response to alendronate treatment in Chinese women with postmenopausal osteoporosis. GGPS1 is an important candidate gene for predicting the impact of bisphosphonates on bone turnover markers and BMD.

### OP 03

#### The Kidney Tonic Formula for the Treatment of Osteopenia: A Double-Blind, Randomized, Placebo-Controlled Trial

Feng Yang<sup>1,2</sup>, Dezhi Tang<sup>1</sup>, Xuejun Cui<sup>1</sup>, Qin Bian<sup>3</sup>, Qi Shi<sup>1,3</sup>, Yongjun Wang<sup>1,3</sup>

<sup>1</sup>Longhua Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China; <sup>2</sup>No. 1 Affiliated Hospital, Shanxi University of Traditional Chinese Medicine, Xianyang, China; <sup>3</sup>Spine Research Institute, Shanghai University of Traditional Chinese Medicine, Shanghai, China

**Background:** Osteoporosis is a growing problem worldwide, with the greatest burden resulting from fractures. Nevertheless, the majority of fractures occur in those people with 'osteopenia' (bone mineral density (BMD) only moderately lower than young normal individuals). As long-term drug therapy is an expensive option with uncertain consequences and side effects, natural herbal therapy offers an attractive alternative. In traditional Chinese medicine (TCM), bone and kidney is closely related. Kidney deficiency could result in bone mass loss. Thus, the kidney tonic formula (Chinese herbal medicines) have been used to prevent bone loss for thousands years. But the evidence intensity of the herbal therapy is poor in evidence-based medicine. The purpose of this study is to evaluate the effect on BMD and safety of the kidney tonic formula for treatment of osteopenia by randomized controlled trial (RCT) and to investigate the mechanism by which this efficacy is achieved.

**Methods/design:** We proposed a multi-center double-blind, randomized, placebo-controlled trial to evaluate the efficacy and safety of the kidney tonic formula for the treatment of osteopenia. Participants aged 55–75 years with low BMD (T-score between  $-1.0$  and  $-2.5$ ) and kidney deficiency syndrome

in TCM were included and randomly allocated into two groups: treatment group and control group. Each group had 100 participants. Participants in the treatment group were treated with the kidney tonic granule for 6 months, whereas the controlled group received placebo. All participants received 600 mg element calcium on a daily basis. Primary outcome was BMD of the lumbar spine and proximal femur measured by the dual-energy X-ray absorptiometry. Secondary outcomes included pain intensity measured with visual analog scales, quality of life, serum markers of bone metabolism and safety. BMD and serum markers of bone metabolism were measured at baseline and at 6 months. Other outcomes were measured at baseline, at 1 month, at 3 months and at 6 months. The outcomes were compared between treatment group and control group using analysis of variance for repeated measures.

**Results:** One hundred and eighty-two participants completed the trial. In the experimental group, eight cases withdrew and three cases had gastrointestinal side effects. In the control group, 10 cases withdrew and 2 cases had gastrointestinal reactions. Four cases of adverse reactions didn't need special treatment and completed the trial. No serious adverse events occurred in any group. The BMD were increased in the treatment group compared with the placebo control group ( $P=0.042$  for interaction between time and group at femoral neck;  $P=0.056$  for interaction between time and group at lumbar spine). The lumbar BMD of the treatment group was increased 4.6% compared with baseline, whereas that of the control group was decreased 3.5% compared with baseline. The kidney tonic formula was statistically significant in relieving pain ( $P=0.014$ ) and improving quality of life ( $P=0.000$ ) compared with placebo. BGP ( $P=0.005$ ) and PINP ( $P=0.008$ ) were significantly increased in the treatment group, whereas CTX ( $P=0.008$ ) was significantly decreased in the treatment group compared with the control group.

**Conclusion:** The kidney tonic formula is superior to the placebo in preventing bone mass loss, as demonstrated by increasing BMD, ameliorating the life quality and the kidney deficiency symptoms, relieving pain and regulating bone metabolism indices. Those results imply that the kidney tonic formula could be an attractive therapy for the treatment of osteoporosis.

### OP 04

#### Age-Related Changes in Body Composition and Their Relationship with Decreases in Bone Mineral Density in Central South Chinese Postmenopausal Women

Hongbing Zhang<sup>1,2</sup>, Xiangping Chai<sup>3</sup>, Shuang Li<sup>2</sup>, Zhimin Zhang<sup>2</sup>, Lingqing Yuan<sup>2</sup>, Hui Xie<sup>2</sup>, Houde Zhou<sup>2</sup>, Xiyu Wu<sup>2</sup>, Zhifeng Sheng<sup>2,4</sup>, Eryuan Liao<sup>2</sup>

<sup>1</sup>Binjiang Hospital, Second Affiliated Hospital, Zhejiang University, Hangzhou, China; <sup>2</sup>Institute of Metabolism and Endocrinology, Second Xiang-Ya Hospital, Central South University, Changsha, China; <sup>3</sup>Department of Emergency, Second Xiangya Hospital, Central South University, Changsha, China; <sup>4</sup>Key Laboratory of Protein Chemistry and Developmental Biology of Education Ministry of China, College of Life Science, Hunan Normal University, Changsha, China

**Purposes:** The objective of this study was to investigate the age-related changes in body composition and their relationship

with decreases in bone mineral density (BMD) in central south Chinese postmenopausal women.

**Methods:** A cross-sectional study was conducted on 779 healthy postmenopausal women, aged 50–77 years. Lumbar spine, total hip, and femoral neck BMD and body composition were measured by dual-energy X-ray absorptiometry.

**Results:** In women under 65 years, lean mass levels showed a stable downward trend and were significantly higher than those in the age group of the 65–70 years and >70 years. However, the fat mass levels showed no significant difference between the age groups. After controlling for age, age at menopause and height, both fat mass and lean mass positively correlated with BDR at the lumbar<sub>1–4</sub> spine, the femoral neck and the total hip. When BDR at the lumbar<sub>1–4</sub> spine was used as the dependent variable, a higher  $R^2$  change and partial  $R^2$  in fat mass were seen than when age, age at menopause or lean mass were used as the dependent variable. When BDR at the femoral neck or total hip was used as the dependent variable, lean mass was a more significant determinant than that of fat mass.

**Conclusion:** We found that with advancing age, lean mass begins to decrease in women aged over 65 years, but fat mass levels show no significant difference between the age groups. Both fat mass and lean mass positively correlate with BDR, with site-specific differences. Fat mass is the most significant determinant of BDR at the lumbar spine, whereas lean mass is the most significant determinant of BDR at the femoral neck and total hip.

#### OP 05

##### Polymorphisms in the WISP3 Gene Are Associated with Low Lumbar Spine Bone Mineral Density in Chinese Postmenopausal Women

Jing Sun, Weibo Xia, Min Nie, Xin Zheng, Qiuping Wang, Xiran Wang, Wenbo Wang, Zhiwei Ning, Wei Huang, Wei Yu, Qiang Lin, Yingying Hu, Mei Li, Yan Jiang, Ou Wang, Xiaoping Xing, Xueying Zhou, Xunwu Meng

Department of Endocrinology, Key Laboratory of Endocrinology, Ministry of Health, Peking Union Medical College Hospital, Chinese Academy of Medical Science, Beijing, China

**Objective:** The participation of Wnt-1-inducible signaling pathway protein 3 (WISP3) in chondrogenesis and osteogenesis may affect susceptibility to osteoporosis. The aim of this study was to explore the association between WISP3 polymorphisms and bone phenotypes consisting of bone mineral density (BMD), osteoporotic fractures and vertebral fractures in Chinese postmenopausal women.

**Methods:** A total of 1570 postmenopausal women of the Han ethnic group were randomly selected from the Peking Vertebral Fracture study in Beijing by a multi-stage and age-stratified sampling. BMD of the lumbar spine, femoral neck and total hip were measured by dual energy X-ray absorptiometry. Fracture phenotypes were obtained by questionnaire, and vertebral fracture phenotypes were ascertained by vertebral X-ray reading. All tagging and potential functional single-nucleotide polymorphisms (SNPs) in WISP3 were determined by Taqman Allelic discrimination assay. Differences in BMD associated with genotype or haplotype were calculated using

multiple linear regressions. The odds ratio for the case and control groups associated with genotype or haplotype were calculated using binary logistic regression.

**Results:** The lumbar spine BMD was clearly associated with rs1230345 (G to T mutation). Individuals carrying the TT genotypes had a 55% increased incidence of osteopenia and osteoporosis in the lumbar spine (OR, 1.554; 95% CI, 1.116–2.165;  $P=0.009$ ) and had lower BMD in femoral neck than those carrying the GT ( $P=0.028$ ) and GG genotypes ( $P=0.027$ ). Vertebral fracture was associated with rs4947163. Individuals homozygous for the minor allele had a 3.8-fold increased incidence of vertebral fracture (OR, 4.833; 95% CI, 1.022–22.85;  $P=0.009$ ). Vertebral fracture was associated with rs9400518. Individuals homozygous and heterozygous for the minor allele had a 57% increased incidence of vertebral fracture (OR, 1.567; 95% CI, 1.008–2.435;  $P=0.046$ ). The lumbar spine BMD was clearly associated with haplotype b (5'-AGCGTTA-3'). Individuals homozygous for this haplotype had a 50% increased incidence of osteopenia and osteoporosis in the lumbar spine (OR, 1.503; 95% CI, 1.048–2.157;  $P=0.027$ ).

**Conclusion:** We found that the rs1230345 polymorphism and the lumbar spine BMD were significantly associated; rs4947163 and rs9400518 polymorphisms were associated with vertebral fractures. These results require confirmation in other populations and larger samples, but suggest a role for WISP3 in the genetic susceptibility to osteoporosis among the Chinese postmenopausal women.

#### OP 06

##### Predictive Value of the Osteoporosis Screening Tool (OST) and Quantitative Ultrasonography in Detecting Women at High Risk for Low Bone Mass in Riyadh, Saudi Arabia

AlJohara AlQuaiz<sup>1,2</sup>, Ambreen Kazi<sup>2</sup>, Shaik Shafi<sup>1</sup>, Salwa Tai<sup>1</sup>, Othman AlSaleh<sup>3</sup>

<sup>1</sup>Department of Family and Community Medicine-KSU, Riyadh, Saudi Arabia; <sup>2</sup>Princess Nora Chair for Women's Health Research-KSU, Riyadh, Saudi Arabia; <sup>3</sup>Department of Nuclear Medicine-KSU, Riyadh, Saudi Arabia

**Introduction:** Osteoporosis and its associated risk factors are increasing in magnitude, especially in the developed world. Reliable and valid tools are needed to screen the people at risk of osteoporosis during the initial stages so that proper preventive steps can be taken. The osteoporosis screening tool (OST) is found to be a convenient and easy method to identify the high-risk patients. Lately, quantitative ultrasound (Achilles machine) has been found to be moderately beneficial in predicting the risk of osteoporosis among the high-risk women.

**Objective:** This study was conducted in Riyadh, Saudi Arabia, to identify the screening tool with better predictive value, to detect women at high risk of low bone mass.

**Method:** This is a community-based, household cross-sectional survey. A total of 1500 women were enrolled in Riyadh City. Primary health care centers (PHCC) of the five regions of Riyadh city (north, east, west, south and center) were randomly selected. Two-stage cluster sampling technique was used. Randomly selected women were invited to PHCC. A structured questionnaire along with anthropometric measurements was administered. Quantitative ultrasonography was

performed through the Achilles machine. Those whose readings were  $> (-1)$  and/or  $OST > 2$  were referred to the King Khalid University Hospital for DEXA measurements and blood investigations. These included vitamin-D level, bone profile, kidney and liver function tests, thyroid function test and parathyroid hormone level.

**Results:** OST for the lumbar spine had a sensitivity and a specificity of 34.72% and 83.8%, respectively, and the area under the curve was 0.60 with a  $P$ -value of  $<0.05$ . The sensitivity and specificity for femur was 68.6% and 61.1%, respectively, with the area under the curve as 0.70 ( $P < 0.01$ ). The Achilles machine demonstrate the sensitivity and specificity for the lumbar spine as 67.89% and 46.50%, respectively, with an area under the curve as 0.53 ( $P=0.22$ ); for femur the sensitivity was 76.67% and specificity was 48.01%, and the area under the curve being 0.59 ( $P < 0.01$ ). In combination, the sensitivity and specificity for lumbar spine was 76.39% and 40.12%, respectively, and the area under curve was 0.59 ( $P < 0.01$ ), and for femur the sensitivity and specificity was 80.39% and 50.18%, respectively, with the area under the curve being 0.70 ( $P < 0.01$ ).

