

NOT TO BE MISSED

Clinical and Basic Research Papers – February 2010

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Clinical Studies and Drug Effects

◆Cosman F, Lane NE, Bolognese MA, Zanchetta JR, Garcia-Hernandez PA, Sees K, Matriano JA, Gaumer K, Daddona PE. Effect of transdermal teriparatide administration on bone mineral density in postmenopausal women. *J Clin Endocrinol Metab.* 2010 Jan;95(1):151-8. [\[Abstract\]](#)

This multicenter, randomized, placebo-controlled, positive control (TPTD, 20 µg/d, sc), multidose, daily administration assay of synthetic TPTD-coated microneedle patches shows new promise for a more friendly delivery of PTH(1-34). In postmenopausal women with osteoporosis, the transdermal patch produced a PK profile that was actually sharper than sc, resulting in a dose-dependent gain of BMD at the spine and total hip over 6 months. Interestingly, though, bone turnover markers showed very little changes as compared to sc TPTD. Changes in serum calcium were similar between patch and sc groups. The patch causes a mild to moderate erythema in all subjects. —SF

◆Yadav VK, Balaji S, Suresh PS, Liu XS, Lu X, Li Z, Guo XE, Mann JJ, Balapure AK, Gershon MD, Medhamurthy R, Vidal M, Karsenty G, Ducy P. Pharmacological inhibition of gut-derived serotonin synthesis is a potential bone anabolic treatment for osteoporosis. *Nat Med.* 2010 Feb 7. [Epub ahead of print] [\[Abstract\]](#)

This is an unusual yet remarkable paper from Dr. Karsenty's group. Unusual because it does not use a genetic approach, but a bona fide pharmacological approach to test the skeletal effects of inhibiting gut serotonin. As a reminder, this group previously demonstrated that LRP5 regulates Tph1, the initial enzyme in the synthesis of gut serotonin. Here they used an experimental oral Tph1 inhibitor in mice and rats. They repeatedly demonstrate that decreasing circulating serotonin levels markedly improves osteoblast number, bone formation indices, and trabecular and cortical microstructure, and femur strength when the Tph1 inhibitor is administered at various times after ovariectomy (OVX). Of note, rodents were young at the time of OVX (the mice were 6 weeks of age, the rats were 12 weeks of age), hence the effects of serotonin inhibition at older ages, when the osteogenic potential of MSCs is less, remains to be established. —SF

Genetics

◆Dean AK, Harris SE, Kalajzic I, Ruan J. A systems biology approach to the identification and analysis of transcriptional regulatory networks in osteocytes. *BMC Bioinformatics.* 2009 Sep 17;10 Suppl 9:S5. [\[Abstract\]](#)

Osteocytes, the most abundant cells in bone, have recently been shown to be responsible for mechanotransduction. However, it is still poorly understood how osteocytes receive and translate mechanical stimulus information. This group studied osteocyte gene expression profiles. Among the 269 overexpressed osteocyte-specific genes, there were many genes and transcription factors known to control muscle differentiation and contractility. Using bioinformatic methods, the investigators proposed a regulatory network model, which showed that many osteocyte-specific genes, including two well-known osteocyte markers, DMP1 and SOST, had highly conserved clustering of cis-regulatory modules such as muscle-related Mef2c and myogenin. This thought-provoking finding thus supports the concept that the same gene network may have a role in muscle contractility, dynamic movements of the osteocyte, and probably osteocyte mechanosensitivity. —DK

◆ Ermakov S, Toliat MR, Cohen Z, Malkin I, Altmüller J, Livshits G, Nürnberg P. Association of ALPL and ENPP1 gene polymorphisms with bone strength related skeletal traits in a Chuvashian population. *Bone*. 2009 Nov 19. [Epub ahead of print]. [\[Abstract\]](#)

◆ Cheung CL, Livshits G, Zhou Y, Meigs JB, McAteer JB, Florez JC, Cupples LA, Demissie S, Kiel DP, Karasik D. Hip geometry variation is associated with bone mineralization pathway gene variants: the Framingham Study. *J Bone Miner Res*. 2009 Nov 5. [Epub ahead of print] [\[Abstract\]](#)

