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Plenary Talk Abstracts

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PL1
Obesity Is Not Protective Against OP and Fractures

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Until now, many doctors believe that obesity is protective against osteoporosis and osteoporotic fractures; however, this belief has recently come into question.
Significant negative association between percentage of fat mass and BMC was found in many studies, indicating that excessive fat accumulation may not protect against OP. A higher percentage of VAT is the most important risk factor of bone loss because (1) adipokines and a chronic inflammatory state promote OC precursors to differentiate into mature cells by the activation of M-CSF and RANKL, and T-lymphocytes can directly motivate OC formation by IL-1, IL-6 and IL-17; (2) different components of the metabolic syndrome, such as hypertension and increased serum triglycerides, are also potential risk factors for the development of OP; (3) oxidative stress is correlated to osteocyte attenuation; and (4) PPARγ in the bone marrow regulates mesenchymal stem cells toward adipogenesis and produces a number of secretory factors to decrease OB differentiation and subsequent bone formation.
Heavier women do have stronger femurs, but the increment in bone strength is not enough against their higher weight measure, and heavier women are more likely to suffer from hip fractures. The rate of incident hip fractures tended to decline with higher BMI only, owing to the less active and fewer trauma opportunities. From clinical observations a vicious cycle among obesity, OP and fracture can be seen, and fracture and debility severely hinder the physical activity, leading to further fat accumulation, and then complication by hypogonadism, chronic inflammation and oxidative stress. Moreover, bone loss is aggravated by an already established OP and the disused secondary OP. More serious condition was seen in patients with obese type 2 diabetes; and as the follow-up continued the fracture rate was getting higher and higher, reaching to almost 50% by the end of 30 years.

Obesity has both positive and negative impacts on bone metabolism, but as a whole the positive effects are too weak to counteract against the negative influence on bone, heart, arteries, lipids, carbohydrate and so on. From this point of view, we could consider that obesity is not beneficial for bone health, BMD, OP and fractures.

PL2
Overview of Osteoporosis Treatment

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Osteoporosis most commonly occurs in postmenopausal women, substantially contributed to by the estrogen deficiency that develops at menopause. In older men, bone loss is also a significant issue, probably also contributed to by the declining levels of sex hormones with advancing age. Osteoporosis results in fractures in more than one-half of European postmenopausal women, and about one-third of older men. In Asia, the epidemiology of fractures is changing; in particular, hip fractures are becoming more common, possibly related to increasing height and femoral neck length.
The first step in osteoporosis management is an evaluation of fracture risk—this has replaced the concept of diagnosis; osteoporosis really represents a continuous spectrum of risk rather than a discrete diagnosis. Both bone density and clinical risk factors need to be factored into determining fracture risk, and computer-based algorithms are available to facilitate these computations.
Some measures can be recommended to all older individuals, even those with low fracture risk. These would include minimization of risk factors (safety in the home to prevent falls, not smoking, moderate alcohol intake, maintenance of adequate vitamin D status through sunshine exposure or the use of supplements, calcium-rich diet). Recent evidence suggests that calcium supplements increase the risk of cardiovascular events; therefore, there has been a decline in their use, although some experts continue to advocate for them.
In individuals at high fracture risk, pharmaceutical intervention is indicated. The bisphosphonates are the most widely used agents at the present time, and it works by reducing osteoclastic bone resorption. These agents have long durations of action and can be administered by mouth every week to months, or intravenously at intervals of up to several years. The intravenous agent, zoledronate, substantially reduces bone resorption for at least 5 years after a single dose of 5 mg, and much smaller doses may have almost a comparable efficacy to the 5-mg dose. Denosumab is a monoclonal antibody directed against RANKL. It is administered as a subcutaneous injection every 6 months and has comparable efficacy to zoledronate.
Parathyroid hormone preparations are anabolic to bone, producing more dramatic and sustained changes in bone density

than bisphosphonates do. Animal studies raised concern that they might cause osteosarcomas, but this has not been borne out from clinical experience. However, their use is usually restricted to periods of less than 2 years.
New anti-resorptives and anabolics are currently under development; therefore, an increasing range of options is likely to be available in the future.

