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Oral Presentations

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OR1

Pdgf-Bb Secreted by Preosteoclasts Induces Cd31^{hi}emcn^{hi} Vessel Subtype in Coupling Osteogenesis

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Background: CD31^{hi}Emcn^{hi} vessel, a specific vessel subtype, couples angiogenesis and osteogenesis. This study aims to investigate the critical mechanisms involved in the regulation of CD31^{hi}Emcn^{hi} vessel subtype in coupling osteogenesis during bone modeling and remodeling.
Methods: We cultured monocytes/macrophages to differentiate into preosteoclasts and osteoclasts in order to collect conditioned medium (CM). We crossed hemizygous *TRAP-Cre* mice with *Pdgfb*$^{fl/fl}$ mice in order to generate TRAP-positive cell specific *Pdgfb* gene knockout mice. We performed bilateral ovary removal surgery in order to obtain osteoporotic ovariectomized (OVX) mice. We also treated OVX mice with a cathepsin K (CTSK) inhibitor orogastrically or with intra-bone marrow injection of recombinant PDGF-BB.
Results: Preosteoclasts but not mature bone resorptive osteoclasts secreted PDGF-BB. Preosteoclast CM significantly increased migration and angiogenesis of epithelial progenitor cells (EPC) and mesenchymal stem cells (MSCs). These effects were abolished by a PDGF-BB neutralizing antibody. Furthermore, we found that preosteoclast CM induced migration and angiogenesis of EPCs and MSCs via FAK signaling pathway. Mice with deletion of PDGF-BB in the TRAP-positive cell lineage showed significant decrease of both trabecular and cortical bone. Interestingly, PDGF-BB levels in bone marrow and peripheral blood were significantly decreased. Particularly, CD31^{hi}Emcn^{hi} vessels were significantly decreased in those mice. In OVX-induced osteoporotic mice, levels of PDGF-BB were significantly decreased in bone marrow and peripheral blood, and the CD31^{hi}Emcn^{hi} vessels were also decreased. CTSK inhibitor, as a novel anti-osteoporosis drug with well-known anti-osteoclast bone resorption activity, effectively increased preosteoclast numbers and PDGF-BB levels and stimulated CD31^{hi}Emcn^{hi} vessels and bone formation in OVX mice. Intra-bone marrow injection of recombinant PDGF-BB also stimulated CD31^{hi}Emcn^{hi} vessels and bone formation in OVX mice.
Conclusion: Preosteoclasts secrete PDGF-BB to induce CD31^{hi}Emcn^{hi} vessels during bone modeling and remodeling. Pharmacotherapies that increase PDGF-BB from preosteoclasts to promote angiogenesis for bone formation offer a novel therapeutic target for osteoporosis.

OR2

Aryl Hydrocarbon Receptor Catabolic Activity in Bone Metabolism Is Osteoclast Dependent *in Vivo*

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Background: Aryl hydrocarbon receptor (AhR) is known as a dioxin receptor and is responsible for various pathological and physiological processes. However, the role of AhR in bone homeostasis remains elusive because the cell type specific direct function of AhR has never been explored *in vivo*. The purpose of this study is to investigate the function of AhR in bone metabolism by analyzing abnormal bone phenotypes of total AhR KO mice, osteoblast specific AhR KO mice and osteoclast specific AhR KO mice.
Methods: Osteoclast culture, Quantitative real-time PCR, Dual energy x-ray absorptiometry (DEXA), Micro-computed tomography *(μCT)* analysis and Bone histomorphometric analysis were performed.
Results: Increased bone mass with decreased resorption and decreased formation in total AhR KO mice (*AhR*$^{-/-}$) mice, suggested that AhR might affect bone metabolism. No significant changes were found in the parameters including bone mineral density, bone mass, bone formation or bone resorption parameters in 12 weeks old osteoblast specific AhRKO mice (*AhR*$^{\Delta Ob/\Delta Ob}$), and suggesting that AhR in osteoblasts does not possess significant role in bone metabolism. An increased bone mass with reduced bone resorption were showed in osteoclast specific AhRKO mice (*AhR*$^{\Delta Oc/\Delta Oc}$) compared with that in control mice. Even under pathological conditions, *AhR*$^{\Delta Oc/\Delta Oc}$ mice are resistant to sex hormone deficiency-induced bone loss resulting from increased bone resorption. Furthermore, 3-Methylcholanthrene, an AhR agonist, induces low bone mass with increased bone resorption in control mice, but not in *AhR*$^{\Delta Oc/\Delta Oc}$ mice.

Conclusion: Our results not only demonstrated that osteoclast-specific AhR is a physiologically relevant regulator of bone resorption, but also highlighted the need for further studies on the skeletal actions of osteoclast-specific AhR inhibitors commonly associated with bone diseases, especially diseases linked to environmental pollutants known to induce bone loss.

OR3

Adverse Effects of Osteocytic Constitutive Activation of ß-Catenin on Bone Strength and Bone Growth

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Background: It has been proven that activation of canonical Wnt/β-catenin signaling pathway in both mesenchymal stem cells and osteoblasts can increase bone mass, showing promise in the treatment of low bone volume conditions such as osteoporosis. However, the possible side effects of manipulation of this pathway were not fully addressed. Previously we reported that constitutive activation of β-catenin in osteoblasts impaired the vertebral linear growth. In the present study, the effects of constitutive activation of β-catenin in osteocytes, the main cell type in bone, on bone mass, bone growth and bone strength were observed. Special attention was paid to the adverse effects of the activation.
Methods: β-catenin was constitutively activated specifically in osteocytes in Catnblox (ex3); Dmp1-Cre mice, and histology, microCT, immunohistochemistry, Masson staining, real-time PCR, and mechanical test et al were employed to assess bone mass, bone strength and bone linear growth.
Results: It was found that cancellous bone mass was dramatically increased as expected, almost filling the entire bone marrow cavity in long bones. However, the mice showed shorter stature with impaired linear growth of the long bones, and died mostly at 5 or 6 weeks after birth. Furthermore, bone strength decreased significantly and the long bones were easily broken. A thinner and more porous cortical bone together with impaired mineralization were responsible for the decrease in bone strength in Catnblox (ex3); Dmp1-Cre mice. Elevated expression of FGF23, down-regulated expression of DKK1 and hypophosphatemia contributed to the decreased mineralization level and impaired linear growth.
Conclusion: Constitutive activation of β-catenin in osteocytes could increase cancellous bone mass, however, the activation also had adverse effects on bone strength and bone growth. These adverse effects should be addressed before any therapeutic clinical application involving adjustment of Wnt/β-catenin signaling pathway.

OR4

Tanshinol Attenuates Osteoporosis Mediated by Oxidative Stress via FoxO/Wnt Signaling

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Background: Oxidative stress has been recently considered the deleterious factor in the development and progression of osteoporosis. This study aims to investigate the action of natural antioxidant Tanshinol on bone metabolism associated with regulation of FoxO3a/Wnt pathway under oxidative stress elicited by long-term exposure to excessive Glucocorticoids.
Methods: 4-month-old female Sprague-Dawley rats were randomly divided in four groups with 8 rats per group, and were administrated prednisone acetate orally once daily for 14 weeks: normal control group (Con), model group (GC, 5mg/kg), GC+Tanshinol group (Tan, 20mg/kg) and GC+Resveratrol group (Res, 5mg/kg).
Results: Tanshinol blocked the decline of body weight and adjusted the unbalance of biomarkers of bone turnover detected by ELISA assay in GIO rats. Importantly, Tanshinol counteracted a series of deleterious consequence related to damaged bone micro-architecture and impairment of bone quality determined by Micro-CT technology, histomorphometery methods and biomechanical assay, respectively. Furthermore, Tanshinol abated the boost of oxidative stress, including the elevation of phosphorylated $p66^{Shc}$ protein and ROS accumulation, and the decreased antioxidants, as well as the increased number of apoptosis cells of bone tissue stained by TUNEL in rats treated with GC. According to the evidence measured using qRT-PCR method and Western blot assay, Tanshinol showed beneficial effects, contributing to suppression of increased PPARγ2 level, and to activation of decreased mRNA level of *Runx2*, *Osteorix*, *OCN* and *Col1α1* in GIO rats. Additionally, Tanshinol reversed down-regulation of the expressions of *Axin2* gene and β-catenin protein, and simultaneously counteracted up-regulation of the induction of *Gadd45α* mRNA and FoxO3a protein triggered by GC.
Conclusion: Tanshinol exerted inhibitory influence on induced oxidative stress in bone tissue elicited by excessive GC treatment through suppression of $p66^{Shc}$/ROS/FoxO3a pathway, and counteracted down-regulation of canonical Wnt/β-catenin/Tcf signaling, consequently leading to suppression of osteoporosis in GIO rats.

OR5

Sensitivity of Renal 25-hydroxyvitamin D 1α-hydroxylase Expression, 1,25(OH)2D3 Formation, and Phosphate Metabolism to Interferon Regulatory Factor-1 (IRF-1) Signaling

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Background: Calcitriol ($1,25(OH)_2D_3$), a powerful regulator of phosphate metabolism and immune response, is generated by 25-hydroxyvitamin D 1α-hydroxylase (1αH) in the kidney and in macrophages. Renal 1αH expression is suppressed by Klotho and FGF23, the expression of which is stimulated by $1,25(OH)_2D_3$. In macrophages, 1αH expression is controlled by transcription factor interferon regulatory factor-1 (IRF-1). Expression of IRF-1 is regulated by Janus kinase-3 (JAK3) and ataxia telangiectasia mutated (ATM) signaling, the main effector kinase of the latter being Checkpoint kinase-2 (Chk2). Thus, the significance of JAK3 and Chk2 for $1,25(OH)_2D_3$ formation and phosphate metabolism was analyzed.
Methods: JAK3-deficient mice ($jak3^{-/-}$) were compared to $jak3^{+/+}$ mice as well as Chk2-deficient mice ($chk2^{-/-}$) to $chk2^{+/+}$ mice. Transcript levels of renal 1αH (CYP27B1) and IRF-1 were determined by qRT-PCR and immunohistochemistry, Klotho expression by Western blotting, serum or plasma $1,25(OH)_2D_3$, PTH, and FGF23 concentrations by immunoassays and serum, fecal, and urinary phosphate concentrations by photometry.
Results: Renal IRF-1 and 1αH transcript levels, serum $1,25(OH)_2D_3$, and FGF23 levels, intestinal phosphate absorption as well as absolute and fractional renal phosphate excretion were significantly higher in $jak3^{-/-}$ mice than in $jak3^{+/+}$ mice. Immunohistochemistry revealed co-expression of renal 1αH and IRF-1. In $chk2^{-/-}$ mice the renal expression of IRF-1 and 1αH as well as serum $1,25(OH)_2D_3$ and FGF23 levels were significantly lower compared to $chk2^{+/+}$ mice. Renal phosphate excretion was significantly higher in $chk2^{-/-}$ mice than in $chk2^{+/+}$ mice despite hypophosphatemia.
Conclusion: Our study reveals that renal IRF-1 expression is controlled by JAK3 and Chk2. Moreover, IRF-1 is a novel regulator of renal 1αH expression. Hence, enhanced renal IRF-1 expression in JAK3 deficiency stimulated whereas decreased IRF-1 expression in Chk2 deficiency lowered 1αH expression in the kidney resulting in marked derangement of phosphate metabolism.