**Conclusion:** The predictive value of OST for both the lumbar spine and femur neck is more than the Achilles machine alone, or in combination with the OST. Hence, it is recommended that OST can be used for screening patients with low bone mass.

#### OP 07

##### Transcriptional Suppressor Foxp1/2/4 Regulate Osteoblast Differentiation in Mesenchymal Progenitor Cells During Endochondral Ossification

Haixia Zhao, Wenrong Zhou, Xizhi Guo

Bio-X Institutes, Shanghai Jiao Tong University, Shanghai, China

**Objective:** Great progress has been achieved in the past years in understanding the transcriptional regulation of skeletal ossification, especially the activators for osteoblast differentiation, such as Runx2, Osterix and so on. Yet, the transcriptional suppressors controlling osteoblast differentiation are still not well defined.

**Methods:** To examine the gene expression pattern, *in situ* hybridization and IHC were performed on sections of embryos ranging from E12.5 to E18.5. For skeletal phenotype analyses, targeted genes were overexpressed in osteochondrogenic progenitor cells driven by the *Col2a1* promoter and were conditionally depleted by *Col2a1-Cre*.

**Results:** Foxp1/2/4, transcriptional factors of the forkhead gene family, are involved in regulating multiple cell differentiation processes. Here we observed that *Foxp1*, *Foxp2* and *Foxp4* were exclusively and similarly expressed in the perichondrium of appendicular skeleton during embryonic development. Overexpression of *Foxp1*, *Foxp2* or *Foxp4* in the chondrocytes driven by the *Col2a1* promoter inhibited chondrocyte hypertrophy and maturation in the growth plate. Conversely, conditional inactivation of *Foxp1* or *Foxp2* in osteochondrogenic progenitor cells resulted in premature osteogenic differentiation in perichondrium and accelerated chondrocyte hypertrophy. The double knockout of *Foxp1* and *Foxp2* exhibited additive defects in osteoblast differentiation. Molecularly, Foxp1/2/4 proteins repressed the transcriptional activity of Runx2 via directly binding to the Runt domain.

**Conclusions:** We speculate that the Foxp1/2/4 proteins act as a suppressor complex controlling the pace of osteoblast differentiation in mesenchymal progenitor cells during endochondral ossification.

#### OP 08

##### Identifying MicroRNA-34a as an Inhibitor of Osteogenic Differentiation in Human Stromal (Mesenchymal) Stem Cells

Li Chen

Odense University Hospital, Denmark

**Objectives:** Osteoblast differentiation and bone formation (osteogenesis) are regulated by transcriptional and post-transcriptional mechanisms. Recently, microRNAs (miRNAs) were identified as novel key regulators of diverse biological processes by mediating translational repression or mRNA degradation of their target genes. In this study we aim to identify novel miRNAs as regulators for osteoblast differentiation (bone formation) in human stromal (mesenchymal) stem cells (hMSCs).

**Methods:** The following methods were used: miRNA array, real-time PCR, *in-situ* hybridization, cell transfection, human stromal stem cell osteogenic differentiation, ectopic bone formation, western blot, miRNA target reporter system, siRNA gene knockdown.

**Results:** We identified miRNA-34a (miR-34a) and its target protein networks that modulate osteogenic differentiation in hMSCs. The miRNA array profiling and further validation by quantitative RT-PCR revealed that miR-34a was tightly regulated during osteoblast differentiation of hMSCs. *In-situ* hybridization also showed that miR-34a is expressed in osteocarcinoma tissues. Overexpression of miR-34a *in vitro* strongly inhibited the differentiation of hMSCs into osteoblasts and mineralization, whereas inhibition of miR-34a function by anti-miR-34a enhanced the expression of osteogenic genes. Furthermore, overexpression of miR-34a reduced the final levels of ectopic bone formation by hMSCs by 60% *in vivo*, and conversely, *in vivo* bone formation was enhanced by around 300% when miR-34a was antagonized. Target prediction analysis and experimental validation by western blots confirmed Jagged1, a ligand for the receptor notch 1, as well as Sirtuin 1, as *bona fide* targets of miR-34a in osteogenesis of hMSCs. Depression of JAG1 and SirT1 regulated the networks of cell proliferation, apoptosis and Wnt pathways, and mediated the attenuation of miR-34a in bone formation.

**Conclusion:** Our findings identified miRNA-34a as an inhibitor of osteoblast differentiation in hMSCs. Pharmacological inhibition of miR-34a by anti-miR-34a could represent a therapeutic strategy for enhancing bone formation in human bone-relative diseases.

#### OP 09

##### miR-17-92 Regulates Osteoblast Differentiation

Xijie Yu, Mingliang Zhou, Xiang Chen, Ma Junrong

Laboratory of Endocrinology and Metabolism, West China Hospital, Sichuan University, Chengdu, China

The miR-17-92 cluster encodes six miRNAs (miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92-1), which are

highly conserved in all vertebrates. Loss-of-function of the miR-17-92 cluster resulted in smaller embryos and immediate postnatal death of all animals owing to severely hypoplastic lungs and ventricular septal defects in the hearts. Germline hemizygous deletions of *MIR17HG* accounted for microcephaly, short stature and digital abnormalities in a few cases of Feingold syndrome. These reports indicate that miR-17-92 may have an important function in growth and skeletal development. However, the precise roles of the miR-17-92 cluster in skeletal development and growth are largely unknown. To determine the functional roles of miR-17-92 in osteoblast differentiation, embryonic stem cells and bone marrow stromal cells were induced to differentiate into osteoblasts in osteogenic medium; the expression of miR-17-92 was assayed by quantitative real-time RT-PCR. The expression of miR-17-92 was downregulated along with osteoblast differentiation; the lowest level was found in mature osteoblasts. To determine the systemic function of miR-17-92 in skeleton, the bone mineral density (BMD) and bone volume were assayed in miR-17-92<sup>+/-</sup> mice by DXA and micro-computed tomography, respectively. Compared with wild-type controls, miR-17-92<sup>+/-</sup> mice showed significantly lower trabecular and cortical BMD, bone volume and trabecular number at 10 weeks old. To determine possibly the direct function of miR-17-92 in bone cells, osteoblasts from miR-17-92<sup>+/-</sup> mice were investigated by *ex vivo* cell culture; miR-17-92 was further conditionally deleted in osteoblasts by a 2.3-kb *Col1a1-Cre* (miR-17-92<sup>ΔOB/ΔOB</sup>). Osteoblasts from miR-17-92<sup>+/-</sup> mice showed lower ALP activity and less calcification. The miR-17-92<sup>ΔOB/ΔOB</sup> mice demonstrated lower BMD, bone volume and trabecular number. Taken together, our results suggest that the miR-17-92 cluster critically regulates skeletal development, and this regulation is mostly through its function in osteoblasts.

#### OP 10

##### Constitutive Activation of $\beta$ -Catenin Impaired the Terminal Differentiation of Osteoblasts and Odontoblasts

Sixu Chen, Zhaowen Zong, Min Jia, Yue Shen, Yufeng Zhao, Qingshan Guo, Jerry Feng

Department of Trauma Surgery, Daping Hospital, Third Military Medical University, Chongqing, China

**Background and Objective:**  $\beta$ -catenin has an important role in bone and teeth development, and both knockout and constitutive activation of  $\beta$ -catenin (ca- $\beta$ -catenin) lead to severe defect in bone and teeth development. However, the role of ca- $\beta$ -catenin in the terminal differentiation of osteoblasts and odontoblasts remains unclear. In this study we aimed to observe the effect of ca- $\beta$ -catenin on the terminal differentiation of osteoblasts and odontoblasts, and to observe the contribution of these effects to the phenotype of ca- $\beta$ -catenin mice.

**Methods:**  $\beta$ -Catenin exon 3 FX<sup>+/+</sup> mice was crossed with a 3.2-kb *Col 1-Cre* ERTM1 mice to get a 3.2-kb *Col 1-Cre* ERTM1  $\beta$ -catenin exon 3 FX<sup>+/+</sup> (or <sup>+/-</sup>) mice, and then exon 3 of  $\beta$ -catenin was knocked out by tamoxifen injection, leading to constitutive activation of  $\beta$ -catenin. X-ray, HE staining, Trap staining and TUNNEL staining were employed to observe the phenotype of ca- $\beta$ -catenin mice. Immunohistochemistry (IHC) and real-time PCR were used to investigate the expression of

bone and teeth development-related genes, such as *RunX2*, *Osterix*, bone sialoprotein (*BSP*), dentin sialophosphoproteins (*DSPP*), tissue non-specific alkaline phosphatase (*TNAP*), human phosphate-regulating neutral endopeptidase (*PHEX*), *FAM20C*, *Biglycan* and dentin matrix protein 1 (*DMP1*) in long bone and teeth. Masson staining was used to observe the content of collagen.

**Results:** Bone volume increased in the tibia and femur of ca- $\beta$ -catenin mice; however, tibia and femur became fragile and they were prone to breakage when dissecting. The point-bending test showed the mechanical strength of tibia and femur in ca- $\beta$ -catenin mice was significantly lower than that in wild-type mice. Masson staining showed the content of collagen decreased in ca- $\beta$ -catenin mice. The expression of *RunX2*, *Osterix*, *TNAP* and *Biglycan* increased in ca- $\beta$ -catenin mice, and Masson staining showed that the content of collagen decreased significantly in ca- $\beta$ -catenin mice.

Teeth development was retarded and the root became extremely short in ca- $\beta$ -catenin mice. HE staining showed that the thickness of dentin decreased and the thickness of predentin increased in ca- $\beta$ -catenin mice. IHC and real-time PCR revealed that the expression of *FAM20C*, *DSPP* and *PHEX* decreased significantly in ca- $\beta$ -catenin mice when compared with wild-type mice, whereas the expression of *Biglycan* increased in ca- $\beta$ -catenin mice. TRAP staining showed the activity and the number of osteoclasts decreased significantly in ca- $\beta$ -catenin mice.

**Conclusions:** Constitutive activation of  $\beta$ -catenin impaired the terminal differentiation of osteoblasts and odontoblasts, contributing to bone and teeth development defect.

#### OP 11

##### Wntless Regulates Bone Development by Affecting Canonical and Noncanonical Wnts

Zhendong Zhong, Cassandra R, Zylstra Diegel, Bart O Williams

Van Andel Research Institute, Grand Rapids, MI, USA

**Objectives:** Wnt signaling has a pivotal role in bone development. However, which cell type(s) produce Wnt ligands in the bone microenvironment to regulate bone acquisition and homeostasis has yet to be determined. *Wntless/Gpr177* is a protein that specifically escorts Wnt ligands for secretion, and is reported to be significantly related to bone mass. We are investigating the roles of Wnt ligands from different tissues in the bone.

**Methods:** In this work we will specifically delete *Wntless* from mature osteoblasts (*Osteocalcin-Cre*), osteochondral cells (*Col2-Cre*) or osteoprogenitors (*Dermo1-cre*). Using microCT and histology, we will compare embryonic skeleton at different stages and examine the molecular mechanisms underlying any phenotype we observe.

**Results:** Without Wnt secretion from mature osteoblasts (*Osteocalcin-Cre*), mice show a severe low bone-mass phenotype after 20 days of age. Without Wnt secretion from osteochondral cells (*Col2-cre*), embryos have a reduced proliferating cartilage zone and display ectopic cartilage. In addition, these mutants have dramatically reduced trabeculae and mineralization, and die shortly after birth. Without Wnt secretion from a

broader region in bone (Dermo1-cre), embryos do not survive beyond E14.5 and show even more severe phenotypes.

**Conclusion:** We demonstrate that Wntless controls canonical and noncanonical Wnts to regulate cartilage development and endochondral ossification.