PL3
Emerging Novel Therapies for Unmet Needs
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Current anti-resorptive therapies substantially reduce the risk of vertebral fractures and are—or soon will be—inexpensive. However, they have limited efficacy for reducing the risk of non-vertebral fractures. A few new treatments have the potential for increasing bone mass and reducing the risk of non-vertebral fractures.
Odanacatib inhibits cathepsin-K, which modestly inhibits the resorption of bone proteins and shifts the balance of remodeling toward bone formation. It increases cortical bone mass in the hip, and a large anti-fracture trial was stopped early, as it significantly reduced the risk of hip fracture.
Sclerostin, produced by osteocytes, blocks bone formation; antibodies to sclerostin promote large increases in bone formation, combined with modest anti-resorptive effects. This produces dramatic increases in bone mass within few months. The efficacy for preventing fracture is being tested in large trials.
Daily administration of nitroglycerin probably acts like an exercise on bone, decreasing the expression of sclerostin with substantial increases in the cortical and trabecular bone mass, which are sustained for at least 2 years. Testing the efficacy of nitroglycerin on fracture risk will require a large clinical trial, but this will be challenging because nitroglycerin therapy has no large pharmaceutical sponsor.
The availability of more potent bone-forming therapies will enable treatment to achieve goals of acceptably low risk of fractures, even in patients with severe osteoporosis.

PL4
Reversing Human Osteoporosis: Anabolic Agents
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Increasing bone mass in children and decreasing bone loss in adults can prevent or delay osteoporosis, and greatly reduce its social and economic costs. But these measures do not reverse bone loss and cure established osteoporosis in adults or children, because bone formation is too slow. In addition, interventions that decrease bone loss usually decrease bone formation ('coupling'). Skeletal anabolic drugs circumvent this limitation by stimulating bone formation. PTH and PTHrP, and their synthetic analogs stimulate bone formation and dramatically improve bone mass, bone architecture and bone strength in humans and animals, but they cure osteoporosis only in rodents. In humans and most animals, their dramatic skeletal benefits are limited by cortical bone remodeling, which these anabolic drugs increase along with increases in bone formation. Osteopetrotic mutants and animal experiments suggest that administering PTH, PTHrP or their synthetic analogs with an anti-resorptive drug (concomitant anabolic anti-resorptive therapy) can overcome this limitation. Initial clinical trials of this concept used PTH plus alendronate and were discouraging, but subsequent clinical trials using more selective anti-resorptive drugs are generating more interesting results, and this approach warrants additional animal and clinical studies.
An alternative is to administer drugs that increase bone formation without increasing bone resorption and cortical bone remodeling (pure anabolic therapy). A monoclonal antibody that blocks the actions of sclerostin seems to have this property in early human studies, and improves bone mass and bone architecture more dramatically and more rapidly than PTH does in animals and humans. Other monoclonal antibodies to sclerostin, and to Dkk, are also being investigated for their ability to cure osteoporosis in humans.
Another promising approach is to administer drugs that decrease bone resorption without decreasing bone formation (pure anti-resorptive therapy). Odanacatib, a cathepsin-K inhibitor, has this interesting property, and improves bone mass and bone architecture more dramatically and more rapidly than bisphosphonates do in animals and humans.
Once considered an inevitable consequence of aging, osteoporosis is now considered preventable and treatable, and may soon be considered curable.

PL5
When to Stop Bisphosphonate Therapy
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The optimal duration of treatment for established osteoporosis is unknown, but increasing concerns about possible long-term side effects with prolonged anti-resorptive therapy have prompted many to consider interrupting or stopping bisphosphonate therapy after 3–5 years. The decision to stop the therapy should be influenced by the evidence that treatment continues to be beneficial and that the side effects can be avoided by discontinuation, preferably using data from clinical trials in which previously treated individuals are randomized to continue bisphosphonate therapy or to receive placebo, and followed for fracture outcomes and potential side effects. Two such trials among older women have been reported: (1) the FLEX study, which evaluated discontinuation of daily alendronate, and (2) the Horizon Extension trial, which studied yearly injections of zoledronic acid. The results of both trials were qualitatively similar; in comparison with those who continued bisphosphonates, discontinuation was not associated with an overall increased risk non-spine fracture, but was associated with a 50% increased risk of spine fractures. A *post-hoc* subgroup analysis among women without vertebral fracture in the FLEX study suggested that non-spine fracture risk was higher (RR=2.0; 95% CI: 1.04, 3.84) among women with femoral neck BMD below −2.5 at the time of discontinuation. Although too uncommon to study even in large trials, it is the side effects,

such as osteonecrosis of the jaw and atypical femur fractures. Thus, after 5 years of alendronate or 3 years of zoledronic acid, it is reasonable to consider withholding the therapy for 3–5 years among low-risk women (those with hip BMD above –2.5 and no previous hip or spine fractures). The utility of serial BMD or biochemical monitoring after bisphosphonate discontinuation is unknown, and rigorous data for other bisphosphonates, as well as other anti-resorptives, are not available.