OR6

Osteoblast Derived-Neurotrophin-3 (NT-3) Promotes Osteoclastogenesis *In Vitro* and Bony Repair and Remodelling at Injured Growth Plate in Rats

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Background: Neurotrophic factors have been implicated in bone fracture healing. Recently, we observed the most prominent induction of neurotrophin-3 (NT-3) among all members of the neurotrophin family during osteogenesis *in vitro* and during bony repair (Su YW *et al*, 2013 ICMRC-ASBMR). The current study investigated potential roles of osteoblast-derived NT-3 in regulating osteoclast formation *in vitro* and bony repair and remodeling in rats.
Methods: NT-3 protein (rhNT-3) or conditioned media from rat bone marrow stromal cell (rBMSC) osteogenic cultures were examined for their abilities in promoting osteoclast formation and resorptive activity in rat bone marrow cells or osteoclast precursor RAW264.7 cells. NT-3 was also examined in NF-kB or NFATC1 activation in RAW264.7 cells and in expression of osteoclastogenesis-regulatory molecules in rBMSC culture. rhNT-3 or anti-NT-3 treatment effects were examined in bony repair and remodeling in a rat tibial drill-hole injury model.
Results: rhNT-3 or rBMSC osteogenic conditioned medium increased osteoclast formation in rat bone marrow cells and elevated expression of osteoclast markers (RANK, OSCAR and cathepsin-K). rhNT-3 or osteogenic conditioned medium synergistically promoted osteoclastogenesis or resorptive activity with exogenous RANKL in RAW264.7 cells. The osteoclastogenic ability of the osteogenic conditioned medium was blocked by the presence of anti-NT-3 antibody. While rhNT-3 did not affect NF-κB or NFATC1 activation in RAW264.7 cells, rhNT-3 increased, but anti-NT-3 reduced, mRNA expression of osteoclastogenic cytokines (TNF-α, IL-1β, and IL-6) and RANKL/OPG ratio during osteogenesis, suggesting an indirect mode of action of NT-3 in promoting osteoblast support for osteoclastogenesis. During bony repair of the injured tibial growth plate, NT-3 was found expressed strongly in callus osteoblasts, and rhNT-3 treatment enhanced, but anti-NT-3 treatment attenuated, the bony repair. rhNT-3-treated rats had higher, but anti-NT-3-treated rats had lower, levels of osteoclast presence and expression of osteoclastogenic molecules at the injury site compared to vehicle or IgG controls.
Conclusion: Osteoblast-derived NT-3 promotes osteoclastogenesis by inducing expression of osteoclastogenic molecules in osteoblasts. During growth plate bony repair, NT-3 enhances bone formation and remodelling.

OR7

Clinical and Genetic Analysis in 126 Chinese Patients With Hypophosphatemic Rickets and PHEX Gene Function Study *in Vitro*

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Background: Hypophosphatemic rickets (HR) is a disorder of phosphate homeostasis characterized by rachitic, growth retardation, hypophosphatemia and renal phosphate wasting. X-linked dominant HR (XLHR), caused by inactivating mutations in phosphate-regulating gene with homologies to endopeptidases on the X chromosome (*PHEX*), is the most common form of HR, but the phenotype-genotype relationship remains unclear.

Methods: We summarized the clinical information of 126 patients with hypophosphatemic rickets and performed gene mutation analysis in 79 patients. *PHEX* genotype-phenotype correlation analysis was performed in 67 patients with *PHEX* mutation. We constructed the wild and different mutational (C85S, C406S, R567X, or T615P) PHEX expression vectors and transfected the vectors into HEK293T cells to observe if there is any change regarding the cellular localization, protein expression, glycosylation and cellular targeting.

Results: Skeletal deformities and bone pain were most common characteristic features in the 126 patients with HR. All patients presented elevated levels of FGF23 and hypophosphatemia, low levels of 1,25-dihydroxy vitamin D ($1,25(OH)_2D_3$) and impaired proximal renal tubular reabsorption of phosphate. Several patients suffered from nephrolithiasis or nephrocalcinosis after active therapy, independent of hypercalcemia or hypercalciuria. 27 *PHEX* mutations were identified in 34 XLHR patients, including 4 missense mutations (12%), 8 nonsense mutation (24%), 6 splicing mutation (18%), 9 deletion mutation (14%), and 2 insertion mutation (6%). 17 of these mutations were novel. The phenotype-genotype association study showed no significant relationship between the age of onset, PTH, FGF23, $1,25(OH)_2D_3$, bone deformity or dental problems with gene mutations. *In vitro* study showed that wild-type and mutant (C85S, C406S, R567X, T615P) PHEX proteins were all detected on the cell membrane, while C85S, C406S, T615P mutant proteins might be incompletely glycosylated.

Conclusion: Mutational analysis of the PHEX gene in 79 unrelated Chinese patients with HR revealed 34 mutations, including 17 novel mutations. No genotype-phenotype correlation was found.

OR8

Genetic Association Study Identifies DDR2 as a New Gene For Bone Mineral Density and Osteoporotic Fractures in Chinese Population

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Background: Bone mineral density (BMD), a diagnostic parameter for osteoporosis and a clinical predictor of fracture, is a highly heritable trait, but the genetic variants involved are largely unknown. DDR2 gene is expressed in osteocytes and osteoblastic cells. *In vivo* studies found that DDR2 is required for normal bone development. *In vitro* studies reported that DDR2 plays an important role in regulating osteoblast differentiation and chondrocyte maturation. Given the important function of DDR2 in bone, it is reasonable to hypothesize that DDR2 could be a new candidate gene for BMD and osteoporosis, but the genetic variations leading to the association remain to be elucidated. Therefore, the aim of this study was to investigate whether the genetic variations of DDR2 are associated with BMD and fracture risk.

Methods: This study was performed in three samples from two ethnicities, including 1,300 Chinese Han subjects, 700 Chinese Han subjects (350 with osteoporotic hip fractures and 350 healthy controls) and 2,286 US white subjects. 30 SNPs in DDR2 were genotyped and tested for associations with hip BMD and fractures.

Results: Eighteen SNPs showed nominal association with hip BMD in the Chinese population. After multiple testing adjustments, 3 SNPs remained significant, which were rs7521233 ($P=1.06\times10^{-4}$, β: –0.018), rs7553831 ($P=1.30\times10^{-4}$, β: –0.018), and rs6697469 ($P=1.59\times10^{-3}$, β: –0.015), separately. SNP rs6697469 was also associated with hip fractures (($P=0.043$, OR: 1.27) in the Chinese population. However, in the white population, we didn't detect significant associations with hip BMD, by presenting some extent of ethnic difference. eQTL analysis revealed that SNPs associated with BMD also affected DDR2 mRNA expression levels, and the most significant SNP was rs3738807 ($P_{eQTL}=2.97\times10^{-8}$).

Conclusion: We provide novel evidence that SNPs in DDR2 are associated with BMD and fractures in Chinese populations, suggesting that DDR2 could be a new susceptibility gene for osteoporosis. Our findings also reveal ethnic difference of DDR2, highlighting the need for genetic studies in each ethnic group.

OR9

Familial Isolated Primary Hyperparathyroidism/ Hyperparathyroidism-Jaw Tumor Syndrome Caused by Germline Gross Deletion or Point Mutations of CDC73 Gene in Chinese

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Background: Hyperparathyroidism–jaw tumor syndrome (HPT-JT) and familial isolated primary hyperparathyroidism (FIHP) are two subtypes of familial primary hyperparathyroidism, which are rarely reported in Chinese population. Here we reported three FIHP families and one HPT-JT family with long-term follow-up and genetic analysis.

Methods: A total of 22 patients, from four FIHP/HPT-JT families of Chinese descent, were recruited and genomic DNA was extracted from their peripheral blood lymphocytes. Direct sequencing for MEN1, CDC73, CASR gene was conducted. Reverse transcription PCR (RT-PCR) and quantitative real-time PCR (qRT-PCR) were used to study the effect of splice site mutations and gross deletion mutations. Immunohistochemistry was performed to analyze parafibromin expression in parathyroid tumors. Genotype–phenotype correlations were assessed through clinical characteristics and long-term follow-up data.

Results: Genetic analysis revealed four *CDC73* germline mutations that were responsible for the four kindreds, including two novel point mutation (c.157 G>T and IVS3+1 G>A), one recurrent point mutation (c.664 C>T) and one deletion mutation (exons 4, 5, 6) (Figure 1 and 2). RT-PCR confirmed that IVS3+1 G>A generated an aberrant transcript with exon3 deletion (Figure 3). Immunohistochemical analysis demonstrated reduced nuclear parafibromin expression in tumors supporting the pathogenic effects of these mutations (Figure 4).

Conclusion: This study supplies information on mutations and phenotypes of HPT-JT/FIHP syndrome in Chinese. Screening for gross deletion and point mutations of *the CDC73* gene is necessary in susceptible subjects.