Regulation of inorganic phosphate (Pi) and inorganic pyrophosphate (PPi) is essential to maintain proper bone mineralization. In two independent studies, genes coding central regulators of phosphate balance, namely alkaline phosphatase (ALPL) and ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), were studied. Thus, in the paper from Ermakov et al., 40 SNPs in ALPL and 14 SNPs in ENPP1 were tested for association with radiographic bone size and hand BMD. Results suggest that both the ALPL and ENPP1 genes harbor polymorphisms affecting bone size. In the study from Cheung et al., modest associations were observed between SNPs in or near ALPL and several DXA-derived bone traits; however, the authors found strong association of ENPP1 with several hip geometric indices, and predicted that there exists a potential binding site for the transcription factor HOXA7. Taken together, these 2 studies attest to the merits of a biological candidate gene approach and prioritize the phosphate-regulator genes for functional studies. —SF

◆ Ichikawa S, Koller DL, Padgett LR, Lai D, Hui SL, Peacock M, Foroud T, Econs MJ. Replication of previous genome-wide association studies of bone mineral density in premenopausal American women. *J Bone Miner Res*. In press.

◆ Zhang YP, Deng FY, Chen Y, Pei YF, Fang Y, Guo YF, Guo X, Liu XG, Zhou Q, Liu YJ, Deng HW. Replication study of candidate genes/loci associated with osteoporosis based on genome-wide screening. *Osteoporos Int*. In press.

These 2 papers attempted to replicate results of three recent genome-wide association studies (GWAS) of BMD, published during 2007-2008. Both used samples of US adults. Thus, Zhang et al. found, in white adults (average age of 50 yrs), associations of BMD with NR5A2 and LRP5, and with/around the ESR1 gene. Ichikawa et al. confirmed association of the ESR1 region with BMD, and also provided suggestive evidence for CTNBL1 and LRP5. The latter study is also remarkable due to its sample of premenopausal black women, therefore extending the associations found in the original GWAS to younger-age and non-Caucasian subjects. —DK

◆ Lee YH, Woo JH, Choi SJ, Ji JD, Song GG. Associations between osteoprotegerin

polymorphisms and bone mineral density: a meta-analysis. *Mol Biol Rep.* 2010 Jan;37(1):227-34. [\[Abstract\]](#)

◆Jurado S, Nogués X, Agueda L, Garcia-Giralt N, Urreizti R, Yoskovitz G, Pérez-Edo L, Saló G, Carreras R, Mellibovsky L, Balcells S, Grinberg D, Díez-Pérez A. Polymorphisms and haplotypes across the osteoprotegerin gene associated with bone mineral density and osteoporotic fractures. *Osteoporos Int.* 2010 Feb;21(2):287-96. [\[Abstract\]](#)

Two studies analyzed associations of polymorphisms in the osteoprotegerin gene (TNFRSF11B, or OPG) with BMD. Thus, in a meta-analysis from Lee et al., the OPG polymorphism rs2073618 (G1181C) was examined in eight studies, representing European ancestry (US, Spain, Norway, and Denmark) and Asian ancestry (Korea, China, and Japan) populations. This SNP was found to be associated with lumbar (LS) BMD in both Caucasians and Asians, and with femoral neck (FN) BMD in Caucasians. Furthermore, in 964 postmenopausal Spanish women in the study from Jurado et al., another SNP was associated with BMD, as well as with osteoporotic fractures. These two studies therefore substantiate results of the recent GWAS meta-analysis by GEFOS, which showed the osteoprotegerin gene's association with both LS and FN BMD. —DK

◆Wilson SG, Jones MR, Mullin BH, Dick IM, Richards JB, Pastinen TM, Grundberg E, Ljunggren O, Surdulescu GL, Dudbridge F, Elliott KS, Cervino AC, Spector TD, Prince RL. Common sequence variation in FLNB regulates bone structure in women in the general population and FLNB mRNA expression in osteoblasts in vitro. *J Bone Miner Res.* 2009 Dec;24(12):1989-97. [\[Abstract\]](#)

◆Li GH, Kung AW, Huang QY. Common variants in FLNB/CRTAP, not ARHGEF3 at 3p, are associated with osteoporosis in southern Chinese women. *Osteoporos Int.* 2009 Sep 1. [Epub ahead of print] [\[Abstract\]](#)

Chromosomal region 3p14-25 has been shown to be linked to BMD in multiple cohorts. One gene in the region, filamin B (FLNB), is known for being involved in several rare human skeletal disorders such as atelosteogenesis, spondylo-carpo-tarsal syndrome, and Larsen's syndrome. Two studies explored positional candidate genes in FLNB. In the study by Wilson et al., 13 common variants were tested in 767 female sibs from the UK (Discovery cohort) for association with BMD. Four SNPs associated with BMD were replicated in 2 additional female samples, of younger UK twins and older Australians (in the latter sample, also with prevalent fractures). Importantly, the study estimated SNP associations with mRNA expression in human osteoblast cell lines.