PL6

The Difference of the Bone Microstructure Between Chinese and White American Women

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Despite lower areal bone mineral density (BMD), Chinese–American women have fewer fractures than white women. We hypothesized that the better skeletal microstructure in Chinese–American women, in part, could account for this paradox. Individual trabecula segmentation, a novel image-analysis technique, and micro-finite element analysis were applied to high-resolution peripheral quantitative computed tomography images, to determine bone microarchitecture and strength in premenopausal (n=95) and postmenopausal (n=97) Chinese–American and white women. At the distal radius, premenopausal Chinese–American women have 63% higher trabecular plate versus rod ratio (P:R ratio), 38% higher plate bone volume fraction (pBV/TV) and 23% greater plate–plate junction densities (P–P Junc.D, indicating intactness of Tb network) compared with premenopausal white women (P<0.01 for all). Despite smaller bone size (–10%, P=0.009), greater cortical thickness and volumetric BMD (Ct.Th and Dcort, 18% and 4%, respectively, P<0.001), and more number of trabecular plates led to 14% greater whole-bone stiffness (P<0.01) in premenopausal Chinese–American versus white women. In contrast, postmenopausal Chinese–American women had similar pBV/TV and P–P Junc.D, yet 36% higher P:R ratio (P=0.01) compared with postmenopausal white women. Again despite the smaller bone size (–10%, P=0.009), postmenopausal Chinese–American women had greater Ct.Th and Dcort (18% and 6%, respectively, P<0.01) and relatively intact Tb plates, resulting in similar bone stiffness compared with white women. In both the racial groups, Ct.Th and Dcort were significantly low in the post- versus premenopausal women, and the intraracial age-related differences were similar between Chinese–American and white women. Whole-bone stiffness, Dtrab and trabecular plate parameters were also significantly low in the post- versus premenopausal women. Moreover, the intraracial age-related differences in pBV/TV was significantly greater (P<0.05) in Chinese–American women, and the differences in whole-bone stiffness, P:R ratio and P–P Junc.D tended to be greater in Chinese–American versus white women. In conclusion, there are microstructural advantages in both cortical and trabecular bone in premenopausal Chinese–American women. However, within-race cross-sectional differences between pre- and postmenopausal women suggest that there may be greater loss in plate-like trabecular bone with aging in Chinese–American women. Nevertheless, advantages, such as thicker cortices and more plate-like trabecular bone, persist in postmenopausal Chinese–American versus white women.

PL7

Bone–Muscle Interactions

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The mammalian musculoskeletal system evolved to enable several functions required for terrestrial life, including locomotion, breathing, protection of internal organs and coordination of global energy expenditure. Bone and muscle tissue are derived from a common mesenchymal precursor and accumulate peak tissue mass in close association, according to genetic information and environmental stimuli. The close linkage of bone and muscle development is evident even before birth. For example, the varying circumferential shape of different long bones occurs through asymmetric mineral deposition, apparently in response to site-specific mechanical strains applied to the newly forming bones by their associated muscle groups *in utero*. A fundamental determinant of peak bone and muscle mass is a genetic background. Bivariate linkage analysis in large human populations has identified significant quantitative trait loci shared by leg lean mass with shaft cross-sectional area. Superimposed on these genetic determinants of bone and muscle mass are the anabolic stimuli that occur postnatally, the most dominant of which is puberty. During the pubertal growth spurt, bone and muscle mass accumulate rapidly under the influence of GH, IGF-1 and sex hormones. Finally, aging results in the progressive and parallel loss of bone (osteopenia) and skeletal muscle (sarcopenia), with profound consequences to quality of life. Age-associated sarcopenia results in reduced endurance, poor balance and reduced mobility that predispose elderly individuals to falls, which more frequently result in fracture because of concomitant osteoporosis. This talk will highlight recent advances in our understanding of mechanisms coupling bone and skeletal muscle mass and identify critical areas where further work is needed.

PL8

Overview of Bone Cells and Bone Biology

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Cells of the osteoblast lineage have the responsibilities of synthesizing and mineralizing the matrix of bone. These activities respond to developmental programs, to mechanical forces and

to hormonal directions to maintain bone strength and participate in calcium/phosphate homeostasis. These osteoblastic cells, of mesenchymal origin, also regulate the generation and activity of osteoclasts, the cells with the responsibility for resorbing bone matrix, and also regulate the fates of hematopoietic stem cells and more differentiated hematopoietic cells. To accomplish these missions, mesenchymal cells differentiate from marrow progenitors, proliferate and form post-proliferative osteoblasts, bone-lining cells and osteocytes. Each of these cell types has distinct functions, and movement from one differentiation state to another is carefully regulated.

Although osteoprogenitors are central to renewal of the osteoblastic lineage cells, little is known about the identity of these cells *in vivo*. We and others have been using genetically marked cells in intact mice to track the fates of cells of the osteoblast lineage. Mice in which the nestin promoter directs the production of the cre recombinase, in a way that can be regulated by administration of tamoxifen, have allowed the identification of large numbers of osteoblast precursors in fetal and early postnatal bone. These cells in fetal life become marrow stromal cells and osteoblasts. These osteoblasts are first found in the perichondrium, but move into the marrow space over time to form the primary spongiosa. These cells become less numerous postnatally and resemble pericytes found adjacent to blood vessels in many tissues. *In vitro*, these cells have characteristics of mesenchymal stem cells.