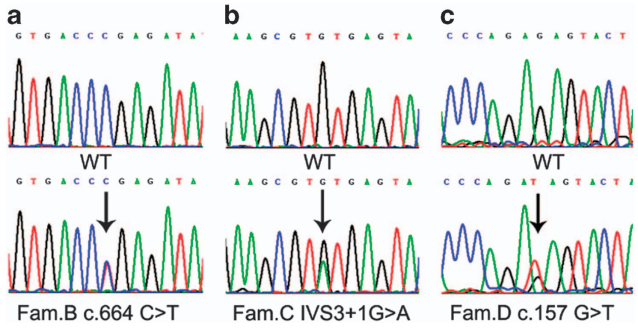

Figure 1 Direct sequence of wild type and mutant *CDC73* gene.

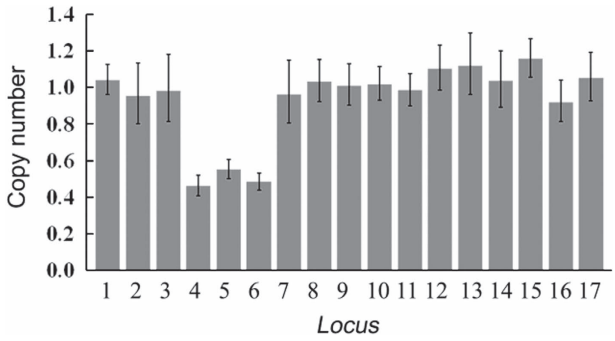

Figure 2 Gene copy number of the seventeen *CDC73 loci* of the proband of Family A.

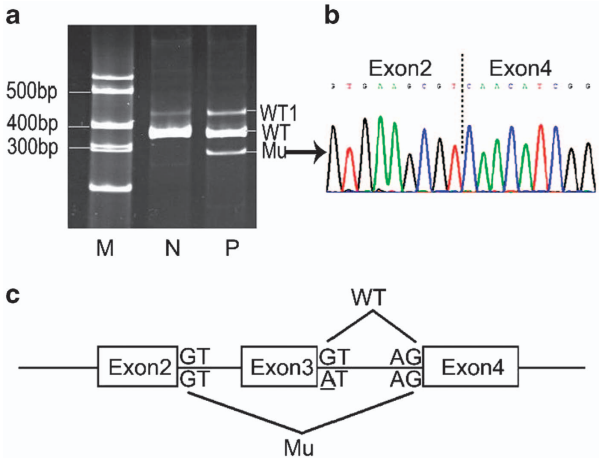

Figure 3 Abnormal mRNA splicing due to intron 3 donor splice site mutation (IVS3+1 G>A).

OR10

Genome-Wide Interaction Analysis Between Mitochondrial SNPs and Nuclear SNPs on Osteoporosis

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Background: Osteoporosis is a typical complex disease diagnosed by the measurement of bone mineral density (BMD), which is a highly heritable and multifactorial trait. Previous studies have demonstrated that mitochondrial single nucleotide polymorphisms (mtSNPs) and nuclear SNPs (nSNPs) are involved in the pathogenesis of osteoporosis. However, possible mtSNP-nSNP interactions of most non-significant SNPs were usually ignored and led to the missing heritability of osteoporosis. The aim of this study was to explore the potential nuclear-cytoplasmic interactions of osteoporosis at genome-wide scale, hunting for the genetic mechanisms of osteoporosis from a new perspective.

Methods: We performed a genome-wide association study (GWAS) to explore potential mtSNP-nSNP interactions contributing to osteoporosis susceptibility in a sample of 2,286 unrelated Caucasian subjects by using the Affymetrix Genome-Wide Human SNP Array 6.0. Interaction analyses were carried out for 44 mtSNPs and 562,011 nSNPs on spine and hip BMD, respectively.
Results: We found significant interactions involving 5 mtSNPs and 26 nSNPs that correlated with BMD (Without interaction analysis, these SNPs did not show any significant correlation with BMD). Four pairs of the most significant interaction SNPs with BMD were: mtSNP *ND5-rs3135030* and nSNP *RORB-rs950058* (P=8.05×10^{-14}, spine BMD), mtSNP *Cytb-rs28357375* and nSNP *TOMM20-rs4920161* (P=1.28×10^{-12}, spine BMD), mtSNP *Cytb-rs28357375* and nSNP *VRK2-rs2678919* (P=2.61×10^{-13}, spine BMD), mtSNP *Cytb-rs28573847* and nSNP *CAMTA1-rs873433* (P=3.85×10^{-12}, hip BMD). Besides, there were three newly reported genes: *ZNF518B*, *RMST*, and *APPADC1A*, with more than one significant nSNPs.
Conclusion: We provide novel evidence that genetic interactions between mtSNPs and nSNPs influence susceptibility to osteoporosis. Our findings demonstrated that association analyses that take gene-gene interactions into account may enhance detection of genetic variants that can be missed by routine single-SNP association analyses.

OR11

Gene-Based Genome-Wide Association Analysis Identified Novel Susceptibility Genes Associated with Bone Mineral Density

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Background: Genetic factors contribute to the susceptibility of bone mineral density (BMD), which is a major risk factor of osteoporosis. The aim of this study was to identify more susceptibility genes for BMD.
Methods: Based on the public available SNP-based P values, we performed an initial gene-based genome-wide association study (GWAS) in a total of 32,961 individuals. Furthermore, we performed differential expression, gene set enrichment and protein-protein interaction analyses to find supplementary evidence to support the significance of the identified genes.
Results: About 21,695 genes for femoral neck (FN)-BMD and 21,683 genes for lumbar spine (LS)-BMD were analyzed in gene-based GWAS. A total of 35 FN-BMD associated genes and 53 LS-BMD associated genes were identified (P<2.3×10^{-6}) after Bonferroni correction. Among them, 64 genes have not been reported in previous SNP-based GWASs. Differential expression analysis further supported the significant association of 14 genes with FN-BMD and 19 genes with LS-BMD. Especially, 4 novel HOXB cluster genes (HOXB1, HOXB3, HOXB4 and HOXB5) for LS-BMD, and novel WNT3 and WNT9B in the Wnt signaling pathway for FN-BMD were further supported by gene set enrichment analysis and protein-protein interaction analysis.
Conclusion: The present study highlighted the high power of gene-based association analysis in finding BMD-associated genes. The evidence taken together supported the importance of Wnt signaling pathway and HOXB cluster genes on determining osteoporosis. Our findings provided more insights into the genetic basis of osteoporosis.

OR12

Suppressed Bone Turnover Was Associated With Increased Osteoporotic Fracture Risks in Non-Obese Postmenopausal Chinese Women with Type 2 Diabetes Mellitus

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Background: The aim of this study was to investigate the association of T2DM with osteoporotic fracture in postmenopausal Chinese women.
Methods: 1410 postmenopausal women were included and stratified into non-obese population (body mass index [BMI]<25kg/m^2) and obese population (BMI≥25 kg/m^2). Each type of population was classified into diabetic group, impaired fasting glucose (IFG) group, and normal glucose group. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry. Serum C-terminal telopeptide of type I collagen (β-CTX) and serum N-aminoterminal prepeptide of type I procollagen (P1NP) were quantified. Vertebral fractures (VFs) and non-VFs were assessed by vertebral X-ray and questionnaire, respectively.
Results: Comparing to normal glucose group, diabetic group and IFG group both had lower levels of P1NP and β-CTX, despite population types. Despite having non-decreased BMD, non-obese diabetic patients had higher risks of total fracture and VF than BMI-matched normal glucose subjects (both P<0.05). Non-obese population was further classified by mean value of P1NP or β-CTX. Non-obese diabetic patients with low P1NP or high β-CTX had higher fracture risks (both P<0.05), comparing to non-obese normal glucose subjects with high P1NP or high β-CTX, respectively.
Conclusions: Type 2 diabetic patients had suppressed bone turnover, which might explain the increased fracture risks, independent of BMD. IFG patients might also have poor bone quality and need early prevention.

OR13

Association of Weight-Adjusted Body Fat and Fat Distribution with Bone Mineral Density in Middle-Aged Chinese Adults: A Cross-Sectional Study

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Background: Although it is well established that a higher body weight is protective against osteoporosis, the effects of body fat and fat distribution on bone mineral density (BMD) after adjustment for body weight remains uncertain. We examined the relationship between body fat and fat distribution and BMD beyond its weight-bearing effect in middle-aged Chinese adults.
Methods: The study had a community-based cross-sectional design and involved 1,767 women and 698 men aged 50-75 years. The BMD of the lumbar spine, total hip, and whole body, and the fat mass (FM) and percentage fat mass (%FM) of the total body and segments of the body were measured by dual-energy X-ray absorptiometry. General information on the participants was collected using structured questionnaire interviews.
Results: After adjusting for potential confounders, an analysis of covariance showed the weight-adjusted (WA-) total FM (or %FM) to be negatively associated with BMD in all of the studied sites ($P<0.05$) in both women and men. The unfavorable effects of WA-total FM were generally more substantial in men than in women, and the whole body was the most sensitive site related to FM, followed by the total hip and the lumbar spine, in both genders. The mean BMD of the lumbar spine, total hip, and whole body was 3.93%, 3.01%, and 3.65% (in women) and 5.02%, 5.57%, 6.03% (in men) lower in the highest quartile (vs. lowest quartile) according to the WA-total FM (all $P<0.05$). Similar results were noted among the groups for WA-total FM%. In women, abdominal fat had the most unfavorable association with BMD, whereas in men it was limb fat.
Conclusion: FM (or %FM) is inversely associated with BMD beyond its weight-bearing effect. Abdominal fat in women and limb fat in men seems to have the greatest effect on BMD.