In addition to this study of Caucasian women, 7 common SNPs were tested in adult southern Chinese women in the study by Li et al., and some were significantly associated with BMD at several skeletal sites. These independent studies promote the FLNB gene as a novel candidate gene, which is biologically relevant for osteoporosis and musculoskeletal health: it is involved in actin binding, and thus is active in skeletal muscle development and cytoskeletal functions. Finally, the gene seems to be pleiotropic, as it was recently associated with adult stature. —DK

◆Zhang L, Guo YF, Liu YZ, Liu YJ, Xiong DH, Liu XG, Wang L, Yang TL, Lei SF, Guo Y, Yan H, Pei YF, Zhang F, Papasian CJ, Recker RR, Deng HW. Pathway-based genome-wide association analysis identified the importance of regulation-of-autophagy pathway for ultradistal radius BMD. *J Bone Miner Res.* 2010 Jan 29. [Epub ahead of print]

◆Chen Y, Xiong DH, Guo YF, Pan F, Zhou Q, Zhang F, Deng HW. Pathway-based genome-wide

association analysis identified the importance of EphrinA–EphR pathway for femoral neck bone geometry. *Bone*. 2010 Jan;46(1);129-36.

In these 2 papers, Dr. Deng's group used a pathway-based analysis approach to analyze genome-wide association study data in a novel way. Instead of focusing only on top-ranking polymorphisms/genes and considering each of these SNPs/genes independently, the authors applied multiple genetic pathways, pre-defined by bioinformatic methods, to the association results of ~1000 US Caucasians. 963 tested pathways were narrowed down to several "candidate" pathways. Thus, for ultradistal radius BMD, they pinpointed a regulation-of-autophagy (ROA) pathway, and for the femoral neck section modulus, an EphrinA-EphR pathway. (Notably, not one of the ephrin/ephrin receptor pathway genes attained association significance). In both studies, results of the discovery cohort were further confirmed using the publicly available Framingham Osteoporosis Study dataset. The authors hypothesize that autophagy might be involved in endochondral ossification at the wrist; it is less clear what role the EphrinA-EphR pathway genes may play in bone biology. Therefore, functional evaluation of these pathways is awaited, to provide new insights into the mechanisms underlying statistical associations. —DK

Bone Modeling, Remodeling, and Repair

◆Aleksyniene R, Thomsen JS, Eckardt H, Bundgaard KG, Lind M, Hvid I. Parathyroid hormone PTH(1-34) increases the volume, mineral content, and mechanical properties of regenerated mineralizing tissue after distraction osteogenesis in rabbits. *Acta Orthop*. 2009 Dec;80(6):716-23. [\[Abstract\]](#)

This study shows very large differences in callus size, strength and energy absorption achieved with 25 µg/kg/day of PTH(1-34). No real difference was found if PTH was started before or after distraction. This study thus documents a very good effect but the dose remains about 70 times that of a 20 µg/day dose in humans, begging the point of relevance to translation. —DGL

◆Baldock PA, Lee NJ, Driessler F, Lin S, Allison S, Stehrer B, Lin EJ, Zhang L, Enriquez RF, Wong IP, McDonald MM, During M, Pierroz DD, Slack K, Shi YC, Yulyaningsih E, Aljanova A, Little DG, Ferrari SL, Sainsbury A, Eisman JA, Herzog H. Neuropeptide Y knockout mice reveal a central role of NPY in the coordination of bone mass to body weight. *PLoS One*. 2009 Dec 22;4(12):e8415. [\[Abstract\]](#)