At the other end of the spectrum, we can mark some bone-lining cells using transgenic mice in which cre recombinase is driven by the dentin matrix protein-1 promoter. We have shown that cells marked when this cre recombinase is activated with tamoxifen can once again become active osteoblasts after PTH administration.

Thus, transgene markers are now allowing us to identify different categories of osteoblast precursors and to study the regulation of movement from one cellular compartment to another.

PL9

Coupling Bone Resorption with Formation

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In the adult skeleton, bone is continuously being formed and resorbed. This process, called bone remodeling, is accomplished by precise coordination of the activities of two cell types: (1) osteoblasts, which deposit the calcified bone matrix, and (2) osteoclasts, which resorb bone. During bone remodeling, osteoclasts resorb the bone, followed by recruitment of bone marrow mesenchymal stem cells (MSCs) for subsequent bone formation. We have shown that TGFβ1 recruits MSCs to the bone-resorptive sites in the coupling of bone resorption with bone formation. The recruited MSCs at bone resorption sites then undergo differentiation for bone formation. However, the osteogenic nature of the microenvironment at bone-resorptive sites is not known. We found that IGF-1 released from the bone matrix stimulates osteoblast differentiation of marrow MSCs for bone formation during bone remodeling. Mice with deletion of IGF-I receptor (*Igf-Ir–/–*) in the osteoblastic lineage of MSCs exhibited low bone mass and reduced mineral apposition rates, and the MSCs recruited at the bone surface were unable to differentiate into osteoblasts. Moreover, injection of IGF-I with IGF-I-binding protein, not IGF-I alone, increases the level of IGF-I in the bone matrix and stimulates new bone formation in aged rats. Thus, IGF-I stimulates osteoblast differentiation of MSCs for the new bone formation at bone remodeling surface by depositing in the bone matrix. TGFβ1 and IGF-I released during bone remodeling changes bone marrow microenvironment.

PL10

Glucocorticoid-Induced Diabetes is a Bone Disease

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Long-term glucocorticoid treatment is associated with numerous adverse outcomes, such as osteoporosis, weight gain, insulin resistance and diabetes. The pathogenesis of most of these adverse effects remains obscure. It has long been known that even small doses of exogenous glucocorticoids profoundly suppress osteoblast function, and in particular the synthesis and release of osteocalcin, an osteoblast-specific peptide reported to be involved in normal murine fuel metabolism.

Our recent studies in genetically modified mice now demonstrate that osteoblasts, most likely mediated by osteocalcin, have a central role in the pathogenesis of glucocorticoid-induced weight gain, insulin resistance and glucose intolerance. Thus, osteoblast-targeted overexpression of the glucocorticoid-inactivating enzyme, 11β-hydroxysteroid dehydrogenase type-2, and hence the disruption of glucocorticoid signaling, exclusively in murine osteoblasts, were found to attenuate the suppression of osteocalcin synthesis and to prevent the development of insulin resistance, glucose intolerance, dyslipidemia and pathological weight gain in mice treated with high doses of exogenous glucocorticoids. Very similar effects were observed in glucocorticoid-treated wild-type mice, following the heterotopic expression of both carboxylated and uncarboxylated osteocalcin through gene therapy. Hepatic expression of osteocalcin in these mice not only led to a normalization of glucose metabolism and insulin sensitivity, but also to a reduction in hepatic lipid deposition and improved phosphorylation of the insulin receptor. These data demonstrate that the adverse effects of exogenous high-dose glucocorticoids on systemic energy metabolism are mediated, at least in part, through the skeleton.

PL11

New Insight into Control of FGF23 Revealed Through Disorders of Hypophosphatemia

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Fibroblast growth factor-23 (FGF23) is central for the normal regulation of phosphate homeostasis. The Mendelian disorder autosomal dominant hypophosphatemic rickets (ADHR)

is unique among the disorders involving FGF23, as individuals with R176Q/W and R179Q/W mutations in the FGF23 176RXXR179/S180 proteolytic cleavage motif can cycle from unaffected status to delayed onset of disease. These changes from normal to clinically affected status may occur in physiological states associated with iron deficiency, including puberty and pregnancy. To test the role of iron status in development of the ADHR phenotype, wild type (WT) and novel R176QFgf23 knock-in (ADHR) mice were placed on control- or iron-deficient diets. WT mice on low-iron diet maintained normal, serum intact Fgf23 and phosphate metabolism, with elevated serum C-terminal Fgf23 fragments. In contrast, the iron-deficient ADHR mice had elevated intact Fgf23, and C-terminal Fgf23 with hypophosphatemic osteomalacia. To isolate the effects of iron deficiency on Fgf23 expression, iron chelation *in vitro* resulted in a significant increase in Fgf23 mRNA that may rely upon specific transcriptional regulation. Finally, our studies show that age may have an effect on FGF23 processing and stability. Thus, unlike other syndromes of elevated FGF23, these collective findings support the concept that ADHR can be the product of the gene–environment interactions, which have important implications for the normal and pathogenic control of FGF23.