## OP 12

### Indian Hedgehog (Ihh) is Not Required for Fibular Fracture Healing in Col2a1-CreER; Ihhf1/fl and WT Mice

Lei Wei<sup>1</sup>, Shaowei Wang<sup>1</sup>, Kai Li<sup>1</sup>, Pengcui Li<sup>1</sup>, Douglas C Moore<sup>1</sup>, Ge Zhang<sup>2</sup>, Qian Chen<sup>1</sup>, Xiaodu Wang<sup>3</sup>, Xiaochun Wei<sup>4</sup>, Hongbin Li<sup>1</sup>

<sup>1</sup>Medical School of Brown University, Providence, RI, USA;

<sup>2</sup>Chinese University of Hong Kong, Hong Kong, China;

<sup>3</sup>University of Texas at San Antonio, San Antonio, TX, USA;

<sup>4</sup>Shanxi Medical University, Shanxi, China

**Aims:** Ihh signaling has a critical role in endochondral bone formation during growth plate development and postnatal bone formation. However, it is not clear whether Ihh also has a role in fracture healing. The objective of this study was to determine whether Ihh is required for fracture healing, using Ihh-deletion mice (Col2a1-CreER; Ihhf1/fl) and by using hedgehog pharmaceutical inhibitor Cyclopamine in wild-type (WT) C57/B6 mice.

**Methods:** A total number of 114 mice were used in this study. Two-month-old female Col2a1-CreER; Ihhf1/fl mice were randomized into the experimental group ( $n=45$ ; deleted Ihh by injecting tamoxifen (TM): 1 mg per mouse per day for 5 days) and the control group ( $n=45$ ; injected corn oil). To keep the activity of the TM-inducible Ihh deletion, additional TM (1 mg) was given every week until the end point. To validate the genetic loss-of-function results, C57/B6 WT mice ( $n=24$ ) were randomized into Ihh inhibition group by Cyclopamine (25 mg kg<sup>-1</sup> body weight per day for 3 weeks) and the control group.

**Fracture model:** a transverse fibula fracture was made in all the mice 2 weeks after treatment.

**X-ray,  $\mu$ CT and histological analysis:** X-ray was taken 2 and 3 weeks immediately after the operation to monitor the fracture healing. Animals were killed 1, 2 and 3 weeks post injury ( $n=15$  per time-point). Three-dimensional volume images were generated of the fibulae with micro-computed tomography system ( $\mu$ CT40), after which the bones were embedded and stained with Safranin-O/Fast Green for histological analysis. The volume of the endochondral bone formation was quantified from the histological slides (NIS-Elements AR 3.10), and bone volume fraction (BV/TV) and bone mineral density (BMD) were calculated from the  $\mu$ CT image data set.

**Mechanical test:** Nanoindentation testing was performed on the fracture calluses from 3-week-old specimens.

**Real-Time PCR:** RT-PCR was used to quantify the changes of mRNAs isolated from fracture callus from 1 and 2 weeks time points.

**Results:** Deletion of Ihh was confirmed by the occurrence of growth plate closure in the Col2a1-CreER; Ihhf1/fl mice by X-ray 3 weeks after treatment of TM 1. The fracture healing was confirmed by X-ray and  $\mu$ CT in the 3 weeks post-fracture period in all animals. Histological analysis with cartilage staining by Safranin-O (red) indicated that compared with the control, cartilage area was less in the fracture sites in the

Ihh-deficient animals by either genetic deletion or drug inhibition at 1 and 2 weeks post fracture. The Ihh immunostaining was diminished in the fracture callus of the Ihh-deletion mice. The mRNA level of Gli-1, a downstream target of the Ihh pathway, was reduced by 70% and 60% in the mice treated with TM or Ihh inhibitor, respectively. This indicated that the Ihh pathway was attenuated in the Ihh-deletion and -inhibition mice. No cartilage staining was found at 3 weeks in all animals. Results of the  $\mu$ CT showed that there was no significant difference in the bone volume fraction (BV/TV) and BMD ( $P>0.05$ ) between the experimental and the control groups. The mechanical test showed a similar result in the elastic modulus and the hardness at 3 weeks after surgery ( $P>0.05$ ).

**Conclusions:** Our data indicated that interruption of the Ihh pathway during endochondral bone formation by either genetic or pharmaceutical approach did not affect fibula fracture healing in the mice. This surprising finding implicates that at least in fibula, Ihh is not required for fracture healing.

## OP 13

### Vertebral Fractures in Chinese Women Aged 50 Years and Older, Prevalence and Potential Risk Factors: Peking Vertebral Fracture (PK-VF) Study

Ling Chen, Weibo Xia, Yan Jiang, Yu Pei, Xin Zheng, Qiuping Wang, Wenbo Wang, Zhiwei Ning, Wei Huang, Xiran Wang, Mei Li, Ou Wang, Xiaoping Xing, Shuli He, Wei Yu, Qiang Lin, Ling Xu

Department of Endocrinology, Key Laboratory of Endocrinology, Ministry of Health, Peking Union Medical College Hospital, Chinese Academy of Medical Science, Beijing, China

**Objective:** Vertebral fracture is one of the most commonly seen symptoms of osteoporosis, and causes considerable mortality, morbidity and economic burden. The aim of this study was to investigate the prevalence of vertebral fracture in women over the age of 50 years, in Beijing, and assess the potential risk factors.

**Methods:** General information, including past medical history and exercise habits, serum biochemical parameters for liver and kidney function, bone turnover markers, bone mineral density (BMD) and lateral spine radiographs were assessed in a random community-based sample of 1834 women over the age of 50 years in Beijing. Vertebral fractures were diagnosed using Genant's semiquantitative technique. The age-specific prevalence was computed, and binary logistic regression was used to analyze the association between BMD, potential risk factors and vertebral fracture.

**Results:** The prevalence of vertebral fractures increased with age, from 1.2% (95% CI, 0.4–2.0%) at ages 50–59 years to 20.3% (11.4–29.2%) for women aged 80 years and older. Multiple vertebral fractures were seen in 30 (26.8%) women. The vertebrae of T<sub>12</sub> and L<sub>1</sub> together accounted for 54.5% of the total fractures. Age, BMD and serum N-terminal procollagen of type 1 collagen (P1NP) were associated with vertebral fracture. Each s.d. decrease in L<sub>2-4</sub>, femoral neck and total hip BMD was associated with a 1.4-fold (1.07–1.75), 1.3-fold (1.02–1.58) and 1.7-fold (1.23–2.30) increased odds, respectively, of having a vertebral fracture. Each s.d. increase in P1NP increased the risk about 1.2-fold (1.02–1.51). Long-duration daily housework was probably a protective factor for vertebral fractures. We did not find an association between other potential

risk factors and vertebral fracture, such as height, weight, menopause, non-spine fracture history, smoking, jobs involving heavy physical labor and serum biochemical parameters.

**Conclusion:** The age-specific prevalence of vertebral fractures for women over the age of 50 in Beijing increased with age steeply. Reduced BMD was associated with increased risk for vertebral fractures, and increased bone turnover markers such as P1NP was associated with increased risk of vertebral fractures.

#### OP 14

##### **Vertebroplasty Versus Kyphoplasty for the Treatment of Thoracolumbar Vertebral Compression Fracture in Female Asian Patients Above 65 Years: Matched-Pair Analysis of Prospective Outcomes**

Justin Boey

National University of Singapore, Singapore

**Introduction:** The aim of this study was to compare the outcomes of vertebroplasty (VP) and kyphoplasty (KP) in 125 consecutive female Asian patients above 65 years with L1 osteoporotic vertebral compression fractures.

**Methods:** A total of 57 patients underwent VP, whereas 68 patients underwent KP from 2004 to 2008. All patients had minimum follow-up of at least 2 years. Informed consent and ethics board approval was obtained. The inclusion criteria was the first incidence of osteoporotic wedge VCF as determined by clinical and radiographic findings. Exclusion criteria were previous thoracolumbar spine surgery, metabolic bone disease, malignancy and trauma. Outcomes were measured prospectively at pre-operation, 1 month, 6 months and 2 years post operation by blinded assessors at an independent diagnostic center. Anterior, middle and posterior vertebral heights of the L1 vertebral body were measured on lateral thoracolumbar spine radiographs using a digital measurement tool with accuracy up to 0.01mm. Three assessors measured each parameter three times on separate days. Functional outcomes measured were short-form 36 (SF-36) score and visual analog scale (VAS) score for back pain. Statistical analysis was conducted using Mann-Whitney *U*-test on SPSS 16.0 statistical software. Intra-observer and inter-observer variability were also assessed.

**Results:** There was no significant difference in the intra-observer and inter-observer variability for radiographic measurements. There was greater improvement of anterior and middle vertebral heights immediately post surgery for the KP group as compared with the VP group ( $P < 0.001$ ).

At 2 years post surgery, the KP group had better maintenance of L1 vertebral body height with percentage loss of 16.4%, 17.3% and 8.84% of anterior, middle and posterior vertebral height, respectively, as compared with the VP group who had a greater loss of 29.2%, 42.3% and 17%, respectively ( $P < 0.001$ ). Back pain improved post-operatively in both groups with no significant difference in VAS back pain score between the two groups at each follow-up time point ( $P > 0.05$ ). The SF-36 score improved post-operatively in both groups ( $P = 0.001$ ). At 2 years post surgery, the physical functioning (SFPF) domain of SF-36 was better in the KP group ( $P = 0.01$ ).

**Conclusion:** At 2 years post surgery, both KP and VP provided back-pain relief. KP provides better restoration and maintenance of anterior and middle vertebral heights with better physical function outcome.

#### OP 15

##### **Appendicular DXA BMC and Lean Mass Strongly Associated to Hip Fracture Risk**

John A Shepherd<sup>1,2</sup>, Serghei Malkov<sup>1</sup>, Kathleen Peters<sup>3</sup>, Jane Cauley<sup>4</sup>, Steve Cummings<sup>3</sup>, Tamara Harris<sup>5</sup>, Rachel Murphy<sup>6</sup>, Suzanne Satterfield<sup>7</sup>, Marjolien Visser<sup>8</sup>, Joseph Wilson<sup>1,2</sup>, Joseph Zmuda<sup>4</sup>, Peggy Cawthon<sup>3</sup>

<sup>1</sup>Department of Radiology and Biomedical Imaging, University of California–San Francisco, San Francisco, CA, USA; <sup>2</sup>UC Berkeley–UCSF Graduate Program in Bioengineering, San Francisco, CA USA; <sup>3</sup>California Pacific Medical Center, San Francisco, CA, USA; <sup>4</sup>Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA; <sup>5</sup>National Institute on Aging, Bethesda, MD, USA; <sup>6</sup>Division of Human Nutrition, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada; <sup>7</sup>Department of Preventive Medicine, University of Tennessee, Memphis, TN, USA; <sup>8</sup>Institute for Research in Extramural Medicine, VU Medical Center and Institute of Health Sciences, VU University, Amsterdam, The Netherlands

**Objective:** We aim to determine the utility of quantifying DXA-derived surrogates of CT measures, including thigh cross-sectional muscle area (CSA) and muscle attenuation.

**Methods:** We used a retrospective case-cohort study design using data from the Health Aging and Body Composition study. Cases were 111 participants that had a hip fracture during the 8.7-year study period. A cohort of 212 non-fracture participants was randomly selected from the entire study population. Each participant received a DXA whole-body scan and a single-slice mid-thigh CT scan. Thigh muscle CSA and attenuation were estimated from DXA using in-house algorithms and geometric modeling to CT measures. Strength testing and an extensive questionnaire were also available from each visit.

**Results:** In a fully adjusted model (age, race, site, gender, chronic disease, physical activity, self-rated health, MMSE, drinking, smoking, education, hip BMD), we found BMC and lean mass variables were associated ( $P < 0.05$ ) to fracture risk (relative hazard/s.d., 95% confidence interval, *P*-value), including appendicular BMC (4.1, 1.72–9.69, 0.001) and arm lean mass (2.5, 1.12–5.49, 0.03). DXA and CT thigh-muscle attenuation values were similar and not significant in the model: (0.75, 0.5–1.11, 0.15) and (0.79, 0.55–1.12, 0.18), respectively.

**Conclusions:** Strong associations for DXA BMC and lean mass variables, but not thigh muscle attenuation, to hip fracture risk were found in a fully adjusted model.

#### OP 16

##### **Balloon Kyphoplasty Versus Percutaneous Vertebroplasty in Treating Osteoporotic Vertebral Compression Fracture: A Meta-Analysis**

Dan Xing<sup>1</sup>, Xin Long Ma<sup>1,2</sup>, Jian Xiong Ma<sup>1</sup>, Jie Wang<sup>1</sup>, Tao Ma<sup>1</sup>, Yang Chen<sup>1</sup>

<sup>1</sup>Tianjin Medical University General Hospital, Tianjin, China;

<sup>2</sup>Tianjin Hospital, Tianjin, China

**Object:** The objective of this study is to assess the safety and efficacy of balloon kyphoplasty compared with percutaneous vertebroplasty (VP), in the treatment of osteoporotic vertebral compression fractures (OVCF).