OR14

Weak Correlation between Body Mass Index and Bone Mineral Density in Overweight Population

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Background: Low BMI is a risk factor for osteoporosis, and is positively related to BMD. This belief has been questioned by recent epidemiologic studies about the relation between obesity and osteoporosis. Our aim was to investigate the association between BMI and BMD at different BMI levels, and between trunk fat percent and BMD.
Methods: Cross-sectional study included 18266 subjects (8971 men, 9295 women) who received routine physical examination at The Second Hospital Affiliated to Suzhou University between 2008 to 2013. BMD in lumbar spine (LS) and femoral neck (FN) and trunk fat percent were performed using DXA (LUNAR DPX-NT). Subjects were categorized into four groups: women and men below 50 years, and women and men above 50 years. Subjects were further divided into different BMI levels: (1) Into three categories according to WHO criteria: Underweight (<18.5 kg/m^2), normal (18.5 kg/m^2 –24.9 kg/m^2) and overweight (≥25 kg/m^2); (2) 7 categories for each 2 kg/m^2 as a division: <19 kg/m^2, 19.1–21 kg/m^2, 21.1–23 kg/m^2, 23.1–25 kg/m^2, 25.1–27 kg/m^2, 27.1–29 kg/m^2, >29 kg/m^2.
Results: (1) Overweight women and men above 50 had higher average BMD and lower risk of low BMD (T-score<–1.0) than normal and underweight. Nevertheless, the correlation between BMI and BMD was very weak in overweight elders, and much smaller than normal or underweight elders. There was no significant difference of average BMD value between adjacent BMI divisions above 27 kg/m^2. (2) Overweight women and men below 50 years had higher average BMD value but higher risk of low BMD (Z-score<–1.0) than normal weight. The correlation between BMI and vertebrae BMD was weak. (3) In all four groups, BMD was negatively correlated with trunk fat percent, and the best correlation located in overweight.
Conclusion: A higher BMI was not always associated with higher BMD in overweight subjects. Excessive trunk fat mass may partially contribute to this phenomenon. There was no gender differences in the association between BMI and BMD.

OR15

Tissue Mineral Density Dependent Mechanical Properties of Individual Trabecular Plates and Rods Do not Differ in Anatomic Directions but Individual Trabecular Directions

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Background: Trabecular bone, susceptible to osteoporosis, comprises individual trabecular plates and rods. They grow distinctly along the longitudinal, transverse, or oblique anatomic directions of the skeleton (Fig.1). In each anatomic direction, tissue mechanical properties of individual trabecula are expected to differ in (axial) or against (lateral) the direction, i.e., anisotropic mechanical properties. However, anisotropic mechanical properties of individual trabecula along various anatomic directions are currently not available. Aims of this study are to measure anisotropic tissue modulus and tissue mineral density (TMD) of individual trabeculae; to examine their dependence on trabecular type and anatomic directions; to determine the relationship between anisotropic tissue modulus and TMD.
Methods: Twelve cylindrical human trabecular bone samples from proximal femurs were imaged at 25 μm resolution by uCT. Individual trabecular types and their anatomic directions were determined using individual trabecula segmentation technique. On the embedded samples, microindentation tests were performed on both axial and lateral cross-sections (Fig. 1c) of selected plates and rods in longitudinal, transverse, and oblique directions, respectively (Fig. 1d). The point-by-point registered grayscale values of the uCT image at the indentation sites were converted to TMD using calibration phantoms.
Results: The tissue modulus and co-localized TMD of trabecular plates were significantly higher than trabecular rods

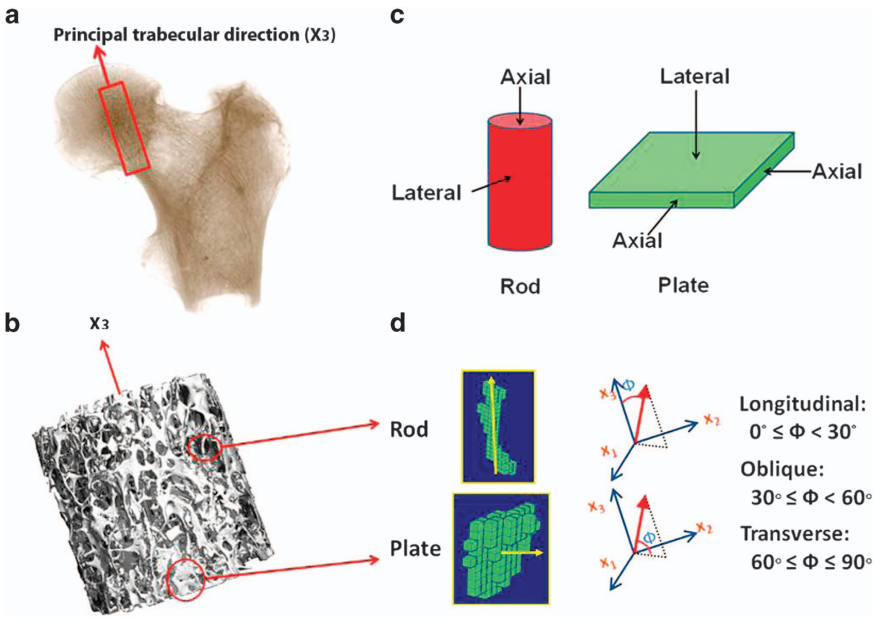

Figure 1 Illustration of the anatomic directions, the axial and lateral trabecular directions of individual trabecular plates and rods in proximal femur and TMD measurements. a. Principle trabecular direction of femoral neck trabecular bone (X-ray radiography); b. μCT illustration of the trabecular structure of a cylinder sample corresponding to the red box in Fig. 1a; c. Axial and lateral directions of trabecular plates and rods; d. Classification of anatomic directions of plates and rods.

(Fig. 2a,b). Tissue modulus was higher in the axial direction for all three anatomic directions (Fig. 2c). The tissue modulus correlated strongly with TMD. This correlation does not differ between trabecular types or anatomic directions. However, the correlation in axial direction was significantly different from the lateral direction (Fig. 2d).

Conclusion: We measured anisotropic elastic modulus of individual trabecular plates and rods of different anatomic directions. Surprisingly, the heterogeneous tissue modulus correlated with TMD similarly regardless of trabecular types and anatomic directions. The correlation only differs in the axial and lateral trabecular directions.

OR16

The Polymorphisms of Wnt Signaling Pathway Genes Are Associated both With Peak Bone Mineral Density and Obesity Phenotypes in Chinese Male Nuclear Families

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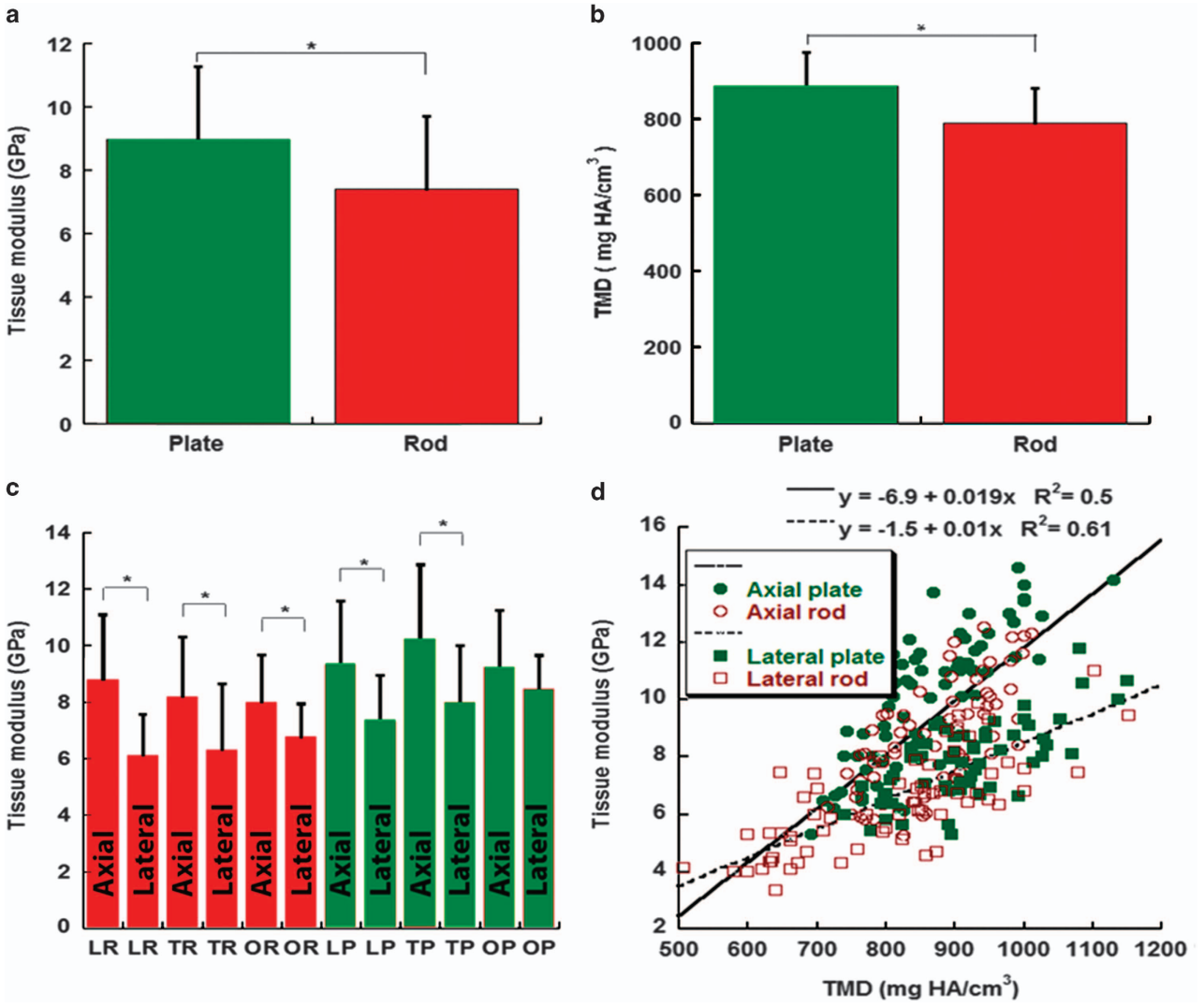
Background: Peak bone mass is an important determinant of the risk of osteoporosis later in life. Previous researches conducted in older species have showed that the 6 Wnt signaling pathway genes (including *WNT4, WNT5B, WNT10B, WNT16*, *CTNNB1* and *CTNNBL1*) were osteoporosis candidate genes. Muscle and fat are tightly linked to bone metabolism. To investigate the contribution of these candidate genes polymorphisms to the variation of peak bone mineral density (BMD) and obesity phenotypes in young Chinese men, we examined genotype, BMD and obesity phenotypes of all subjects.