PL12

Therapeutic Challenges in Hypophosphatemic Rickets

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X-linked hypophosphatemia (XLH) was first described as a form of vitamin-D-resistant rickets in the 1930s. It was not until the 1950s that phosphate wasting was identified as the salient pathophysiological feature, which prompted the use of phosphate therapy. This approach was variably successful, but complications, in particular hyperparathyroidism, were common. Therapy was modified to a combination regimen using phosphate together with pharmacological vitamin D. This approach often resulted in vitamin D intoxication. With the later development of activated vitamin D compounds, calcitriol in combination with phosphate have been the most widely accepted form of therapy for XLH since the early 1980s.
Recently, the study of XLH and its related disorders has led to the discovery of a novel homeostatic system by which total body phosphate is regulated. At the core of this system is the novel fibroblast growth factor, FGF23, a unique FGF with endocrine properties. FGF23 leads to reduced expression of type II sodium–phosphate cotransporters in the renal tubule, necessary for reabsorption of phosphate, and to reduced expression of CYP27B1, which encodes the vitamin D 1α-hydroxylase. The excess FGF23 levels (owing to mutations in PHEX in XLH) explain the characteristic hypophosphatemia and inadequate circulating $1,25(OH)_2D$, typical of XLH and its related conditions. Renal tubular-selective action of FGF23 is mediated by the transmembrane protein klotho, an essential coreceptor for FGF23, converting generic FGF receptors to specific FGF23 receptors. Phosphate wasting occurs with activating mutations in FGF23, in a unique mutation that leads to overexpression of klotho, and in other osteocyte genes (for example, *DMP1*) that result in increased FGF23 expression. This novel phosphate regulatory system serves as a mechanism by which the skeleton can communicate mineral abundance or demand to the kidney and, in turn, signal the elimination or retention of phosphate. Thus, FGF23-mediated hypophosphatemia represents an aberration in a novel bone-kidney axis, which regulates the phosphate and vitamin D status.
Simply replacing the phosphate and activated vitamin D does not completely heal rachitic deformities; many patients require surgical correction of the bony abnormalities. The therapy can generate hyperparathyroidism and renal calcinosis. In addition to its complications and burdens, currently available therapy does not address other features of XLH that are debilitating in later life, such as development of osteophytes and calcification of tendons and ligaments, hearing loss and osteoarthritis. The new understanding of the FGF23/vitamin D/phosphate system provides new potential therapeutic targets for XLH.

PL13

Clinical Features and Gene Mutation Analysis of Chinese Patients with Hypophosphatemic Rickets

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Hypophosphatemic rickets (HR) is a dominant disorder of phosphate homeostasis, characterized by growth retardation, rachitic bone disease, hypophosphatemia and renal phosphate wasting. The genes responsible for these disorders were identified as *PHEX* (formerly *PEX*), *FGF23*, *DMP-1* and *SLC34A3*. As HR is an uncommon disease, its clinical features were not well described in literature. Recently, extensive gene mutation analysis has revealed a wide variety of gene defects in HR. The ethnic distribution of the mutations is very broad. Only a few mutations in the Chinese patients were reported previously. To analyze the molecular basis of Chinese patients with HR, we determined the nucleotide sequence of the genes *PHEX* (formerly *PEX*), *FGF-23*, *DMP-1* and *SLC34A3*.
The clinical features showed that 80 patients with HR came from 70 unrelated families, the male-to-female ratio was 33:47 and 30 patients were familial. Their median age at the time of diagnosis was 11 years and the duration of follow-up ranged from 0.1 to 37 years (median: 2.5). The mean birth length of nine patients with related records available was 49.3±2.9 cm. The ricket manifestations, such as lower limb deformities, gait abnormality and growth retard, were all found in patients below the age of 4 years, mostly during the period of learning to walk. Low-trauma fractures (all sites were long bone of extremities) occurred in 7 out of 80 individuals; 70 out of 80 patients had lower limb deformities; among them, 10 patients had ever undergone osteotomies on at least one occasion. Six out of sixteen individuals had experienced dental problems. The height of the group of combination treatment with active VitD metabolite and phosphate (n=9) was significantly higher than that of the untreated group (n=37; –1.71±1.00 and –3.04±1.11, respectively; P<0.05). During the treatment, PTH was markedly elevated to be higher than 150 pg ml^{-1} in 4 out of 26 patients and nephrolithiasis was found in 2 patients.
The *PHEX* gene analysis of the 70 Chinese pedigrees revealed 49 different mutations, including 26 novel mutations and 23