**Methods:** A systematic search of studies published between 2000 and March 2012 was conducted using MEDLINE, EMBASE, OVID, Science Direct and Cochrane Review databases. Randomized controlled trials and prospective comparative studies of comparing KP with VP performed at one center or multi-centers providing data on safety and clinical effects were identified. Two authors independently reviewed the 231 articles that were originally identified, and selected 10 studies for analysis. Study title, demographic characteristics, numbers of vertebral body, adverse events and outcomes were extracted manually from all the selected studies. Rev-Man 5.1 software was used for meta-analysis.

**Results:** Ten studies encompassing 783 patients met the inclusion criteria. Overall, the result of meta-analysis indicated that there were significant differences between two groups in long-term kyphosis angle (WMD=-2.64; 95% CI, -4.66, -0.61;  $P=0.01$ ), anterior height of vertebral body (WMD=3.67; 95% CI, 1.40, 5.94;  $P=0.002$ ) and cement leakage rates (RR=0.70; 95% CI, 0.52, 0.95;  $P=0.02$ ). However, there were no significant differences in short-term visual analog scale (VAS) scores (WMD=-0.57, 95% CI, -1.33, 0.20;  $P=0.15$ ), long-term VAS scores (WMD=-0.99, 95% CI, -2.29, 0.31;  $P=0.14$ ), short-term Oswestry Disability Index (ODI) scores (WMD=-6.54; 95% CI, -14.57, 1.48;  $P=0.11$ ), long-term ODI scores (WMD=-2.01; 95% CI, -11.75, 7.73;  $P=0.69$ ), operation time (WMD=4.47; 95% CI, -0.22, 9.17;  $P=0.06$ ), short-term kyphosis angle (WMD=-2.25; 95% CI, -5.14, 0.65;  $P=0.13$ ) and adjacent-level fracture rates (RR=1.52; 95% CI, 0.76, 3.03;  $P=0.24$ ).

**Conclusions:** The results of this meta-analysis demonstrate that KP is a safe and effective surgical procedure in treating OVCF. Compared with VP, KP can relieve long-term kyphosis angle, improve height of vertebral body and reduce the incidence of cement leakage, whereas it cannot improve pain and function scores, and decrease adjacent-level fracture rates.

#### OP 17

##### Correlation Between Constitution in Chinese Medicine and Bone Stiffness Index of Calcaneum in the Changfeng Community of Shanghai

Zhonghua Liu, Ling Shi, Xia Wu, Yishun Hu, Dan Liu, Yuhua Chen, Guohua Wang, Li Qiu, Yan Hu, Xiangrong Feng  
Shanghai Changfeng Community Health Service Center, Shanghai, China

**Aim:** According to the traditional Chinese medicine (TCM), there is some relation between occurrence and development of disease and different characteristics of bodily constitution. In this study, the correlation between constitution in Chinese medicine and health-related bone stiffness index was explored so as to provide reference for TCM to adjust biased constitution and enhance people's bone stiffness index.

**Methods:** By using the Constitution in the Chinese Medicine Questionnaire, 2780 residents (including 1187 men and 1593 women) were surveyed with cross-section locale investigation from September 2009 to October 2011 with informed consent. They were all over 45 years old and from the Changfeng communities in Shanghai, China. The type of constitution in TCM was evaluated with the diagnosis standard of the Constitution Classification in Chinese Medicine published by China Association of Chinese Medicine.

The bone mineral density was examined by SONOST-2000 supersonic bone density instrument to calculate the bone stiffness index of the right calcaneum ( $SI=0.67 \times BUA+0.28 \times SOS-420$ ).

The correlation between constitutional types and bone stiffness index was analyzed with SPSS 11.5 software.

**Results:** The bone stiffness index was higher in males than that in females, and decreased with increasing age ( $F=22.462$ ;  $P=0.000$ ). The constitutional types were in an order as, from high to low in bone stiffness index, damp-heat, phlegm-dampness, normal, qi stagnation, yin deficiency, special, qi deficiency, yang deficiency and blood stasis constitutions. The differences between constitutions were significant ( $F=5.776$ ;  $P=0.000$ ).

**Conclusion:** Constitutional type is one of the significant influencing factors for bone stiffness index. Adjusting biased constitution could improve the bone stiffness index and promote health. In particular, the constitution of blood-stasis-type adjustments should be paid more attention.

#### OP 18

##### Age and Fat Distribution Effects on the Relationship Between Body Composition and Hip Structure in Healthy Chinese Men

Yanping Du, Qun Cheng, Hanmin Zhu, Xiaoying Zhu, Xuemei Zhang, Sihong Xue, Huilin Li, Wei Hong  
Department of Huadong Hospital Affiliated to Fudan University, Shanghai, China

**Objective:** We have previously shown age effects on the association between body composition and bone mineral density in healthy Chinese men and women. Motivated by these findings, we sought to examine (1) the characteristics of the distribution of body composition (LM, FM and BMD) and hip geometry parameters at different ages in Chinese men, (2) to determine whether age affects the association between body composition and hip geometry and (3) whether fat distribution has a role in this association.

**Methods:** The current study included 1168 men aged 20–96 years. To examine the effect of age, the participants were divided into groups based on age: young (age, 20–30 years), middle-aged (age, 30–65 years) and older group (age, >65 years). The BMD of the lumbar spine (L2–4), left femoral neck and hip geometry parameters were measured by dual-energy X-ray absorptiometry (DXA). In this study, three variables were studied: (1) the cross-sectional area (CSA,  $\text{cm}^2$ ) (2) the average cortical thickness (ACT, cm) and (3) the Bucking ratio (BR). TFM, arm FM, leg FM, trunk FM, TLM, arm LM, leg LM, trunk LM and body fat percentage (Fat%) were also measured by DXA.

**Results:** Aging was accompanied by an increase in FM, CSA and ACT, and a decrease in LM, BMD and BR. In the young group, LM showed significantly positive correlations with CSA and cortical thickness ( $r=0.165-0.712$ ; all  $P<0.01$ ), and negative correlations with BR at all three sites. The relationship between LM and hip geometric parameters is similar in young, middle-aged and older men. The correlations between LM and CSA were the strongest in all models of hip geometric parameters at three sites. FM produced a negative contribution to CSA and ACT at all sites in the young group. But the negative effect became weak in the middle-aged group and the older group. The contribution produced by trunk LM



became the largest positive contribution to CSA and ACT at all three regions in the older group. Trunk FM was the principal negative determinant of CSA and ACT at NN, IT and FS in the young group. But the negative contribution produced by trunk FM became weaker in the middle-aged and older groups.

**Conclusions:** Our findings showed that among the body composition parameters LM was the best predictor of hip geometry, because it was almost significantly correlated to all hip geometric parameters at the three regions in the young, middle-aged and older groups. The impact of FM on hip geometry changed with aging.

#### OP 25

##### **A Novel FGFR3-Binding Peptide Inhibits FGFR3 Signaling and Reverses the Lethal Phenotype of Mice Mimicking Human Thanatophoric Dysplasia**

Min Jin, Ying Yu, Huabing Qi, Lin Chen

Center of Bone Metabolism and Repair, State Key Laboratory of Trauma, Burns and Combined Injury, Institute of Surgery Research, Daping Hospital, Third Military Medical University, Chongqing, China

Gain-of-function mutations in FGFR3 lead to several types of human skeletal dysplasia syndromes, including achondroplasia, hypochondroplasia and thanatophoric dysplasia (TD). Currently, there are no effective treatments for these skeletal dysplasias. Here we screened, using FGFR3 as bait, a random 12-peptide phage library and obtained 23 positive clones, which share identical amino acid sequences (VSPPLTLGQLLS), named as peptide P3. This peptide had high binding specificity to the extracellular domain of FGFR3. P3 inhibited tyrosine kinase activity of FGFR3 and its typical downstream molecules, ERK/MAPK. P3 also promoted proliferation and chondrogenic differentiation of cultured ATDC5 chondrogenic cells. In addition, P3 alleviated the bone growth retardation in bone rudiments from mice mimicking human TD type II (TDII). Finally, P3 reversed the neonatal lethality of TDII mice. Thus, this study identifies a novel inhibitory peptide for FGFR3 signaling, which may serve as a potential therapeutic agent for treatment of thanatophoric dysplasia.

#### OP 26

##### **Epidermal Growth Factor Receptor Regulates Cartilage Matrix Remodeling During Endochondral Ossification Through $\beta$ -Catenin-Dependent and -Independent Pathways**

Xianrong Zhang<sup>1,2</sup>, Ji Zhu<sup>1</sup>, Valerie A Siclari<sup>1</sup>, Motomi Enomoto-Iwamoto<sup>3</sup>, Frank Beier<sup>4</sup>, Ling Qin<sup>1</sup>

<sup>1</sup>Department of Orthopaedic Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; <sup>2</sup>Department of Physiology, School of Basic Medical Science, Wuhan University, Wuhan, Hubei, China; <sup>3</sup>Department of Surgery, The Children's Hospital of Philadelphia, Philadelphia, PA, USA; <sup>4</sup>Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada

Loss of epidermal growth factor receptor (EGFR) activity in mice alters growth plate development, impairs endochondral ossification and retards growth. However, the detailed mechanism

by which EGFR regulates endochondral bone formation is unknown. We constructed a pharmacological rat model and a transgenic mouse model to study such mechanisms. Administration of an EGFR inhibitor, gefitinib, into 1-month-old rats for 7 days produced profound defects in long-bone growth plate characterized by thickening of epiphyseal growth plate (2.0-fold), massive accumulation of hypertrophic chondrocytes (2.3-fold) and decreased mineralization of hypertrophic cartilage matrix. We observed similar growth plate phenotypes along with a delayed formation of secondary ossification center (SOC) in 1-week-old mice with chondrocyte-specific inactivation of EGFR (collagen2a1 promoter driven-Cre  $Egfr^{Wa5/flox}$ ). Immunostaining for Sox9, p57 and Ki67, BrdU labeling and mRNA expression profile of chondrocyte differentiation markers revealed that EGFR inactivation did not alter growth plate chondrocyte proliferation, differentiation and matrix synthesis. Vascular invasion into growth plate cartilage was not impaired. However, we observed a 50% decrease in the number of TRAP-positive osteoclasts at the chondro-osseous junction, owing to decreased RANKL expression in the growth plate. Moreover, EGFR inactivation strongly inhibited the expression of matrix metalloproteinases (MMP9 and MMP13), increased the amount of collagen fibrils and decreased degraded extracellular matrix products in the growth plate. At the SOC of Col-Cre $Egfr^{Wa5/flox}$  mice, we observed similar decreases in the chondrogenic expression of MMPs and RANKL, the number of TRAP-positive cells and matrix mineralization, suggesting that cartilage matrix degradation was also suppressed in this region. *In vitro*, the EGFR ligand transforming growth factor- $\alpha$  strongly stimulated MMP9 and RANKL expression in primary chondrocytes, which was partially abolished by Wnt/ $\beta$ -catenin signaling inhibitors (DKK1 and IWR-1-endo). Further study showed that EGFR signaling phosphorylated LRP6 and activated nuclear translocation of  $\beta$ -catenin. As Wnt/ $\beta$ -catenin signaling itself elevates these gene expression, we conclude that EGFR signaling cross-talks with the Wnt/ $\beta$ -catenin pathway in regulation of MMP9 and RANKL. In contrast, EGFR-induced MMP13 expression is independent of the Wnt/ $\beta$ -catenin pathway. Together, we demonstrated that EGFR signaling regulates matrix degradation directly by stimulating chondrocytes to express MMPs and indirectly by activating chondrogenic RANKL expression to support osteoclastogenesis. EGFR signaling has an essential role in the remodeling of cartilage extracellular matrix into bone through Wnt/ $\beta$ -catenin-dependent and -independent pathways during endochondral ossification.