Methods: Male nuclear families, which consisted of 1214 Chinese Han subjects from 399 families, were recruited. 51 single-nucleotide polymorphisms (SNPs) located in the 6 genes were screened. BMD and total fat mass (TFM) and total lean mass (TLM) were measured using dual energy X-ray absorptiometry (DXA). The associations between 51 SNP of the 6 genes with peak BMD and obesity measurements (including body mass index [BMI], TFM, TLM, percentage fat mass [PFM], and percentage lean mass [PLM]) were analyzed using quantitative transmission disequilibrium tests (QTDTs).

Results: In peak BMD, rs10917157 in *WNT4* gene, three SNPs (rs2240506, rs7308793 and rs4765830) in *WNT5B* gene, and two SNPs (rs6126098 and rs8126174) in *CTNNBL1* gene were significantly associated with lumbar spine BMD, four SNPs (rs6091103, rs238303, rs6067647 and rs4811144) in *CTNNBL1* gene were significantly associated with femoral neck BMD, rs8126174 in *CTNNBL1* gene was significantly associated with total hip BMD. In obesity phenotypes, five SNPs in *CTNNBL1* gene (rs6091103, rs238303, rs6067647, rs8126174 and rs4811144) were significantly associated with TFM, four SNPs in *CTNNBL1* gene (rs238303, rs6067647, rs8126174 and rs4811144) were significantly associated with PFM and PLM, rs3809269 in *WNT5B* gene and two SNPs in *CTNNB1* gene (rs22405061 and rs22405064) were significantly associated with TLM.

Conclusion: For Chinese man, genetic polymorphisms in *WNT5B* and *CTNNBL1* genes may be a major contributor to variability in both peak BMD and obesity, genetic polymorphisms in *WNT4* gene may be a major contributor to variability in peak BMD, genetic polymorphisms in *CTNNB1* gene may be a major contributor to variability in obesity.



[OR15] Figure 2 Tissue modulus and TMD vary in different trabecular types, anatomic directions and individual trabecular directions. (Plate: n=216; rod: n=216). a. Plates have higher tissue modulus than rods. *P<0.05; b. Plates have higher TMD than rods. *P<0.05; c. Tissue modulus in axial direction is higher than in lateral direction. *P≤0.05. P and R denote plate and rod; L, T, O denote longitudinal, transverse and oblique; d. Tissue modulus closely correlates with TMD. However, the correlations differ between axial and lateral indentation directions, *P<0.05.

OR17

Aberrant Subchondral Bone TGF-β Activation Causes Rheumatoid Arthritis Joint Degeneration

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Background: Rheumatoid arthritis (RA) is an autoimmune inflammatory polyarthritis that often leads to joint destruction, deformity, and loss of function. The immune system has been the primary target of disease modifying anti-rheumatic drugs. However, the joint destruction is not effectively halted with these treatments. Accumulating evidence has shown that bone marrow lesions occur during the early stage of RA are closely associated with the prognosis, similar to osteoarthritis (OA).

Methods: The TGF-beta1 ligand and its down-stream p-smad2/3 in the subchondral bone of both collagen-induced arthritis (CIA) mice and TNF-alpha mice were determined. The dynamic subchondral bone alterations during the disease were investigated by microCT. Histological analyses and MRI were employed to characterize the subchondral pathology and the consequent cartilage degeneration. TGF-beta type I receptor kinase inhibitor (systemic injection) or pan-specific TGF-beta neutralizing antibody (local embed) was used to rescue the subchondral bone pathology in CIA mice or rats, respectively. TGF-beta receptor II (Tgfbr2) was further conditionally deleted

in nestin$^+$ cells to delineate the cellular mechanisms of cartilage degeneration during RA.
Results: Aberrant activation of TGF-beta by immune response-stimulated osteoclastic bone resoprtion recruited mesenchymal stem cells (MSCs)/progenitors as clusters in the subchondral bone marrow to initiate abnormal bone formation and consequently caused cartilage degeneration. Importantly, either systemic or local blockade of TGF-beta signaling attenuated articular cartilage degeneration in RA. Moreover, conditional deletion of Tgfbr2 in nestin$^+$ cells also effectively halted progression of RA joint destruction.
Conclusion: Our data demonstrate that subchondral bone and articular cartilage work in concert as a functional unit. The pathogenesis of the cartilage degeneration of both RA and OA converges on the aberrant activation of TGF-beta in the subchondral bone. Modulation of TGF-beta activity represents a promising therapy for joint degeneration of both RA and OA.

OR18
A Peptide-Modified Delivery System to Target Osteoclasts

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Background: Dysregulated miRNAs in osteoclasts caused osteoclasts dysfunction contributing to skeletal disorders and modulating dysregulated miRNA in osteoclasts could regulate their dysfunction. But there is lack of systems for delivering therapeutic miRNAs to osteoclasts *in vivo*. To develop a delivery system specifically approaching bone resorption surfaces to target osteoclasts to facilitate modulating miRNAs in osteoclasts.
Methods: The D-Asp$_8$ can selectively bind to bone resorption surfaces for meeting osteoclasts nearby. The D-Asp$_8$ was conjugated to the surface of liposome confirmed by HPLC. (D-Asp$_8$)-modified liposome encapsulated the miR-148a antagomir, which suppress osteoclastogenesis to inhibit bone resorption. Its hydrodynamic diameter, encapsulation efficiency and serum stability were detected. Its tissue- and cell-selective delivery of miR-148a antagomir *in vivo* were analyzed by biophotonic imaging system and immunohistochemistry, respectively. The therapeutic effect evaluation of miR-148a antagomir delivered by (D-Asp$_8$)-liposome in ovariectomized mice was analyzed by microCT.
Results: The D-Asp$_8$ was successfully conjugated to the liposome surfaces. The delivery system has a average diameter of 180 nm and encapsulation efficiency of 80%. The miR-148a antagomir in (D-Asp$_8$)-liposome was detectable at 24 hours after incubation in serum, while the free miR-148a antagomir was vanished at 1 hour after incubation in serum. The fluorescence signals of the labeled miR-148a antagomir were relatively stronger in bone tissues and lower in liver and kidney from the (D-Asp$_8$)-liposome group in comparison with liposome group. More instances of co-localization of the labeled miR-148a antagomir with OSCAR$^+$ cells were found in (D-Asp$_8$)-liposome group compared with liposome group. The microCT data showed that the bone mineral density of the 5th vertebrae body from ovariectomized mice were significantly higher in (D-Asp$_8$)-liposome-miR-148a antagomir group compared to other groups.
Conclusion: The D-Asp$_8$ could facilitate miR-148a antagomir encapsulated within liposome to selectively target osteoclasts.

OR19
Substrate Stiffness Regulates Annulus Fibrosus-Derived Stem Cells in Cellular, Gene Expression of Extracellular Matrix and Biomechanical Characteristics

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Background: Annulus fibrosus (AF) regeneration is critical to intervertebral disc repair, yet remains challenging due to the remarkable heterogeneity of AF tissue. Since the stiffness of substrate determines stem cell lineage commitment (1), this study aimed to explore whether the differentiation of AF-derived stem cells (AFSCs), a newly identified cell source for AF tissue engineering, could be regulated by substrate stiffness.
Methods: AFSCs were cultured on polyacrylamide gels (PAGs) of different Young's modulus (2.5, 10, and 35KPa). After 14 days, the cells were harvested and their gene expression and cell traction forces (CTFs) were measured using RT-qPCR and cell traction force microscopy (CTFM), respectively.
Results: AFSCs proliferated well on all PAGs, but showed distinct morphology. Cells on soft (2.5KPa) gels maintained rounded shape instead of spindle or polygon shape on hard (35KPa) gels. AFSCs on soft gels showed the least expression of collagen type I gene, but the greatest expression of collagen type II and aggrecan genes. In contrast, AFSCs on hard gels exhibited exactly the opposite case (Figure 1). The CTF of cells gradually decreased with gel stiffness although cell size increased (Figure 2).
Conclusion: Substrate stiffness effectively regulates AFSCs in the cellular, extracellular matrix gene expression, and biomechanical characteristics. Findings from this study may help better understand the pathophysiological changes at cellular level during disc development and degeneration.

Reference

1. Engler *et al*., *Cell* 2006;126:677–689.

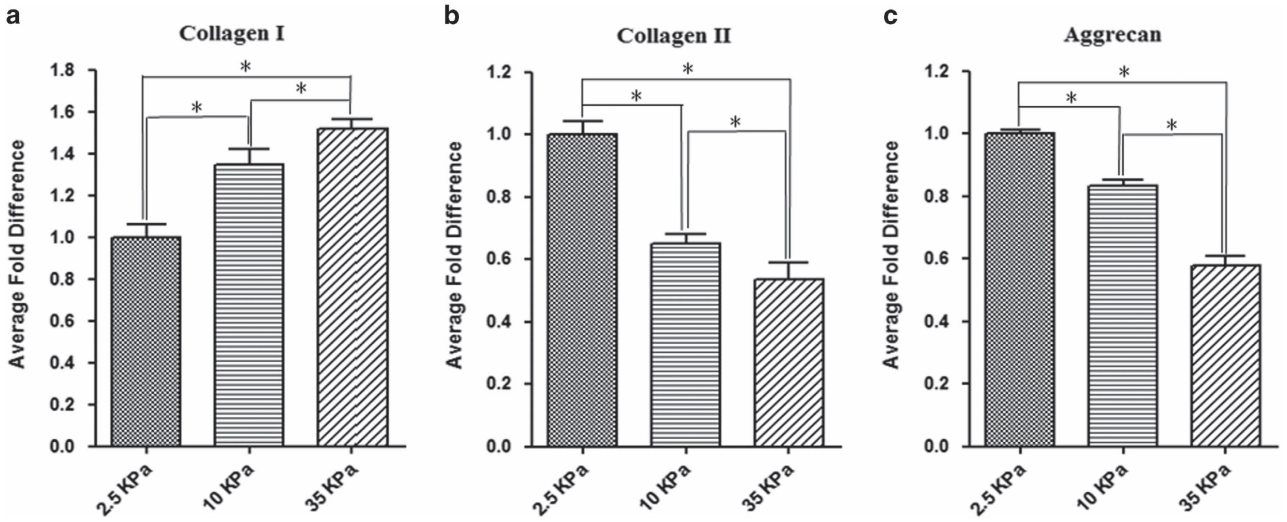

Figure 1 Gene expression of AFSCs cultured on PAGs of different stiffness for 14 days (*, $p < 0.01$).