mutations that have been previously described in the literature or entered in PHEXdb database as personal submission.
The phenotype–genotype association study showed no significant relationship between severity of bone deformities or dental problems and gene mutations.
One family of Chinese ethnic with ADHR was diagnosed by molecular genetic analysis. A single heterozygous c.527G>A (p.R176Q) mutation in the *FGF23* gene was detected in three of the family members, including the proband, her brother and their mother.
Typical manifestations of rickets can occur in most patients with HR if effective therapy is not given in time. With age, bone pain and arthralgia can occur more frequently and obviously. The combination therapy with active VitD metabolites and phosphate can be effective to improve the height. We found the *PHEX* gene mutations in 70 Chinese pedigrees with XLH. A family with ADHR carrying a heterozygous c.527G>A (p.R176Q) mutation in the *FGF23* gene was also identified among our HR patients.

PL14

A Novel Bone-Targeting Delivery System Carrying Bone-Forming Phytomolecule Icaritin for Prevention of Osteoporosis

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Our recent multicenter and randomized clinical trial demonstrated both efficacy and safety of a herb Fufang with rich *Epimedium* flavonoids (EF) as the main compound for treatment of postmenopausal osteoporosis, yet it did not show dose dependency, that is, a high dose (12 capsules per day) did not generate additional benefits as compared with a conventional dose (6 capsules per day) used in our clinics. Our recent experimental studies provided an important clue to explain such 'dose-independent' effect, that is, the concentration of bone-forming phytomolecule icaritin, the most potent bioactive metabolite of EF, did not increase in serum of the high-dose group because of the short retention time in circulating blood, where most of the herbal metabolites distributed to liver and then excreted into intestinal track through the bili-hepatobiliary circulation without being utilized to execute its bone-forming effect. To improve the efficiency and efficacy of EF treatment, how to maintain serum concentration of icaritin and its targeting to bone-formation surface is one of the key pharmacokinetic and biotechnological breakthroughs in modern herbal medicine. Our multidisciplinary team has just established a relevant concept and innovative biotechnology to maximize the efficiency of icaritin for bone formation, that is, (1) to maintain its longer retention time in blood circulation and (2) to facilitate targeting icaritin to bone-formation surface. Our team has successfully adopted liposome as a pharmacokinetic strategy to prolong the retention time of icaritin in blood circulation, and has published a novel delivery system, that is, aspartate–serine–serine $(DSS)_6$ liposome system, targeting bone-formation surfaces to facilitate anabolic therapy. Accordingly, we developed our novel injectable $(DSS)_6$–liposome–icaritin therapy to maintain bioactive and bone-forming phytomolecule icaritin in blood circulation for longer time, with or without less biliary excretion of icaritin into the intestinal tract and facilitate icaritin targeting to bone-formation surface for effective prevention of ovariectomy (OVX)-induced osteoporosis.

PL15

New Insight on FGFR3-Related Chondrodysplasias

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There are 23 kinds of fibroblast growth factors (FGFs). FGFs execute their function through binding FGF receptors (FGFR1–4). The major downstream signaling pathways of FGF/FGFR signaling include MAPK, PLCγ, Stats and so on. Mutations in FGFRs have been found to cause 15 kinds of human skeletal genetic diseases. Indeed, both gain-of-function and loss-of-function mutations in FGFR1, -2 and -3 can lead to multiple skeletal genetic diseases in humans, including craniosynostosis, achondroplasia (ACH), CATSHL syndrome and so on.
During the limb bud development, FGFR3 is first expressed in chondrocytes differentiated from the center core of the condensed mesenchymal cells. During late bone development, FGFR3 is mainly expressed in the resting and proliferative zones of the growth plates. In addition, FGFR3 also has mild expressions in osteoblasts and osteoclasts. In 1994, Shiang found that point mutation of FGFR3 lead to ACH, and subsequently several other point mutations of FGFR3 were found to lead to ACH, hypochondroplasia and TD (Thanatophoric dysplasia).
ACH is the most common form of human short-limb dwarfism. ACH patients have short stature, especially short upper and lower limbs. Moreover, ACH patients exhibit central facial dysplasia and dome-shaped skull. TD patients have more severe clinical manifestations than ACH. The development of their rib cartilage is severely hampered, resulting in narrow rib cage and insufficient oxygen exchange; therefore, most TD patients died at perinatal period. In 2006, Toydemir reported that R621H mutation of FGFR3 can lead to partial loss-of-function of FGFR3, resulting in the CATSHL syndrome in which patients have tall stature, scoliosis and hearing loss.
Point mutations of FGFR3 leading to retarded skeletal development can occur at the extracellular, transmembrane or the intracellular domains; all these mutations cause enhanced activity of FGFR3. The severity of dwarf phenotype is associated with the level of FGFR3 activation, that is, stronger activation of FGFR3 caused is associated with more severe dwarf phenotypes. Patients with TD normally will die at perinatal period. To study the role of FGFR3 in skeletal development and related human skeletal dysplasia, and to find measures to prevent or cure these diseases, researchers have generated a variety of FGFR3-related mouse models using gene knockout (knockin) technology.
The FGFR3 knockout mice have bone overgrowth, wider proliferative and hypertrophic zones of growth plates, and increased chondrocyte proliferation, mimicking patients with the CATSHL syndrome. Besides, mice mimicking human ACH have smaller body size, shortened round head, retarded long bones, decreased chondrocyte proliferation activity, reduced