#### OP 27

##### **Novel Chondroprotective Matrilin-3 Hybrid Nanotubes for Cartilage Tissue Engineering**

Yupeng Chen<sup>1</sup>, Kevin Koopman<sup>1</sup>, Chathuraka Jayasuriya<sup>1</sup>, Jack Cossman<sup>1</sup>, Thomas Webster<sup>1,3</sup>, Hicham Fenniri<sup>4</sup>, Qian Chen<sup>1</sup>

<sup>1</sup>Department of Orthopaedics, Brown University, Providence, RI, USA; <sup>2</sup>Division of Biology and Medicine, Brown University, Providence, RI, USA; <sup>3</sup>School of Engineering, Brown University, Providence, RI, USA; <sup>4</sup>Department of Chemistry, University of Alberta, Edmonton, AB, Canada

**Aims:** Numerous biomaterials have been developed for cartilage tissue engineering. However, significant obstacles still

remain, including the adhesion of neo-cartilage tissue constructs with native tissues and the release of catabolic matrix metalloproteinase during wound healing.

To develop chondroprotective nanomaterials for cartilage repair, novel hybrid nanotubes were developed from the self-assembly of rosette nanotubes (RNTs) and a native cartilage-specific extracellular matrix protein, matrilin-3 (MATN3). This strategy is based on the unique chondroprotective properties of RNTs and MATN3: RNTs improve chondrocyte adhesion by forming a collagen-like matrix, and MATN3 enhances chondrogenesis and inhibits expression of catabolic genes such as *MMP-13*. Our hypothesis is that a novel hybrid nanotube containing both RNTs and MATN3 may possess advantages from both the RNTs and MATN3, thereby enhancing chondrocyte adhesion and matrix integration, as well as inhibiting *MMP-13* expression during cartilage repair.

**Methods:** For material characterization, MATN3/RNT hybrid nanotubes were characterized by transmission electron microscopy and energy-dispersive X-ray spectroscopy. Cell adhesion study was performed by seeding chondrocytes (ADTC5) with or without MATN3/RNT. After 4 h, adherent cells were then fixed, stained and counted. Gene expression was quantified using real-time RT-PCR for aggrecan and *MMP-13* mRNA levels, which was normalized to 18S rRNA. Biomechanical test of the adhesion strength of cartilage plugs coated with the MATN3/RNT was performed on articular cartilage from young adult pigs. MATN3/RNT was applied to 4-mm-diameter explants of 10-mm-diameter full thickness cartilage specimens. After healing for 3 days, a push-out test was performed and the maximum load was recorded.

**Results:** Electron microscopy analysis showed that MATN3 co-assembled with RNTs generated larger hybrid nanotube bundles. MATN3/RNT hybrid nanotubes significantly increased the percentage of chondrocyte adherent to the substrate *in vitro*. Gene expression analysis showed that *MMP-13* expression decreased more than 60%, whereas aggrecan expression increased 60% after it was cultured with MATN3/RNT. Mechanical testing indicated that the cartilage plugs coated with MATN/RNT significantly enhanced their adhesive strength to the native cartilage tissues.

**Conclusions:** Our data showed that the hybrid MATN3/RNT matrix enhanced chondrocyte adhesion *in vitro*, and cartilage explant biointegration to the native tissues *ex vivo*. Therefore, the novel matrix is not only chondroconductive by enhancing chondrocyte adhesion and tissue integration, but are also chondroprotective by stimulating chondrocyte anabolic gene expression and inhibiting catabolic gene expression. These synergistic effects achieved by the hybrid nanomaterials may be highly beneficial to enhancing the tissue repair process for advanced cartilage tissue engineering.

## OP 28

### Inflammation and Bone Loss in Collagen Antibody-Induced Arthritis Are Attenuated by Transgenic Disruption of Glucocorticoid Signaling in Osteoblasts

Jinwen Tu<sup>1</sup>, Shihani Stoner<sup>1</sup>, Yaqing Zhang<sup>1</sup>, Julian Kelly<sup>1</sup>, Cornelia M Spies<sup>1,2</sup>, Frank Buttgerit<sup>2</sup>, Markus J. Seibel<sup>1,3</sup>, Hong Zhou<sup>1</sup>

<sup>1</sup>Bone Research Program, ANZAC Research Institute, University of Sydney, Sydney, NSW, Australia; <sup>2</sup>Department of Rheumatology and Clinical Immunology, Charité University Medicine, Berlin, Germany; <sup>3</sup>Department of Endocrinology and Metabolism, Concord Hospital, Sydney, NSW, Australia

**Objective:** Transgenic (tg) overexpression of the glucocorticoid (GC)-inactivating enzyme, 11beta-hydroxysteroid dehydrogenase type 2 (HSD2), under the control of a 2.3-kb collagen type I promoter (Col2.3-HSD2), abrogates intracellular GC signaling exclusively in mature osteoblasts and osteocytes. Using the T-cell-independent K/BxN serum transfer model of autoimmune arthritis, we previously reported that osteoblast-targeted disruption of endogenous GC signaling attenuated arthritis in Col2.3-HSD2-tg mice. In the present study we aimed to further elucidate the role of endogenous GCs in a T-cell-dependent model of inflammatory joint disease, namely collagen antibody-induced arthritis (CAIA).

**Methods:** CAIA was induced in 6-week-old male Col2.3-HSD2-tg mice (tg-CAIA,  $n=8$ ) and their wild-type (WT) littermates (WT-CAIA,  $n=10$ ). Eight tg and eight WT mice receiving carrier only served as controls (CTR). Body weight and the degree of arthritis (clinical score of paw swelling) were measured daily from induction to endpoint (day 14). Skeletal changes were determined by micro-CT and histomorphometry of the tibia.

**Results:** Both tg-CAIA and WT-CAIA mice developed acute arthritis with significant swelling and redness of all paws. However, based on clinical scores the inflammatory response was significantly blunted in tg-CAIA mice from day 9 onward ( $P<0.05$ ). Histologically, synovial inflammation was less pronounced in the wrist and ankle joints of tg-CAIA compared with WT-CAIA mice. As for micro-CT analysis, tibial trabecular separation was significantly increased ( $P<0.05$ ) with lower trabecular number ( $P=0.05$ ) in WT-CAIA mice, but not in tg-CAIA mice (compared with WT-CTR mice), consistent with accelerated bone resorption. In contrast, trabecular thickness was increased in WT-CAIA ( $P<0.05$ ) but not in tg-CAIA mice. Similar results were obtained by tibia histomorphometry, and the significantly increased osteoclast surface in WT-CAIA mice ( $P<0.01$  compared with tg-CAIA mice) confirmed the known effects of inflammation on systemic bone turnover. At the same time, inflammatory activity was found attenuated in tg-CAIA mice compared with WT-CAIA mice ( $P=0.05$ ), with lesser effects on bone structural parameters.

**Conclusions:** Our results demonstrate that disruption of the GC signaling in mature osteoblasts attenuates arthritis not only in T-cell-independent, but also in T-cell-dependent, models of arthritis.

## OP 29

**Dual Origins of Osteoblasts in Bone Formation—Chondrocytes Transdifferentiate into Osteogenic Lineage Cells *In Vivo***Liu Yang<sup>2,1</sup>, Tiffany Tang<sup>1</sup>, Kwok Yeung Tsang<sup>1</sup>, Danny Chan<sup>1</sup>, Kathryn Cheah<sup>1</sup><sup>1</sup>University of Hong Kong, Hong Kong, China; <sup>2</sup>Fourth Military Medical University, Xi'an, China

**Objective:** It is widely accepted that in vertebrates, bone formation occurs via one of the two processes—membranous bone formation in which osteoblasts directly differentiate from mesenchymal cells or via endochondral ossification (EO), and a multistep process wherein chondrocytes differentiate from mesenchymal condensations, to form a cartilaginous template, proliferate and exit the cell cycle to undergo hypertrophy and terminal differentiation. Vascular invasion occurs and hypertrophic chondrocytes (HCs) undergo apoptosis, and the bone is laid down by osteoblasts from the periosteum and bone collar surrounding the hypertrophic zone, replacing cartilages. However, whether *in vivo* all HCs undergo apoptosis in EO or can ‘transdifferentiate’ into osteoblasts has been a subject of considerable controversy.

**Methods:** To address this controversy we have followed the fate of HCs using the Cre-loxP system in mice. We used homologous recombination to generate two cre lines, Col10a1-cre and Col10a1-creER, which express Cre recombinase under the control of HC-specific *Col10a1* gene. We genetically tagged HCs by crossing Col10a1-cre mice to Cre-reporter mice (Rosa-26R-LacZ or Rosa-26R-YFP) and performed cell-lineage analyses to track the fate of HCs in fetal and postnatal stages.

**Results:** These experiments in combination with pulse-chase experiment by using tamoxifen induction of Cre activity in Col10a1-creER Rosa-26R-LacZ mice showed that *in vivo*, HCs transdifferentiate to osteoblasts contributing to trabecular and cortical bone of endochondral bones. These osteoblasts of HC origin (HCObs) contributed to about a third of bone cells in long bones. In a bone-injury model, we further showed that HCObs contributed to new bone formation in the healing process.

**Conclusion:** Our results reveal the plasticity of terminally differentiated chondrocytes and provide new insight into the homeostasis of bone growth and maintenance. The different origins of osteoblasts may be key to assuring proper bone growth and the establishment of niches for endocrine, hematopoietic and immunological functions. We anticipate the elucidation of molecular mechanism regulating chondrocyte to osteoblast lineage extension will advance technology in cellular reprogramming and tissue engineering.

## OP 30

**Functional Study of Smad7 in Bone Development and BM-MSCs Characterization**Nan Li<sup>1</sup>, Gang Li<sup>1,2</sup><sup>1</sup>School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong, China; <sup>2</sup>Department of Orthopaedics and Traumatology, Chinese University of Hong Kong, Hong Kong, China

**Aims:** TGF- $\beta$  (transforming growth factor- $\beta$ ) is a pleiotropic cytokine that controls proliferation, cellular differentiation and

other functions in most cells. Smad7 has been well demonstrated to be a negative regulator of TGF- $\beta$  signaling, which inhibits TGF- $\beta$  signaling through multiple mechanisms in both the cytoplasm and nucleus. It serves as an important cross-talk mediator of the TGF- $\beta$  signaling pathway with other signaling pathways. Hence, the altered expression of Smad7 often leads to human diseases, such as cancer, inflammatory diseases and so on. To investigate the role of the TGF- $\beta$ /Smad7 signaling in the process of bone development and MSCs characterization, we performed a series of *in-vivo* and *in-vitro* experiments using wild-type (WT) and Smad7-null (KO) mice. Furthermore, we will also perform experiments with some disease models to support our study.

**Methods:** We used flow cytometry to detect the cell surface expression of CD90, CD44, Scal, CD34 and CD45. After the confirmation of mBMSCs, multi-differentiation study (including osteogenesis, adipogenesis and chondrogenesis) and specific staining were used to compare the characterization between the KO and WT mBMSCs, the mRNA expressions of relative markers were also detected by quantitative real-time reverse transcription-PCR. The parameters of long-bone development using 6-, 12- and 24-week-old mice were assessed by digital X-ray, micro-CT (trabecular bone near the metaphysis was selected for analysis within a conforming volume of interest; a region 0.6 mm from the distal end, with a height of 1.8 mm was analyzed), three-points bending mechanical test and histology methods using paraffin section and H&E staining. Data analysis was done by SPSS software and Mann-Whitney's *U*-test;  $P \leq 0.05$  was regarded as statistically significant.

**Results:** In our *in-vitro* study, the adipogenic potential detected at day 7, 14 and 21 by Oil Red O staining ( $n=3$ ) showed much more and earlier lipid droplets formation in the KO group, and the mRNA expression detection ( $n=6$ ), including PPAR $\gamma$  and C/EBP $\alpha$ , also showed higher expression. But osteogenic potential detected at day 7 and 14 by Alizarin Red S staining and the acetic acid extraction method ( $n=3$ ) showed less mineralized nodules, and the mRNA expression ( $n=6$ ) of ALP and RUNX2 were also lower. The *in-vivo* study using three-points bending mechanical test showed that the KO group has significant increase of slope/stiffness and maximum load in the 6-, 12- and 24-week-old mice ( $n=12$ ). Micro-CT and histomorphometric measurements also showed significant increase in trabecular number and trabecular thickness, and showed decrease in trabecular spacing with the growth of age in the KO group. There is no significant difference in digital X-ray figures.