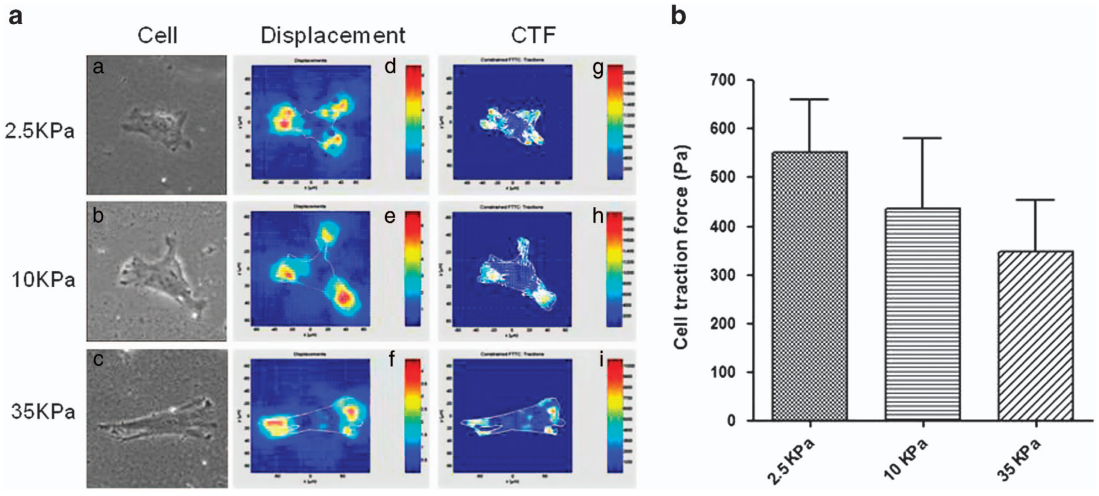

Figure 2 CTFM measurements of AFSCs on PAGs of different stiffness. (a) CTF maps. (b) CTFs.

OR20

Bone Metastasis in Lung Cancer: Novel Animal Model and microRNA as Biomarkers ror Early Diagnosis and Targets for Treatment

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Background: Lung cancer is the major cause of malignancy-related death worldwide. Metastases account for 90% of lung cancer deaths, and frequently target the skeleton and lead to rapid deterioration in quality of life and death. The molecular mechanism underlying bone metastases is largely unknown.
Methods: A novel xenograft model was established in NOD scid/scid IL-2rrg –/– (NSG) immunodeficient mice, which lack mature T cells, B cells, and functional NK cells. The target tissue distribution of human small cell lung cancer cell lines SBC-5 and SBC-3 was conducted 30 days post-tail vein injection. miRNA profiles were investigate in SBC-5 and SBC-3. Loss and gain of function techniques based on a lentiviral system were used to permanently restore miRNAs relevant to bone metastasis in SBC5. The miRNA modified SBC5 cells were cocultured with osteoblasts/osteoclasts. The phenotypes as well as communications between SBC5 cells and bone cells were investigated. The miRNA modified SBC5 cells were further implanted into NSG mice to determine the changes in bone metastases.
Results: The SBC-5 cells colonized bone and formed lytic lesions, as well as colonizing liver, spleen, and, less frequently, pancreas, ovary, and kidney. The SBC-3 cell xenografts formed

easily visible tumor foci in liver, pancreas, ovary/uterus, kidney, but not bone metastases. The expression of miR-335 and miR-29awas reduced in SBC-5 cells. miR-335 but not miR-29acorrelated with SBC-5 cell-induced osteoclast formation. Restoring miR-335 expression in SBC-5 cells significantly reduced cellular migration and invasion, and decreased proliferation and colony formation *in vitro*. In NSG mice, increasing miR-335 expression inhibited bone metastases of SBC-5 cell xenografts. IGF-1R and RANKL, considered key factors in bone metastases of other carcinomas, exhibited higher expression levels in SBC-5 cells than in SBC-3 cells. Restoring miR-335 inSBC-5 cells reduced IGF-1R and RANKL expression.
Conclusion: SBC-5 cells in NSG mice provide a suitable xenograft model system for bone metastasis of human lung cancer. miR-335 plays an important role in homing mechanisms of lung cancer metastasis to bone, which may service as a novel biomarker and candidate therapeutic target for bone metastases in lung cancer.

OR21

Aptamer-Functionalized Lipid Nanoparticles (LNPs) Targeting Osteoblasts as a Novel RNA Interference (RNAi)-Based Bone Anabolic Strategy

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Background: Metabolic skeletal disorders associated with impaired bone formation (e.g., osteoporosis) are still major clinical challenges. RNAi provides a promising approach to address the challenges. However, the bottleneck for translating RNAi into clinical application is lack of directly osteoblast-specific delivery systems for osteogenic siRNAs at cellular level. Here, we aimed to develop novel aptamer-functionalized delivery systems directly targeting osteoblasts for RNAi-based bone anabolic therapy.
Methods: Aptamer was screened by cell-SELEX with osteoblasts as target cells and hepatocytes and peripheral blood mononuclear cells (PBMCs) as negative controls. Then, osteoblast-specific aptamer was conjugated with LNPs encapsulating *Plekho1* siRNA, *i.e.*, aptamer-LNPs-siRNA. Flow cytometry, cyto-TEM and confocal imaging were employed to evaluate *in vitro* physicochemical and biological characteristics of aptamer-LNPs-siRNA. Biophotonic imaging, immunohistochemistry, FACS, microCT, bone histomorphometry and mechanical testing were employed to examine the *in vivo* tissue distribution, cell-selectivity, gene knockdown, dose-response pattern, bone anabolic action and toxicity of aptamer-LNPs-siRNA in ovariectomized rodents.
Results: After cell-SELEX, we screened an aptamer (CH6), which targeted rat and human osteoblasts but not hepatocytes and PBMCs. Then, we developed CH6-functionalized LNPs encapsulating *Plekho1* siRNA, *i.e.*, CH6-LNPs-siRNA, which had the particle size less than 90 nm and encapsulating efficiency above 80%. *In vitro* data also suggested that CH6 facilitated osteoblast targeting of siRNA and cellular uptake mainly *via* macropinocytosis. Consistently, *in vivo* data showed that CH6 facilitated skeleton/ osteoblast-specific delivery of siRNA and high gene knockdown efficiency, leading to promoted bone formation, improved micro-architecture, increased bone mass and enhanced mechanical properties in osteopenic rats with no obvious toxicity.
Conclusion: CH6 aptamer-functionalized LNPs are promising targeting system for directly delivering siRNA to osteoblasts and will accelerate the clinical translation of RNAi-based bone anabolic strategy.

OR22

Target Delivery Of Mesenchymal Stem Cells to Bone

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Background: Aging is associated with a reduction in bone marrow MSC numbers and a deficiency in the supportive bone marrow microenvironment that augments the bone formation process. Bone regeneration by the means of induction of osteogenesis from MSCs offers a rational therapeutic option. However, this approach is problematic due to the transplanted MSC do not preferentially home to bone.
Methods: We attached a synthetic peptidomimetic ligand (LLP2A) that has high affinity for activated $\alpha4\beta1$ integrin on the MSC surface, to a bisphosphonates (alendronate) that has high affinity for bone, to direct the MSCs to bone.
Results: We have performed several preclinical studies and demonstrating (1). LLP2A has high affinity against $\alpha4\beta1$ integrin. When the MSCs transition osteoblasts, the cells that form bone, $\alpha4\beta1$ integrin is highly expressed on the cell surfaces. (2). *In vitro*, LLP2A-Ale increases MSC migration in a Transwell assay system. It increased commitment of MSCs to osteoblast differentiation and increased osteoblast maturation and function via Akt-signaling. (3). The new hybrid compound, LLP2A-Ale, directs transplanted MSCs to bone and increases the retention of the transplanted MSCs in bone in an *in vivo* xenotransplantation study. (4). LLP2A-Ale augments bone formation and bone mass in young immune-competent mice. (5). LLP2A + MSCs reverses bone loss in osteoporotic mice and rats induced by estrogen deficiency. (6). LLP2A + MSCs increases bone formation in aged mice. (7). LLP2A-Ale + MSC are anti-inflammatory and prevents bone loss associated with inflammatory arthritis.
Conclusion: These results strongly support MSC transplantation as a novel therapeutic option for the treatment of bone loss related to aging and hormone deficiency if they are directed to bone. Under proper guiding, MSCs transplantation can be used to augment bone mass by increased homing and osteogenic efficacy. The benefices of systemic MSCs transplantation are multi-factorial and shall be considered beyond a simple cell-based therapy but also providing growth factors and paracrine modulating effects to treat or to alter the course of diseases.

OR23

Fracture Risk Assessment Following Hematopoietic Stem Cell Transplantation

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Background: Patients underwent hematopoietic stem cell transplantation (HSCT) experience rapid bone loss after HSCT. The magnitude of fractures and the risk factors remain unknown.
Methods: We performed a retrospective study of adult that underwent HSCT at MD Anderson Cancer Center from January 1, 1997 to December 31, 2011 to identify fractures by physician billing codes, confirmed by clinic notes and/or radiologic reports. Fracture rates were compared with the US general population using estimates from the 1994 National Health Interview Survey (NHIS). Gender, age, indication and type of HSCT, were assessed as risk factors using regression analysis based on the Cox proportional hazard model.
Results: A total of 7,650 patients underwent HSCT in the 15 year period. The mean age at the time of HSCT was 49.29 (±13.51). About 45% were female. The most common reason for HSCT was a hematological malignancy (89.5%), of which 22.2% were multiple myeloma. Approximately 8% (n=631) developed fracture with about 11% (n=440) in those with an autologous transplant and 5% (n=191) in those with an allogeneic transplant. The rate of fracture in both males and females was similar around 8%. Age and gender specific fracture rates following HSCT were significantly greater than that of the US general population in all sub-groups. The striking difference was an approximately 10 times greater risk in 45-64 year old males and females and an even greater risk in males >65 years of age at the time of HSCT. A Cox proportional hazard model showed that patients with autologous HSCT had larger hazard of fracture than those with allogeneic HSCT (HR=1.58, p-value <0.0001).
Conclusion: Both males and females experience a similar increased risk of fractures following HSCT. Our study is the first to determine the burden of the disease. Evaluation of a comprehensive set of risk factors is needed.