hypertrophic zone and less expression of collagen X, which suggest that gain-of-function mutations of FGFR3 lead to inhibited chondrocyte proliferation and differentiation.
Molecular mechanism studies have found that activation of FGFR3 cause inhibited chondrocyte proliferation and differentiation through upregulation of several signaling pathways, including MAPK, cell cycle inhibitors and Stat1. In addition, FGFR3 can regulate cartilage development by regulating the expressions of the molecules related to cartilage development, including Ihh, PTHrPR, BMP and CNP.
Meanwhile, notable progresses have been made in finding the treatment for FGFRs-related skeletal dysplasias, including ACH. This presentation will focus on the molecular and cellular mechanisms of the role of FGFR3 in bone development and FGFR3-related skeletal diseases, the latest progress of targeted therapy for ACH.

PL16
The Role of TGF-β Signaling in the Development of Osteoarthritis
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Osteoarthritis (OA) is a common, joint degenerative disease affecting more than 25% of people over the age of 18 years. The pathogenesis of OA is poorly understood. Several lines of evidence suggest that the TGF-β/Smad3 pathway has a critical role in OA development. However, the downstream target gene(s) for this signaling pathway during OA development remains unknown. In this study, we investigated the role of TGF-β signaling in OA development at postnatal/adult stage by generating chondrocyte-specific type II TGF-β receptor (*Tgfbr2*) conditional knockout (cKO) mice. In 3- and 6-month-old *Tgfbr2* cKO mice, a severe and progressive OA-like phenotype was observed, including articular chondrocyte hypertrophy, tears and clefts in the articular cartilage surface, severe loss of articular cartilage tissue, osteophyte formation and subchondral sclerosis. Histomorphometric analysis confirmed that articular cartilage area on both tibial plateau and distal femur were significantly reduced in *Tgfbr2* cKO mice. *Mmp13*, *Adamts5* and *ColX* expression was significantly increased in articular chondrocytes derived from *Tgfbr2* cKO mice. Through a series of *in vitro* studies, we demonstrated that inhibition of TGF-β signaling upregulates expression of *Mmp13* and *Adamts5* in a Runx2-dependent manner. Although TGF-β treatment significantly downregulated *Mmp13* transcription by over 80%, mutation of the Runx2-binding site in the proximal region of the *Mmp13* promoter strongly reversed the inhibitory effect of TGF-β on *Mmp13* promoter activity. These results indicate that TGF-β signaling represses *Mmp13* expression through Runx2. The ChIP assay further confirmed Runx2 binding to the *Mmp13* promoter in RCS chondrogenic cells. To determine if *Mmp13* and *Adamts5* are critical target genes of TGF-β signaling in articular chondrocytes, we generated *Tgfbr2*/*Mmp13* and *Tgfbr2*/*Adamts5* double KO mice. Results from histological analysis of these mice demonstrated that deletion of the *Mmp13* or *Adamts5* gene under the *Tgfbr2* cKO background significantly reversed articular cartilage defects observed in *Tgfbr2* cKO mice. Histomorphometric analysis demonstrated that articular cartilage areas were significantly increased in 3- and 6-month-old *Tgfbr2*/*Mmp13* double KO mice and 3-month-old *Tgfbr2*/*Adamts5* double KO mice compared with the *Tgfbr2* cKO mice. In addition, subchondral sclerosis and osteophyte formation phenotype was also completely restored in 3- and 6-month-old *Tgfbr2*/*Mmp13* double KO mice and 3-month-old *Tgfbr2*/*Adamts5* double KO mice. These findings indicate that *Mmp13* and *Adamts5* are the key downstream targets of TGF-β signaling in OA development. Further studies demonstrated that deletion of the *Mmp13* gene in *Mmp13* cKO mice significantly reduced cartilage degeneration at 8, 12 and 16 weeks post-meniscus injury compared with the control group, which exhibited OA-like fibrillation, clefting and cartilage loss down to the tidemark 8 weeks post surgery. The cartilage area (tibia and femur) was significantly increased 16 weeks post surgery in *Mmp13* cKO group. Finally, histological and histomorphometric analyses revealed that OA progression, especially articular cartilage degradation, was decelerated with injection of 1, 5 and 10 mg kg^{-1} of an MMP13 inhibitor, particularly with the 10 mg kg^{-1} dose. Comparing with the saline injection group, total cartilage area was increased to 21, 19 and 38%, with the treatment of 1, 5 and 10 mg kg^{-1} of MMP13 inhibitor, respectively. Our studies provide significant insights into the mechanism of OA development induced by TGF-β signaling inhibition.