**Conclusions:** The *in-vitro* experiments showed that mBMSCs from Smad7-null (KO) mice has much better adipogenic but worse osteogenic potential than WT group. Although a series of *in-vivo* assessments showed that loss of Smad7 may increase the stiffness, TbN and TbTh decreased the TbSp of mice femurs. These results also show the dual-directional regulation of TGF- $\beta$  signaling on the osteoblasts and complex, dynamic bone remodeling process, which has already been reported. The precise function of Smad7 on bone development need further study of disease models, such as fractured and ovariectomized mice (OVX).

## OP 31

**Polymorphisms in the GALNT3 Gene Are Associated with Bone Mineral Density and Fracture Risk in Chinese Postmenopausal Women**

Nan Li, Weibo Xia, Yan Jiang, Xiran Wang, Xin Zheng, Qiuping Wang, Wenbo Wang, Zhiwei Ning, Wei Huang, Yu Pei, Chunlin Li, Min Nie, Mei Li, Ou Wang, Xiaoping Xing, Shuli He, Wei Yu, Qiang Lin, Ling Xu

Department of Endocrinology, Key Laboratory of Endocrinology, Ministry of Health, Peking Union Medical College Hospital, Beijing, China

**Aims:** Osteoporosis has strong genetic component, but its exact genetic background is still poorly understood. We studied whether *GALNT3*, a gene associated with hyperphosphatemic familial tumoral calcinosis, is an osteoporosis-risk gene by examining association between its polymorphisms and bone mineral density (BMD), osteoporotic fractures and vertebral fractures in Chinese postmenopausal women.

**Methods:** A total of 1607 postmenopausal women were randomly selected from the Peking Vertebral Fracture study in Beijing. BMD of the lumbar spine, femoral neck and total hip were measured by dual energy X-ray absorptiometry. Vertebral fracture phenotypes were ascertained by vertebral X-ray reading. Osteoporotic fracture phenotypes were obtained from a questionnaire. Single nucleotide polymorphisms (SNPs) of *GALNT3* were determined by TaqMan allelic discrimination assay. We used multiple statistic methods to test the association between SNP genotypes and phenotypes of osteoporosis.

**Results:** Polymorphisms of rs13429321, rs6710518 and rs1863196 were significantly associated with femoral neck BMD (*P*-value was 0.005, 0.003 and 0.020, respectively) as well as total hip BMD (*P*-value was 0.002, 0.000 and 0.002, respectively). Individuals carrying genotype TT of rs13429321, TT of rs6710518 or GG of rs1863196 had higher risk of osteopenia and osteoporosis in femoral neck. Osteoporotic fractures were associated with rs6721582 and rs4667836. Homozygous and heterozygous of minor allele increased the incidence of fractures by 1.444 (95% CI, 1.023–2.037; *P*=0.037) and 1.560 (95% CI, 1.124–2.163; *P*=0.008)-fold, respectively.

**Conclusions:** In our study, polymorphisms of rs13429321, rs6710518 and rs1863196 were significantly associated with femoral neck BMD and total hip BMD. Polymorphisms of rs13429321, rs6721582 and rs4667492 were associated with fracture risk. This is the first report about the association between these allelic variants and the phenotypes of postmenopausal osteoporosis in Chinese population. *GALNT3* may have a role in the genetic susceptibility to osteoporosis and fracture among Chinese postmenopausal women.

## OP 32

**The Association of Serum MMP-3 Levels with Disease Activity and Joint Damage in Female Patients with Rheumatoid Arthritis**

Xia Ou yang, Lie Dai, Donghui Zheng, Langjing Zhu, Yingqian Mo, Jianda Ma

Department of Rheumatology, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, China

**Aims:** Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease with progressive joint destruction. The

generation of matrix metalloproteinase 3 (MMP-3) is upregulated by IL-1 and TNF- $\alpha$  in active RA. Pro-MMP is activated by the MMP-3. The extracellular matrix of joints is degraded by MMP-3 and other matrix metalloproteinases in RA, which causes irreversible joint destruction. This study aims to analyze the relationship of serum MMP-3 levels with disease activity and joint damage in female RA patients.

**Methods:** We recruited female RA patients (*n*=83), including active (*n*=58) and remissive (*n*=25) RA patients, and normal women (*n*=31) as a control group. Parameters of disease activity indicators were collected and DAS28 (3)-CRP scores were counted, serum MMP-3 levels were measured by ELISA and hands X-ray Sharp scores were assessed. We compared the difference of serum MMP-3 levels between different groups and analyzed the correlations between serum MMP-3 levels and disease activity indicators or joint damage indicators.

**Results:** MMP-3 levels of active RA patients, remission RA patients and normal control were 227.35 (19.79–913.40), 77.34 (16.15–308.30) and 49.00 (22.00–73.00) ng ml<sup>-1</sup>, respectively. MMP-3 levels of active or remissive RA patients were higher than normal control. RA patients were grouped according to DAS28 (3)-CRP, and the MMP-3 levels of remission, that is, low activity, medium activity and high activity of the RA patients were 227.35 (19.79–913.40), 77.34 (16.15–308.30) and 49.00 (22.00–73.00) ng ml<sup>-1</sup>, respectively. MMP-3 levels of RA patients were higher in high, medium and low activity groups than in the remission group (*P*<0.05). MMP-3 levels of 83 RA patients were positively correlated with pain VAS scores of patients and doctors, SJC, TJC, functional status score of patients, HAQ, CRP, ESR and DAS28 (3)-CRP (*r*=0.35, 0.38, 0.42, 0.32, 0.34, 0.43, 0.58, 0.48, 0.46, respectively; *P*<0.05). The MMP-3 levels positively correlated with X-ray Sharp narrow erosion and total scores in 48 RA patients (*r*=0.43, 0.42, respectively; *P*<0.05). The MMP-3 levels also positively correlated with X-ray Sharp narrow erosion and total scores in 38 active RA patients (*r*=0.37, 0.33, respectively; *P*<0.05).

**Conclusions:** Female RA patients' serum MMP-3 levels are positively correlated with disease activity, and can be used clinically for monitoring of disease activity; serum MMP-3 levels are related to joint narrowing and erosion, suggesting that serum MMP-3 can be used for monitoring patients with RA joint destruction.

## OP 33

**Investigation and Treatment of Osteoporosis in Patients with Compression Vertebral Fractures After Percutaneous Vertebroplasty in China**

Hui Jin, Ruo-nan Cai, Zhen Zhao, Shi-cheng He, Gang Deng  
Department of Endocrinology, Affiliated Zhongda Hospital of Southeast University, Nanjing, China

**Aim:** The objective of this study is to assess the post-fracture evaluation and pharmacological treatment of osteoporosis among patients with spinal compression vertebral fractures after percutaneous vertebroplasty.

**Methods:** A total of 354 patients who suffered from compression vertebral fractures and received percutaneous vertebroplasty from 2003 to 2012 in Affiliated Zhongda Hospital of Southeast University, Nanjing, China, were involved. Information about their history of fracture, investigation and treatment

of osteoporosis before or after the fracture was collected by telephone interview and case review.

**Results:** Of the 354 patients with osteoporotic vertebral fractures, 269 were female whose mean age was  $71.69 \pm 8.39$  years old, whereas the others were  $76.17 \pm 8.10$  years old. A total of 179 (50.6%) of the 354 vertebral fractures occurred without any signs, except back pain; 109 patients (30.8%) were diagnosed with vertebral fractures after falls; 20 (5.6%) of the osteoporotic vertebral fractures were caused by weight-bearing; 12 (3.4%) of the 354 patients incurred fractures with bending; the rest of the osteoporotic vertebral fractures occurred when coughing or behaving improperly. Among the vertebral bodies with fractures, the number of each spine from number 4 thoracic vertebra to number 5 lumbar vertebra was 3, 11, 21, 28, 25, 32, 28, 65, 121, 130, 100, 48, 48 and 24, respectively. The most frequent sites of vertebral fracture were T12, L1 and L2 (Figure 1). A total of 157 (44.4%) patients reported that they had experienced fracture only in 1 vertebra, and 87 people had experienced fractures in 2 vertebrae. Moreover, 110 (31.1%) had more than 3 fractured vertebrae. One hundred and twenty-seven (35.9%) of the 354 patients received percutaneous vertebroplasty more than once. Furthermore, 17 (9.3%) patients suffered fractures at other sites, such as the distal radius, proximal humerus and proximal femur. Only 3.7% of the patients had undergone dual X-ray absorptiometry, and all of them had the bone densitometry before fractures. The rate of the supplementation of calcium, vitamin D and medications for anti-osteoporosis was 9.5%, 6.7% and 36%, respectively. Among those patients who had taken anti-osteoporosis drugs, 27.5%, 29.7%, 2.2% and 3.3% received bisphosphonate, calcitonin, ossotide and traditional Chinese drug, respectively; there were 37.4% of patients who could not recall the name of the drugs they had taken. Our study also found that many patients do not take drugs regularly to resist osteoporosis.

**Conclusions:** Surgeons do not pay enough attention to the investigation and treatment of osteoporosis in China. More efforts should be taken to prevent, evaluate and treat osteoporosis, especially to prevent secondary fracture.

#### OP 34

##### The Effect of High Versus Low Bone Loading on Musculoskeletal Health in Middle Life

Charles Milgrom<sup>1</sup>, N Constantini<sup>1</sup>, Y Milgrom<sup>1</sup>, D Skera<sup>1</sup>, Y Applbaum<sup>1</sup>, A Finestone<sup>2</sup>

<sup>1</sup>Hadassah University Hospital, Jerusalem, Israel;

<sup>2</sup>Department of Orthopaedics, Assaf Harofeh Medical Center, Zerrifin, Israel

**Objective:** Regular physical exercise is recommended to maximize long-term musculoskeletal health. The aim of this study was to determine the effect of sedentary lifestyle versus extremely demanding physical activity from an early age on bone and musculoskeletal health in middle life.

**Methods:** In 1983, a group of the elite infantry recruits sustained a 31% incidence of stress fractures during basic training. The most frequent site of stress fracture was the tibia. Twenty-five years after the training began, the musculoskeletal health of 25 of these soldiers, 11 of whom had sustained stress fractures, was compared with a group of 20 subjects who received exemption from military service in 1982–1985,

because they were religious scholars. The bone density and geometric strength of the tibia was assessed by quantitative computerized tomography. Degenerate changes in the lower back and knee were assessed by magnetic resonance using the Pfirrmann and WORMS score. The average daily dietary intake and metabolic expenditure were assessed by questionnaires.

**Results:** At the 25 year follow-up, there was no difference in the tibia cortical density between cohorts, but the soldiers had stronger tibias based on geometric engineering criteria, with larger area moment ( $P=0.02$ ) and polar moments of inertias ( $P=0.02$ ). Soldiers had more degenerate disk changes ( $P=0.003$ ), but not knee changes. Religious scholars had lower daily calcium intake ( $P=0.02$ ) and burned less calories daily, in particular because they did less sports activity ( $P=0.001$ ). There was no difference in tibia bone strength parameters between soldiers who did and did not sustain stress fractures in their 1983 basic training.

**Conclusions:** Very high musculoskeletal loading beginning at an early age when compared with very low loading appears to strengthen the tibia by increasing geometric properties and not by increasing cortical density, and increase lumbar spine degeneration but not knee degeneration in middle life. In spite of the great difference in bone-loading history between the two cohorts, the difference in their musculoskeletal health status at middle age was small. Recruits who sustained stress fracture at a young age did not have decreased tibia cortical bone strength in middle life.

#### OP 35

##### Characteristics of Bone Mineral Density in Young Adult Patients with Hyperthyroidism

Jiyan Wang, Xuenong Xing

Department of Endocrinology, Anhui Provincial Hospital, Hefei, China

**Objective:** The objective of this study was to investigate the effect of hyperthyroidism on the bone mineral density (BMD) in young adult patients.

**Methods:** BMDs of forearm, lumbar vertebrae and the neck of femur were measured using dual-energy X-rays absorptiometry in young adult patients ( $n=340$ , aged  $35.1 \pm 8.1$  years) and normal subjects ( $n=60$ , aged  $36.9 \pm 4.6$  years). On the basis of the lowest measure of the three sites (Z-score), the patients were divided into a normal bone mass group (ON,  $-0.156 \pm 0.605$ ), an osteopenia group (OD,  $-1.556 \pm 0.442$ ) and an osteoporosis group (OP,  $-3.607 \pm 0.953$ ). Free triiodothyronine (FT3), free thyroxine (FT4) and thyroid-stimulating hormone (TSH) levels, measured using a chemiluminescence method, were compared among the three groups of patients.