OR24

Greater Serum Carotenoid Concentration Associated with Higher Bone Mineral Density in Chinese Adults

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Background: Carotenoids may positively regulate bone metabolism through their antioxidant properties; however, few studies have examined the relation between serum carotenoids and bone health. We determined the associations between the serum concentration of several carotenoid subclasses and bone mineral density (BMD) in a Chinese population.
Methods: This is a community-based cross-sectional study was conducted in urban Guangzhou, Guangdong Province, China. 1523 women and 595 men aged 59.6 (40-75) years old completed serum β-cryptoxanthin, zeaxanthin+lutein, lycopene and α-carotene concentration analyses at baseline and BMD assessments at 3-year after enrollment. Serum individual carotenoids were assessed by the methods of reverse-phase high-performance liquid chromatography. Dual-energy X-ray absorptiometry was applied to determine BMD at whole body, lumbar spine, total hip, femur neck, trochanter and Wards' triangle. ANCOVA was used to examine the correlations between categorized individual carotenoids and BMD at measured sites.
Results: After adjusting for potential covariates, a monotonic dose-response positive correlation between circulating levels of β-cryptoxanthin, lycopene and α-carotene, and BMD at various skeletal sites was observed in both women and men. Compared with women (men) in the bottom quartiles, women (men) in the top quartiles of serum β-cryptoxanthin, lycopene or α-carotene exhibited 1.23–4.07% (2.65–6.94%), 0.95–3.88% (2.14–4.03%) or 1.99–4.49% (3.54–7.40%) higher BMD at the bone sites with significant results (P-trend: <0.05), respectively. No significant associations between serum zeaxanthin+lutein and BMD were detected in either gender.
Conclusion: These results strongly suggest that serum carotenoids have a favorable association with bone health in the study population. This study provides further evidence to recommend antioxidant-rich foods as a useful tool in bone health promotion and osteoporosis prevention.

OR25

Efficacy and Safety of Alfacalcidol in Chinese Postmenopausal Women with Osteoporosis or Osteopenia: Results of an Open Label, Non-Comparative, Post Marketing Observational Study

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Background: To explore the efficacy and safety of 1μg alfacalcidol daily in Chinese postmenopausal women with osteoporosis or osteopenia.
Methods: In this open-label and uncontrolled clinical trial, 62 postmenopausal women were included. Nine months treatment with daily basic oral calcium (elemental calcium 0.5g) and alfacalcidol (Alpha D3®, 1 μg) were administrated. Efficacy assessment including muscle function tests, bone mineral density and bone turnover markers were carried out at baseline and regular intervals. Renal ultrasound and multiple biochemical indexes such as serum creatinine, serum calcium and 24-hour urine calcium were tested as safety assessment. Treatment adjustment: in case of any value above the normal

range (serum calcium > 2.70 mmol/L or 24-hour urine calcium > 300 mg) and a confirmation 1 week that after, the daily dosage of alfacalcidol were reduced to 0.5 μg. In case of repeated confirmed values above normal range, the patients should be exited from the study.

Results: A. Efficacy assessment: (1) Muscle function tests: A significant improvement in the performance of timed up and go test (TUG) and chair rising test (CRT) was proved already after 3 months of treatment and further improved by the end of the study. There were significant increases in the number of participants able to successfully perform the tests: 91.9% at baseline and 100% at the end of trial for the TUG (*P*=0.0073) and 62.2% at baseline and 81.1% at the end for the CRT test (*P* = 0.0070). (2) Bone mineral density: No significant change. (3) Bone turnover markers: Significant decrease of serum C-terminal telopeptide of type I collagen (CTX) and N-aminoterminal propeptide of type I collagen (P1NP) was observed after 3 months of treatment (P<0.001) and lasted through the study. Serum intact parathyroid hormone had a similar change as CTX and P1NP. B. Safety assement: 37 patients completed the study, 18 of whom adjusted alfacalcidol to 0.5μg daily (29.0%). There were 30 adverse drug reactions in 21 patients out of 62 (incidence 33.9%). No severe drug reactions and hypercalcemia. 17 patients (27.4%) dropped out because of repeated hypercalciuria. An increase of serum creatinine was observed as compared to baseline (P<0.001), but all the values were in normal range.

Conclusion: Treatment with 1μg alfacalcidol daily can significantly improve muscle function and bone metabolism. Although 1μg alfacalcidol is relatively safe, regular and close monitoring of urine calcium and timely adjusting the dosage are very important.

OR26

PTH Inhibition Rate Is Valuable in Distinguishing Different Types of Hyperparathyroidism

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Background: Presently, researches about the intravenous calcium suppression test (CST) have been mainly focusing on the diagnosis of primary hyperparathyroidism (PHPT) but rarely on other types. Therefore, despite exploring the autonomy of parathyroid function of secondary hyperparathyroidism (SHPT), this study was also designed to identify the diagnosis as PHPT or SHPT of patients with elevated parathyroid hormone (PTH), normal renal function and normal or intermittently elevated serum calcium.

Methods: 60 normal control subjects and 61 patients clinically diagnosed as hyperparathyroidism were divided into 6 groups: Control, PHPT, SHPT with mildly impaired renal function (SHPT1), SHPT with severely impaired renal function (SHPT2), tertiary hyperparathyroidism (THPT) and cause unknown hyperparathyroidism (CUH). Intravenous CST was administered to all the subjects; besides, total serum calcium and ICMA PTH levels were measured before and after the start of infusion, respectively.

Results: The most maximum serum PTH inhibition rate (PTH-IR) was 50.11±16.98%(PHPT), 88.24±7.84%(CUH), 88.64±3.27%(SHPT1), 63.18±19.78%(SHPT2), 51.06±4.93% (THPT), 85.06±10.38%(Control), respectively (mean(SEM), *P*=0.000, between groups). Though pairwise comparison, the PTH-IR was similar among PHPT, SHPT2 and THPT (*P*>0.1), while lower compared with Control, CUH and SHPT1, respectively (*P*<0.05). The ratio of PTH-IR/ΔCa was used to reflect the sensitivity of calcium-mediated PTH suppression and the index was significantly higher in Control compared with PHPT and SHPT2, respectively (*P*<0.05). Surprisingly, Receiver Operating Characteristic curve analysis suggests that 68.21% is the cut-point to differentiate PHPT and Control, 75.59% to differentiate PHPT and CUH, 82.26% to differentiate PHPT and SHPT1, 79.85% to differentiate CUH and SHPT2, 82.60% to differentiate SHPT1 and SHPT2.

Conclusion: In this study, it is confirmed that PTH-IR is of great diagnostic value in differentiating PHPT from SHPT. With aggravatingly impaired renal function, the autonomy of parathyroid function of SHPT is enhancing to make it undifferentiated with THPT, indicating that the pathophysiological process from SHPT to THPT is continuous.

OR27

Tumor Induced Osteomalacia: Reports of 15 Cases in China

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Background: Tumor-induced osteomalacia (TIO), or oncogenic osteomalacia, is a rare paraneoplastic syndrome attributed to tumor overproduction of FGF23, increasing renal phosphate excretion and secondary hypophosphatemia. Clinical signs include bone pain, proximal muscle weakness and pathological fractures. Surgical resection leads to good prognosis, but tumors are usually located in bones and soft tissues, and grow slowly causing difficult localization. Our aim was to investigate the clinical symptoms, biochemical data, diagnostic methods, course after tumor resection and histopathological findings.

Methods: We analyzed clinical data, diagnostic methods and pathological results of 15 patients with TIO. After medical history and physical examination, we measured biochemical markers of 43 nonfamilial, adult-onset hypophosphatemia osteomalacia patients from 2009 to 2014 in Shanghai sixth people's hospital, and performed 18-F fluorodeoxyglucose PET and CT (18F-FDG PET/CT) on 15/43 patients with suspected TIO. Based on PET/CT, tumor resection surgery was conducted on 15 patients with TIO.

Results: 6/15 women (40%) and 9/15 men (60%) had TIO. Average age was 47.2±10.7 years (range 25-65). All presented with bone pain, proximal muscle weakness and pathological fractures. 9 had bioconcave or wedged vertebrae (9/15, 60%), 5 had femur fracture (5/15, 33.3%) and 2 had pelvis fracture (2/15, 13.3%). The mean serum phosphate was 0.43±0.12 mmol/L; ALP 267±151 U/L. The results of PET/CT suggested 6 of them had increased accumulation of 18F-FDG. Histopathology on 12/15 (80%) showed phosphaturic mesenchymal tumor (PMT), in bones and soft tissues (11/15, 73.3%).

Conclusion: 18-F FDG PET/CT is playing a considerable role in determining the localization of TIO-associated tumors.

In view of the recovery of biochemical marker and clinical symptoms, tumor resection surgery still remains the treatment of first choice and be treated as kind of radical therapy.

OR28

Clinical Analysis of MEN1-Related Primary Hyperparathyroidism in Chinese

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Background: Primary hyperparathyroidism (PHPT) is the most frequent and initial multiple endocrine neoplasia type 1 (MEN1)-associated endocrine disease. Here, we summarized clinical characteristics of MEN1-related PHPT (MHPT).
Methods: Retrospectively analysis was performed in 40 MHPT patients and 171 consecutive patients with sporadic PHPT (SHPT) diagnosed from 2002 to 2013 in PUMCH.
Results: The mean age at diagnosis of PHPT was 45.0 years in MHPT group, which was younger than SHPT group (50.5 years, $P<0.05$). Compared with SHPT group, the percentages of skeletal disorder and polydipsia or polyuria were significantly lower in MHPT (57.5% vs. 87.3%, $P<0.001$; 20.0% vs. 45.0%, $P<0.05$, respectively), while the percentage of urolithiasis was significantly higher in MHPT (60.0% vs. 40.4%, $P<0.05$). No difference in serum total calcium level was found between the two groups. But elevation of ionized calcium (iCa) level and decline of serum phosphate (P) level were less severe in MHPT than in SHPT group (iCa: 1.40mmol/L vs. 1.50mmol/L, $P<0.05$; P: 0.83mmol/L vs. 0.73mmol/L, $P<0.05$). ALP and PTH level were lower in MHPT group than those in SHPT group (median ALP: 102.5U/L vs. 168.5U/L, $P<0.001$; median PTH: 237.0pg/mL vs. 639.2pg/mL, $P<0.001$). Compared with SHPT, the incidence of parathyroid hyperplasia was higher in MHPT (54.1% vs. 23.7%, $P<0.001$), meanwhile multi-glandular involvement was more common in MHPT group (58.3% vs. 12.9%, $P<0.001$). After parathyroidectomy, performed in 34 MHPT patients, 5 patients showed persistent hypercalcemia and 9 patients showed recurrent hyperparathyroidism during a mean follow-up time of 2.0 years (range 0.6–25 years). Thus, the recurrent/persistent rate was 41.2%.
Conclusion: This is the largest study concerning MEN1-related PHPT in Chinese. In conclusion, MHPT patients show more common kidney involvement but less common skeletal involvement and polydipsia or polyuria manifestation despite a milder biochemical presentation compared with their SHPT counterparts.