PL17
Systematic Solutions for Pedicle Screw Implantation in Osteoporotic Spine Surgery
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In China, there are more than one million patients receiving pedicle screw implantations to treat spinal diseases every year. Among them, many elderly patients have complications owing to osteoporosis. The screw-loosening rate in osteoporotic spinal patients was estimated as high as 20%. Many of the screw-loosening procedure need reoperations. Thus, how to decrease the risks of loosening the screws has become urgent and of much importance for the spinal surgeons. The aim of this study was to establish systematic solutions for pedicle screw implantation in osteoporotic spine surgery and to propose a guideline for the surgeons. To solve this problem, osteoporotic animal and specimen models were first established by OVX and decalcification. Second, new devices, including expandable pedicle screw, cannulated screw tap and so on, were invented and were tested *in vitro* and *in vivo*. According to the enhancement of fixation strength by different methods, a systematic solution for pedicle screw implantation in osteoporotic spine surgery was proposed. The newly invented devices and methods, including expandable pedicle screw, cannulated screw tap, partial augmentation and vertebrae augmentation, were able to increase the fixation strength of the screws in the osteoporotic spine.
Patients with mild osteoporosis can use expandable pedicle screw; patients with moderate osteoporosis can use expandable pedicle screw combined with track partial augmentation; and patients with severe osteoporosis should use expandable pedicle screw combined with vertebroplasty. The rate of screw

loosening can decrease with 0.5% by using these methods. The customized methods for pedicle screw implantation significantly decreased the rate of screw loosening. According to the degree of osteoporosis, different patients should use different methods to increase screw stablility and decrease medical expenses in the meanwhile.

PL18
DXA beyond the BMD
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The primary use of dual-energy X-ray absorptiometry (DXA) systems is to measure bone mineral density to diagnose and monitor osteoporosis. However, there are applications beyond bone density that take advantage of the very precise and accurate quantification of soft tissue masses. The objective of our research group is to extend the utility of DXA into other diseases primarily described as wasting of the functional lean tissues (sarcopenia, cachexia, HIV, protein energy wasting and so on) and diseases, which create distinct patterns in the regional distribution of fat and lean masses. We have developed several novel algorithms applicable to whole body DXA scans from either Hologic or GE systems. First, we have derived a method to quantify regional volumes of the arms, legs and trunk, and have shown that these volumes are accurate to independent measures of the total body volume by air-displacement plethysmography (r^2=0.99, RMSE <0.5 l). Second, we have used the ratio of the trunk to leg (T:L) volumes by DXA as a predictor of metabolic diseases. In a fully adjusted model, the T:L volume ratio was the strongest risk factor for prevalent diabetes with OR=2.3/s.d. and sixfold increase in risk from the fourth to the first quartile in a 9000-person observation study in the United States. (AUC=0.84). High T:L volume was also highly associated with high triglycerides (1.8/s.d.), blood pressure (1.3/s.d.), and low HDL cholesterol (1.6/s.d.).
The T:L volume ratios were highest in men, older adults, and white and Mexican Americans. Third, we show how the DXA total body volume can be combined with simple measures of total body water from bioelectrical impedance to derive an accurate estimate of total body protein, the functional mass of muscle and lean tissue. In a validation study of 192 outpatients, DXA protein was related to protein by neutron activation analysis (NAA) by NAAprotein=1.04×DXAprotein (r^2=0.87, RMSE=0.87 kg). Fourth, DXA can also be used to derive the composition of muscle and visceral adipose tissue (VAT) using algorithms that subtract off the contributions of subcutaneous adipose. The association between DXAVAT and CTVAT has been found to be r^2>0.80. Last, dedicated breast scans using DXA have been used to quantify breast density, an important risk factor for breast cancer risk, with the primary application being studies in young girls. In summary, this is an interesting time for DXA research owing to DXA's extensive and novel applications beyond bone densitometry.