**Results:** The BMDs of forearm (total radius), lumbar vertebrae 2–4 and femur neck in the patients were significantly lower than those of normal subjects ( $0.49 \pm 0.11 \text{ g cm}^{-2}$  vs  $0.59 \pm 0.06 \text{ g cm}^{-2}$ ,  $1.12 \pm 0.17 \text{ g cm}^{-2}$  vs  $1.22 \pm 0.10 \text{ g cm}^{-2}$  and  $0.93 \pm 0.17 \text{ g cm}^{-2}$  vs  $0.97 \pm 0.10 \text{ g cm}^{-2}$ , respectively;  $P < 0.05$ ). Osteoporosis was found in 27.1% of the patients and osteopenia was found in 34.5% of the patients. Among the three measurement sites, forearm has the highest rate of osteoporosis (26.0%), followed by lumbar vertebrae 2–4 (2.1%) and femur neck (0.9%). As compared with patients in the ON group, the OP patients have

significantly higher level of FT3 and FT4 ( $26.92 \pm 15.42 \text{ pmol l}^{-1}$  vs  $21.53 \pm 13.17 \text{ pmol l}^{-1}$  and  $59.64 \pm 26.56 \text{ pmol l}^{-1}$  vs  $51.74 \pm 23.45 \text{ pmol l}^{-1}$ , respectively;  $P < 0.05$ ), and significantly lower level of TSH ( $0.002 \text{ mIU l}^{-1}$  vs  $0.004 \text{ mIU l}^{-1}$ ;  $P < 0.05$ ). Compared with OD patients, the OP patients had significantly higher FT3 and FT4 levels ( $26.92 \pm 15.42 \text{ pmol l}^{-1}$  vs  $21.67 \pm 13.37 \text{ pmol l}^{-1}$  and  $59.64 \pm 26.56 \text{ pmol l}^{-1}$  vs  $51.53 \pm 20.97 \text{ pmol l}^{-1}$ , respectively;  $P < 0.05$ ), and significantly lower TSH level ( $0.002 \text{ mIU l}^{-1}$  vs  $0.004 \text{ mIU l}^{-1}$ ;  $P < 0.05$ ). FT3, FT4 and TSH were not significantly different in OD and ON patients ( $21.67 \pm 13.37 \text{ pmol l}^{-1}$  vs  $21.53 \pm 13.17 \text{ pmol l}^{-1}$ ,  $51.53 \pm 20.97 \text{ pmol l}^{-1}$  vs  $51.74 \pm 23.45 \text{ pmol l}^{-1}$  and  $0.004 \text{ mIU l}^{-1}$  vs  $0.004 \text{ mIU l}^{-1}$ , respectively;  $P > 0.05$ ). There was a significant negative correlation between FT3 and BMD ( $r = -0.190$ ,  $P = 0.001$ ), and between FT4 and BMD ( $r = -0.141$ ,  $P = 0.01$ ) among the patients, whereas there was a significant positive correlation between TSH and BMD ( $r = 0.129$ ,  $P = 0.018$ ). There was no correlation between the duration of the disease and BMD.

**Conclusion:** The rate of osteoporosis in patients with hyperthyroidism is high and the forearm bone is affected the most. High levels of FT3 and FT4, and low levels of TSH can cause disordered bone metabolism.

#### OP 36

##### Mutation Spectrum of Type I Collagen Genes in CHINESE OI Patients

Yanqin Lu<sup>1</sup>, Xiuzhi Ren<sup>1</sup>, Yanzhou Wang<sup>1</sup>, Chao Xu<sup>1</sup>, Gongchao Li<sup>1</sup>, Fei Zhao<sup>1</sup>, Jinxiang Han<sup>1</sup>

Key Laboratory for Rare and Uncommon Diseases of Shandong Province, and Key Laboratory for Biotech-Drugs, Ministry of Health, Shandong Academy of Medical Sciences, Jinan, China

**Aims:** Osteogenesis imperfecta (OI) is an inheritable connective disease of great genetic and phenotypic heterogeneity caused by defective type I collagen or abnormal collagen metabolism. The dominant OI form is caused by the mutation of *COL1A1* and *COL1A2* genes. There are few reports about the mutation spectrum of OI patients in China. The study aims to explore the epidemiology of OI in China.

**Methods:** The study was approved by the Shandong Academy of Medical Sciences Review Board. Peripheral blood was collected under informed consent. Blood genomic DNA was extracted for PCR amplification. The coding region and the exon-intron boundaries of the *COL1A1* and *COL1A2* genes were sequenced based on PCR amplification. Genetic variations were analyzed by Mutation Surveyor software 4.0. Mutations were further confirmed by 200 normal control samples from a healthy population.

**Results:** A total of 61 heterozygous mutations were identified in the *COL1A1* and *COL1A2*, with 30 mutation sites being novel. Mutation incidence of the *COL1A1* and *COL1A2* was nearly equal. In 30 *COL1A1*-related mutations, 24 heterogeneous missense mutations and 2 new frame-shift nonsense and splice site mutations were discovered in the *COL1A1* gene. Twenty-nine *COL1A2*-related mutations were heterozygous missense mutations and two mutations were induced by splice site variation. Glycine substitutions were the main mutation type for missense mutation, and their detection rate in the

*COL1A1* and *COL1A2* gene was 70% and 83.87%, respectively. The percentage of glycine substitution by serine was 40% in the *COL1A1* and 45.06% in the *COL1A2* gene. Substitutions of glycine by cystine were the second most common mutation type in the *COL1A2* gene ( $n=6$ , 19.35%). Alanine for glycine substitutions was detected only in the *COL1A1* gene with 16.67% prevalence. Substitutions of glycine by arginine, asparagine, valine, glutamine and tryptophan were rare, but existed in either the  $\alpha 1$  or  $\alpha 2$  chain. Two new recurrent mutations of c.1913G>C in the *COL1A1* gene and c.3061G>T in the *COL1A2* gene were detected in two and three unrelated OI patients, respectively.

**Conclusions:** The study reveals the *COL1A1* and *COL1A2* mutation spectrum in Chinese OI patients. The identification of a large spectrum will be useful for clinical diagnosis, genetic counseling and prenatal diagnosis. It discovers the epidemiology of OI in China and provides data for phenotype-genotype correlations.

#### OP 37

##### Loss of wnt/ $\beta$ -Catenin Signaling Causes Cell Fate Shift of Preosteoblasts from Osteoblasts to Adipocytes

Lige Song<sup>1,2</sup>, Minlin Liu<sup>1</sup>, Noriaki Ono<sup>1</sup>, Richard F. Bringham<sup>1</sup>, Henry M. Kronenberg<sup>1</sup>, Jun Guo<sup>1</sup>

<sup>1</sup>Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA; <sup>2</sup>Department of Endocrinology, Tongji Hospital, Tongji University School of Medicine, Shanghai, China

**Aims:** The objective of this study is to determine whether postnatal deletion of  $\beta$ -catenin in preosteoblasts causes bone marrow adiposity and whether this increased bone marrow adipogenesis is attributed to cell fate shift of preosteoblasts with the  $\beta$ -catenin gene knockout (KO) from osteoblasts to adipocytes.

**Methods:** To knock out the  $\beta$ -catenin gene from preosteoblasts,  $\beta$ -catenin flox/flox mice ( $\beta$ -cat<sup>f/f</sup>) were mated with osterix-cre transgenic mice (osx-cre), in which the cre recombinase expression is driven by the osterix promoter. During pregnancy, the mice were fed with doxycycline (DOX) to stop the expression of cre recombinase, and thus the  $\beta$ -catenin gene could be expressed almost normally. In contrast, the  $\beta$ -catenin gene could be knocked out when DOX was stopped. Tibiae and femurs of the  $\beta$ -cat<sup>f/f</sup> (control) and osx-cre; $\beta$ -cat<sup>f/f</sup> (KO) mice were dissected, fixed and decalcified for histological analysis, and paraffin sections from the decalcified bone and plastic sections from undecalcified bone were prepared by standard histological procedures.

To determine whether osteogenesis and/or adipogenesis of the bone marrow stem cells (BMSCs) from KO mice were changed, BMSCs were isolated from the tibia and femurs of the control and KO mice, and cultured *in vitro* for 14 or 21 days in adipogenic or osteogenic medium. After culture, the cells were stained with Von Kossa and Oil Red O, and real-time PCR was used to detect the expression of adipogenic and osteogenic genes.

To trace whether the increased adipogenesis of BMSCs from KO mice could be attributed to cell-fate shift of preosteoblasts from osteoblasts to adipocytes after the  $\beta$ -catenin gene KO, osx-cre and KO mice carrying mT/mG transgene were used.

BMSCs were isolated from 8-week-old femurs and tibiae of mT/mG; osx-cre (CON) and mT/mG;osx-cre; $\beta$ -cat<sup>fl/fl</sup> (KO) mice were treated with Dox until killing, cultured under adipogenic condition for 21 days and analyzed by Oil Red O staining, and cellular immunofluorescent confocal detection for FABP4 (red) and GFP (green).

To obtain *in vivo* data of the previous  $\beta$ -catenin KO cell-fate shift, proximal tibiae from 5-month-old mT/mG;osx-cre (CON) and mT/mG;osx-cre; $\beta$ -cat<sup>fl/fl</sup> (KO) mice treated with Dox until 2-month-old were prepared and stained with anti-FABP4 (red), GFP (green), tomato (pink) and DAPI (blue), and photos were taken by confocal microscopy.

**Results:** Postnatal disruption of  $\beta$ -catenin in the preosteoblasts led to extensive bone marrow adiposity and low bone mass in adult mice. In cultured bone marrow-derived cells isolated from the KO mice, adipogenic differentiation was dramatically increased, whereas osteogenic differentiation was significantly decreased.

Using lineage tracing both *in vivo* and *in vitro*, we demonstrated that the loss of  $\beta$ -catenin from preosteoblasts caused a cell-fate shift of these cells from osteoblasts to adipocytes, a shift that may at least partly contribute to the bone marrow adiposity and low bone mass in the KO mice.

**Conclusions:** These novel findings indicate that Wnt/ $\beta$ -catenin signaling exerts control over the fate of lineage-committed early osteoblasts, with respect to their differentiation into osteoblastic versus adipocytic populations in bone, and thus offers potential insight into the origin of bone marrow adiposity.

#### OP 38

##### The CREB-Smad6-Runx2 Axis Contributes to the Impaired Osteogenesis Potential of Bone Marrow Stromal Cells in Fibrous Dysplasia of Bone

Qiming Fan<sup>1</sup>, Bing Yue<sup>1</sup>, Zhenyu Bian<sup>1</sup>, Wenting Xu<sup>1</sup>, Bing Tu<sup>1</sup>, Kerong Dai<sup>1</sup>, Gang Li<sup>2</sup>, Tingting Tang<sup>1</sup>

<sup>1</sup>Shanghai Key Laboratory of Orthopedic Implants, Department of Orthopedic Surgery, Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, Shanghai, People's Republic of China; <sup>2</sup>Department of Orthopedics and Traumatology, The Chinese University of Hong Kong, Hong Kong, People's Republic of China

**Background and Aims:** Fibrous dysplasia (FD) is characterized by the replacement of normal bone with abnormal fibro-osseous tissue. This disorder is due to activating missense mutations in *GNAS* gene and the resultant overproduction of cAMP. However, the signaling pathways that contribute to FD pathogenesis remain unknown. In the current study, we aimed to elucidate the molecular mechanisms underlying how *GNAS* mutation leads to an osteogenesis disorder of BMSCs.

**Methods:** The following methods were used: isolation, culture and osteogenesis induction of BMSCs, cAMP measurement, allele-specific PCR/MTT assay, real-time PCR, lentivirus and adenovirus vectors, luciferase reporter gene assay and chromatin immunoprecipitation assay.