OR29

Effects of Alendronate Versus Zoledronatic Acid in Chinese Children with Osteogenesis Imperfecta: Two-Year Clinical Trial

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Background: Bisphosphonates are beneficial for osteogensis imperfecta (OI) as they can increase bone mineral density and reduced bone fracture incidence. The clinical study of bisphosphonates in patients with OI is limited. We compare the efficacy and safety of oral alendronate with intravenous zoledronic acid in Chinese patients with OI.
Methods: In this open-label, 2 year, prospective study, we randomly assigned children and adolescents aged from 1 month to 18 years old with moderate to severe OI to receive either intravenous zoledronic acid 5mg annually or oral alendronate 70mg weekly. Calcium and vitamin D were supplemented daily. Primary endpoint was changes of bone mineral density (BMD) during treatment. Secondary efficacy endpoint included changes in liner growth speed, fracture incidence, and bone turnover biomarkers.
Results: Of 159 participants, 104 received alendronate and 55 were assigned to zoledronic acid group. After two years of treatment, the mean BMD z score of lumbar spine was increased significantly from -2.3 to 0 in zoledronic acid ($P<0.001$) and –2.9 to –0.1 in alendronate ($P<0.001$). Zoledronic acid induced a significantly larger increase in femoral neck BMD z score than alendronate (0.0±-1.9 vs –4.2±2.5, and –1.7±2.3 vs –4.0±2.8, $P=0.019$). Fracture incidence was reduced more significantly in zoledronic acid group than that in alendronate group (0.16±0.59 vs. 0.43±0.78, $P=0.031$). Serum cross-linked C-telopeptide of type I collagen decreased equivalently in both groups (26% in the alendronate-treated group, 24% in the zoledronic acid group, $P=0.779$). Adverse effects were similar between the two groups.
Conclusion: Both intravenous zoledronic acid and oral alendronate are effective, well-tolerated alternative treatment for OI. Intravenous zoledronic acid is associated with more significant decrease in fracture incidence.

OR30

A Survey Of Osteoporosis Evaluation and Management of Patients Following Fragility Fractures in China

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Background: Data about the evaluation and management of osteoporotic patients following fragility fractures is still lacking in China. This study aims to investigate the outcomes and management of patients with osteoporotic fractures during the post-fracture course in China.
Methods: This retrospective study involved female patients hospitalized for low-trauma hip or vertebral fractures from Jan 1st, 2008 to Dec 31st, 2012 in 18 hospitals of different areas in China. A questionnaire composed of 36 questions was administered to the patients by telephone survey.
Results: The response rates of subjects with hip and vertebral fractures were 66.4% and 73.5%, respectively. 1989 cases aged 73.4±10.0 were analyzed, including 1148 cases of hip fracture and 841 cases of vertebral fracture. Falling was the most common cause of fractures (66.5%). During 3.08(±1.44) years following fractures, 217 cases (10.9%) died including 153(13.3%) and 64(7.6%) cases of hip and vertebral fracture. 3.5% and 26.0% of the living patients are disabled or need help in daily life, respectively. Osteoporosis was found in the discharge diagnoses in 56.8% of all patients. BMD had never been tested in 42.0% of the patients. 142(7.1%) and 20(1.0%) cases experienced one and two refractures, respectively. 69.6% (1385) of the cases initiated anti-osteoporosis pharmacotherapy after fractures. Only 7.0% of the patients knew about osteoporosis very well and 23.3% did not know about osteoporosis. 70.0% of the patients took steps to prevent osteoporosis and fracture including prevention of falling (50%), milk or calcium-fortified food (48.6%).
Conclusion: In the present study, only 59.4% of the patients resumed daily life ability completely following fractures and hip fractures affected living status more significantly. The rates of diagnosis and treatment of osteoporosis in patients with fragility fractures were low and the pharmacotherapy was insufficient in such cases in China.

OR31

Clinical Measurement of Intravertebral Pressure during Vertebroplasty and Kyphoplasty

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Background: Vertebroplasty (VP) and kyphoplasty (KP) are emerging procedures for immediate pain relief when treating osteoporotic or osteolytic fractures. The main complication is polymethylmethacrylate (PMMA) leakage, leading to compression of neural structures or embolism. Intravertebral pressure (IP) is an important factor determining the risk for leakage, but there is limited information from clinical and experimental studies. This study compared the intravertebral pressures of compressed vertebrae and adjacent normal vertebrae, and measured the IP of compressed vertebrae during VP and KP.
Methods: Thirty-five patients (40 compressed vertebrae and 35 adjacent normal vertebrae) were randomly allocated IP measurements. Cannulas were placed bipedicularly into the posterior third of each vertebral body. Either PMMA or a balloon was injected into the vertebral body through the right cannula. A manometer was connected to the cannula in the left pedicle, and heparin was injected to verify the pressure measurement system.
Results: The range, average IP, and SD of compressed vertebrae were 0–39 mmHg and 24.5±11.3 mmHg; and adjacent normal vertebrae were 3–16 mmHg, 7.3±4.2 mmHg. Average IP for Phase 1 (before PMMA injection) for VP was 23±11.9 mm Hg; the maximum IP during injection was 169±46.8 mmHg and the IP for 10 minutes after injection was 33±9.4 mmHg. The highest IP recorded for KP patients was 142±39.6 mmHg. The average IP for Phase 1 (before balloon inflation) was 24±12.7mmHg; Phase 2 (peak IP during inflation) was 63±25.8 mmHg; and Phase 3 (after inflation/before PMMA injection) was 18±10.8 mmHg. The IP 10 minutes after injection in KP was 36±8.5 mmHg.
Conclusion: The IP of compressed vertebrae was significantly higher than that of adjacent normal vertebrae. There was a significant increase in IP during the PMMA filling in VP and KP; the IP of compressed vertebrae was not significantly reduced by the balloon inflation in KP, and no statistically significant differences in IP were found during all common stages of PMMA filling in VP and KP.

OR32

Minimally Invasive Plate Osteosynthesis with a Locking Compression Plate Is Superior to Open Reduction and Internal Fixation in The Management of the Proximal Humerus Fractures

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Background: The use of minimally invasive plate osteosynthesis (MIPO) via anterolateral deltoid splitting has good outcomes in the management of proximal humerus fractures. While using this approach has several advantages, including minimal soft tissue disruption, preservation of natural biology and minimal blood loss, there is an increased risk for axillary nerve damage. This study compared the advantages and clinical and radiological outcomes of MIPO or open reduction and internal fixation (ORIF) in patients with proximal humerus fractures.
Methods: A matched-pair analysis was performed, and patient groups were matched according to age (±3 years), sex and fracture type. Forty-three pairs of patients (average age: MIPO, 63 and ORIF, 61) with a minimum follow-up of 12 months were enrolled in the study group. The patients were investigated radiographically and clinically using the Constant score.
Results: The MIPO technique required less surgery time and caused less blood loss compared to ORIF ($P<0.01$). In addition, MIPO required a smaller incision, resulted in less scarring, and was cosmetically more appealing and acceptable to female patients than ORIF. Following MIPO, patients had better functional results at 3 and 6 months, with better outcomes, less pain, higher satisfaction in activities of daily living, and a higher range of motion when compared to ORIF ($P<0.05$). Fracture configuration, according to the AO/ASIF(Association for the Study of Internal Fixation) fracture classification, did not significantly influence the functional results. The complication rate was comparable between both groups.
Conclusion: The use of MIPO with a locking compression plate in the management of proximal humerus fractures is a safe and superior option compared to ORIF.

OR33

Vertebral Fractures among Breast Cancer Survivors in China

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Background: Women with breast cancer (BC) are at high risk for fracture due to the deleterious impact of BC therapies on bone. In China, BC survival is improving as screening and treatment programs expand, however no guidelines exist to prevent BC treatment induced bone loss. We designed a pilot study to evaluate the scope of this problem among BC survivors at a large cancer referral hospital in Beijing.
Methods: BC survivors receiving care at the Cancer Institute and Hospital of the Chinese Academy of Medical Sciences between April-December 2013 were invited to participate. Women between 50-70 years of age were eligible if they had initiated treatment for BC at least 5 years prior to enrollment, and had no evidence of metastatic bone disease. Study procedures included a questionnaire regarding risk factors for and personal history of fracture and a thoracolumbar x-ray.
Results: 100 women were enrolled with a mean age of 57±5 years, and BMI of 26.6±4.8 kg/m^2. Mean years since BC diagnosis was 6.0±0.8. The majority of cases were stage I or II at diagnosis (79.2%) and estrogen and/or progesterone receptor positive (87%). In total, 12 vertebral fractures (VFs) were identified. Average reported lifetime height loss was 1.7±1.1 cm, 11% reported a parental history of fracture, 9% reported a personal history of fracture, and 22% reported falling within the past year. Forty-five percent of all participants reported taking calcium supplements, but only 4% reported taking vitamin D supplements. Only 25% of women reported having a bone density scan since being diagnosed with BC.
Conclusion: Prevalence of VF among our cohort of Chinese BC survivors was 12%, much higher than reported among age-matched healthy Chinese women in Beijing (less than 5%). Chinese women undergoing BC therapy should be routinely evaluated for osteoporotic fracture risk. Larger studies are necessary to inform screening and prevention guidelines.