**Results:** We isolated BMSCs from excised FD lesions. The R201H mutation in *GNAS* was confirmed by allele-specific PCR and sequencing. The cAMP levels were much higher in the FD-derived BMSCs than in control. Enhanced proliferation

was also observed in the FD-derived BMSCs compared with control. More importantly, osteogenesis potential was impaired in BMSCs from FD lesions. We established two *in vitro* cell models that could mimic the pathological features of FD. The first one was to stably transfer R201H *GNAS* into normal BMSCs. We constructed a lentivirus vector expressing mutated *GNAS* (R201H) and used it to infect normal BMSCs. Another cell model was employed in the current study: normal BMSCs treated with exogenous 2 mM cAMP. The FD phenotype could be transferred to normal BMSCs by lentivirus infection carrying mutated *GNAS* and exogenous cAMP treatment. We screened three key transcription factors in osteogenesis, Runx2, Msx2 and Osx, by exposing normal BMSCs to BMP-2 and excess cAMP, and quantifying their mRNA abundance. Runx2 was downregulated in BMSCs with the FD phenotype. The expression levels of several members in the BMP-Smads signaling pathway were investigated in response to excess cAMP. A remarkable upregulation of Smad6, an inhibitor of the BMP-Smads signaling pathway, was observed in normal BMSCs treated with BMP-2 and exogenous cAMP. Runx2 expression, which was downregulated in LV-R201H BMSCs and cAMP-treated BMSCs, was effectively recovered in response to Smad6 silencing. CREB activated Smad6 expression through binding to the CRE in the Smad6 promoter in BMSCs with the FD phenotype. We confirmed the CREB-Smad6-Runx2 axis in FD lesion-derived BMSCs. What's more? The impaired *in vitro* osteogenesis potential of LV-R201H BMSCs could be recovered through modulation of Runx2 and Smad6.

**Conclusions:** The CREB-Smad6-Runx2 axis contributes to the impaired osteogenesis potential of bone marrow stromal cells in fibrous dysplasia of bone.

#### OP 39

##### The Vitamin D Receptor Promotes Human Prostate Cancer Cell Growth via a Ligand-Independent Pathway

Yu Zheng<sup>1,2</sup>, Difei Deng<sup>1</sup>, Trupti Trivedi<sup>1</sup>, Shu Oi Chow<sup>1</sup>, Julian Kelly<sup>1</sup>, Robert Sutherland<sup>2</sup>, Colin Dunstan<sup>1,3</sup>, Hong Zhou<sup>1</sup>, Markus Seibel<sup>1,4</sup>

<sup>1</sup>Bone Research Program, ANZAC Research Institute, University of Sydney, Sydney, NSW, Australia; <sup>2</sup>Cancer Research Program, Garvan Institute of Medical Research, Sydney, NSW, Australia; <sup>3</sup>Department of Biomedical Engineering, University of Sydney, Sydney, NSW, Australia; <sup>4</sup>Department of Endocrinology and Metabolism, Concord Hospital, Sydney, NSW, Australia

**Aim:** Bone is a frequent site for prostate cancer metastasis. We have previously reported that vitamin D deficiency promotes human prostate cancer cell growth in bone. However, little is known about the role of the vitamin D receptor (VDR) in this context. The present study aimed to define the role of the VDR in human prostate cancer growth *in vitro* and *in vivo*.

**Methods and Results:** VDR expression was knocked down by stable expression of shRNA in PC3 cells (PC3-VDR-KD), with non-target cells (PC3-NT) generated as controls. The VDR mRNA knockdown was 85% and induction of CYP24 mRNA expression by 1,25(OH)<sub>2</sub>D<sub>3</sub>, normally seen in VDR expressing cells, was abrogated in PC3-VDR-KD cells, indicating effective disruption of the VDR signaling.

Treatment of PC3-NT cells with  $1,25(\text{OH})_2\text{D}_3$  significantly reduced cell growth by up to 51% as compared with untreated PC3-NT cells. Surprisingly, growth of PC3-VDR-KD cells in ligand-free cultures was also reduced by 49% (compared with NT cells). Moreover, cell migration was increased by 10% in PC3-VDR-KD cells. Of note, PC3-VDR-KD cells did not respond to treatment with  $1,25(\text{OH})_2\text{D}_3$ .

To further investigate the effects of VDR knockdown *in vivo*, PC3-NT and PC3-VDR-KD cells were implanted subcutaneously in nude mice, and tumor growth was monitored for 69 days. Compared with NT cells, VDR knockdown resulted in significantly smaller tumors from day 12 onwards. Similarly, when PC3-NT or PC3-VDR-KD cells were implanted into the tibiae of vitamin-D-sufficient mice, disruption of VDR signaling resulted in significantly smaller osteolytic lesions from day 17 onwards (X-ray analysis).

**Conclusion:** These results suggest a novel ligand-independent role of the VDR in promoting prostate cancer cell growth and suppressing invasive cell potential (migration). This novel function of the unliganded VDR contrasts with the known anti-proliferative actions of the liganded VDR, and may offer new therapeutic approaches in cancer treatment.

#### OP 40

##### Identification of Five Novel Mutations in the *SLCO2A1* Gene in Four Unrelated Chinese Families with Primary Hypertrophic Osteoarthropathy

Zeng Zhang, Jin-Wei He, Wen-Zhen Fu, Chang-Qing Zhang, Zhen-Lin Zhang

Metabolic Bone Disease and Genetic Research Unit, Department of Osteoporosis and Bone Diseases, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

**Introduction:** Primary hypertrophic osteoarthropathy (PHO) is a rare genetic disease, characterized by digital clubbing, pachydermia and periostosis. We previously described, in addition to HPGD deficiency, deficiency of the prostaglandin transporter (*SLCO2A1*) as another cause of this condition. So far, mutations in *SLCO2A1* were reported in only 20 families. To expand this mutational spectrum and better delineate the *SLCO2A1*-deficiency phenotype, we report the clinical and molecular characterization of another four families with PHO and compare the features to HPGD-deficiency patients.

**Method:** Four affected individuals and all available healthy family members from four unrelated Chinese families with PHO were clinically studied. The *SLCO2A1* gene was screened and analyzed, and the mutations were confirmed using molecular genetic techniques. Urine PGE<sub>2</sub> and PGE-M levels were measured using competitive enzyme-linked immunosorbent assays (Cayman Chemicals).

**Results:** Biallelic *SLCO2A1* mutations (c.855delA/c.855delA in family 1; p.Gly369Asp/p.Gly369Asp in family 2; p.Glu465Lys/p.Glu465Lys in family 3; p.Glu165X/p.Gly379Glu in family 4) were identified in affected individuals in all the four families, confirming a very specific association of this phenotype with the *SLCO2A1* mutations. Urine PGE<sub>2</sub> and PGE-M levels of all of the patients were elevated, further supporting the notion that abnormal PGE<sub>2</sub> metabolism is the pathogenesis in PHO. In comparison with HPGD-deficiency patients, *SLCO2A1*-deficiency

patients had a late onset in puberty or early adulthood, were exclusively males, had severe pachydermia, cutis verticis gyrata and blepharoptosis, and had severe complications, such as multifactorial anemia, myelofibrosis, hypertrophic gastropathy, hypoalbuminemia and hypocholesterolemia.

**Conclusion:** The present findings broaden the allelic spectrum of the *SLCO2A1* mutations. The clinical differences between HPGD-deficiency patients and *SLCO2A1*-deficiency patients suggest they are two clinically distinct PHO subgroups. The skewed sex ratio and the onset in puberty in *SLCO2A1*-deficiency patients indicate GnRH (gonadotropin-releasing hormone) or/and testosterone may have a role in the regulation of prostaglandin metabolism.

#### OP 41

##### Susceptibility Genes of Osteoporotic Fracture in Postmenopausal Chinese Women

Chun Wang, Zeng Zhang, Hao Zhang, Jin-Wei He, Yun-Qiu Hu, Yu-Juan Liu, Zhen-Lin Zhang

Department of Osteoporosis and Bone Diseases, Metabolic Bone Disease and Genetics Research Unit, Shanghai Sixth People's Hospital Affiliated with Shanghai Jiao Tong University, Shanghai, China

**Aims:** To identify the susceptibility genes of osteoporotic fracture in postmenopausal Chinese women, a two-stage case-control association study using joint analysis was conducted in 1046 patients with non-traumatic vertebra, hip or distal radius fractures and 2303 healthy controls.

**Methods:** First, 113 SNPs in 16 potential osteoporosis candidate genes reported in recent genome-wide association studies, meta-analyses studies, large-scale association studies and functional studies were genotyped in a small-sample-size subgroup comprising 541 patients with osteoporotic fractures and 554 healthy controls. Variants and haplotypes in *SPTBN1*, *TNFRSF11B*, *CNR2*, *LRP4* and *ESR1* that have been identified as being associated with osteoporotic fracture were further re-analyzed in the whole case-control group.

**Results:** We identified one SNP in *TNFRSF11B* (rs3102734), three SNPs in *ESR1* (rs9397448, rs2234693 and rs1643821), two SNPs in *LRP4* (rs17790156 and rs898604) and four SNPs in *SPTBN1* (rs2971886, rs2941583, rs2941584 and rs12475342), which were associated with all the broadly defined fractures of any kind. The most significant polymorphism was rs3102734, with an increased risk of osteoporotic fracture (odds ratio: 1.347; 95% CI: 1.173–1.545; Bonferroni  $P=2.6\times 10^{-4}$ ). Furthermore, rs3102734, rs2941584, rs12475342, rs9397448, rs2234693 and rs898604 showed significant allelic, genotypic and/or haplotypic association with vertebral fracture. The rs12475342, rs9397448 and rs2234693 SNPs showed significant genotypic association with hip fracture, whereas the rs3102734, rs2073617, rs1643821, rs12475342 and rs2971886 SNPs showed significant genotypic and/or haplotypic association with distal radius fracture. No significant gene-gene interaction was observed in *TNFRSF11B* and *SPTBN1*.

**Conclusion:** Variants in *TNFRSF11B*, *SPTBN1*, *ESR1* and *LRP4* are susceptibility genetic loci for osteoporotic fracture in addition to the clinical risk factors in postmenopausal Chinese women.



## OP 42

**Pathological Roles of MicroRNAs on Bone Metastasis in Lung Cancer**

Junrong Ma, Meng Gong, Mingliang Zhou, Xijie Yu

Laboratory of Endocrinology and Metabolism, West China Hospital, Sichuan University, Chengdu, China

**Aims:** Lung cancer is the major cause of malignancy-related death worldwide. It is estimated that 90% patients die with metastases. The skeleton is the common site of metastasis. Currently, no curative therapy exists for bone metastasis. MicroRNAs (miRNAs) critically regulate tumor metastasis. However, the pathological roles of miRNAs in bone metastasis in lung cancer are unknown. Our aim was to determine the miRNA profiles relevant to bone metastasis in lung cancer and its molecular mechanisms.

**Methods:** Microarray and real-time qRT-PCR were used to investigate miRNA profiles in lung cancer cell lines with/without bone metastasis. A lentiviral system was used to permanently restore miRNAs relevant to bone metastasis in SBC5, a human small cell lung cancer cell line that can metastasize to bone. The *in vitro* phenotypes as well as *in vivo* bone metastases were investigated.

**Results:** The expression levels of miR-335 and miR-29a were significantly lower in lung cancer cells with bone metastasis.

Restoring miR-335 in SBC5 significantly decreased proliferation and tumorigenesis, but did not affect cellular functions of osteoblasts and osteoclasts cocultured with SBC5. Restoring miR-29a in SBC5 did not affect proliferation and tumorigenesis, but significantly impaired cellular functions of osteoblasts and osteoclasts cocultured with SBC5. Restoring either miR-335 or miR-29a could reduce bone metastasis in NSG immunodeficient mice; the group with miR-335 and miR-29a together showed the maximum reduction. Bioinformatics study showed miR-335 could target IGF1R, and miR-29a could target PTHrP. Intriguingly, significantly high levels of IGF1R and PTHrP were found in SBC5. Furthermore, restoring miR-335 or miR-29a could significantly reduce the expression of IGF1R or PTHrP, respectively, in SBC5.

**Conclusion:** Deregulating PTHrP by miR-29a in lung cancer enhances osteoclastic bone resorption, which releases growth factor IGF1. Low level of miR-335 in lung cancer cells results in a higher level of IGF1R, which enhances IGF1 signaling and facilitates the development of bone metastasis. Restoring miR-335 and miR-29a could block the vicious cycle between tumor cells and bone cells, thus reducing bone metastasis in lung cancer. Targeting miRNAs may represent novel therapies for bone metastasis in lung cancer.