Leptin inhibits bone formation via a hypothalamic relay. Leptin reduces NPY in the hypothalamus, and NPY affects bone formation via the Y2 receptor in the hypothalamus. In this study, NPY(-/-) mice exhibit increased bone mass with enhanced osteoblast activity, while NPY(-/-) mice with over-expression of NPY in the hypothalamus (AAV-NPY+) show a reduction of bone mass with obesity. Thus, bone responds to the low energy state, i.e., high hypothalamic NPY levels, independent of body mass. These results are consistent with the notion that NPY is another important regulator of bone homeostasis, by increasing bone formation during the obese condition with low hypothalamic NPY, and by reducing bone formation under starving conditions with high hypothalamic NPY levels. —TM

◆Brouwers JE, van Rietbergen B, Ito K, Huiskes R. Effects of vibration treatment on tibial bone of ovariectomized rats analyzed by in vivo micro-CT. *J Orthop Res*. 2010 Jan;28(1):62-9. [\[Abstract\]](#)

Whole-body vibration (WBV) has been shown to affect rodent bone. However, effects

over time on 3D bone microstructure in ovariectomized (OVX) animals have not been documented. Eight weeks post-OVX, rats were placed on a vibrating platform (Juvent) for 20 minutes at 0.3 g and 90 Hertz. Frequency of treatment was twice per day, 5 days a week for 6 weeks. *In vivo* μ -CT showed no changes in the metaphysis between 8 to 12 weeks after OVX in the treatment group, compared to the OVX group, with both groups showing continued deterioration of bone structure. WBV had no effects on mechanical testing results. The absence of an effect of the Juvent vibrating platform in the presence of OVX needs confirmation from larger studies. —DGL

- ◆Lynch MA, Brodt MD, Silva MJ. Skeletal effects of whole-body vibration in adult and aged mice. *J Orthop Res*. 2010 Feb;28(2):241-7. [[Abstract](#)]

75 male BALB/c mice (7-month-old/young adult; 22-month-old/aged) were subjected to 5 weeks of daily WBV (15 min/day, 5 day/wk; 90 Hz sine wave) at acceleration amplitudes of 0 (sham), 0.3, or 1.0 g. Whole-body bone mineral content (BMC) increased with time in 7-month-old ($p < 0.001$) but not 22-month-old ($p = 0.34$) mice, regardless of exposure to WBV. Lower-leg BMC increased with time in the 0.3 g and 1.0 g groups ($p < 0.005$) but not in the sham group ($p = 0.09$), indicating a positive WBV loading effect in the limbs. However, in aged 22-month-old mice, there were no changes with time in lower-leg BMC ($p = 0.11$). WBV did not affect tibial trabecular or cortical bone structure as assessed by μ CT. Effects of WBV on aged bone are not supported in this study. —DGL

- ◆Walker EC, McGregor NE, Poulton IJ, Solano M, Pompolo S, Fernandes TJ, Constable MJ, Nicholson GC, Zhang JG, Nicola NA, Gillespie MT, Martin TJ, Sims NA. Oncostatin M promotes bone formation independently of resorption when signaling through leukemia inhibitory factor receptor in mice. *J Clin Invest*. 2010 Jan 4. [Epub ahead of print] [[Abstract](#)]

The authors report that oncostatin M (OSM) is produced by osteoblasts and osteocytes in mouse bone, and that it has distinct effects when acting through the OSM receptor (OSMR) and the leukemia inhibitory factor receptor (LIFR). When acting through LIFR, mouse OSM (mOSM) inhibits sclerostin production in osteocytes and enhances bone formation in *Osmr*^{-/-} mice. In contrast, when acting through OSMR in osteoblasts, mOSM stimulates RANKL production and osteoclast formation, promotes osteoblast differentiation and inhibits adipocyte differentiation. Mice lacking OSMR exhibit osteopetrosis, suggesting a key role for OSMR in bone resorption. These data provide new insights into OSMR and LIFR signaling, and may open up a new therapeutic approach to osteoporosis by selectively modulating LIFR and OSMR signaling. —TM

- ◆Yang P, Jia B, Ding C, Wang Z, Qian A, Shang P. Whole-body vibration effects on bone before and after hind-limb unloading in rats. *Aviat Space Environ Med*. 2009 Feb;80(2):88-93. [[Abstract](#)]

In non-aged rats, after 28 days of hind-limb unloading (HLU), WBV reduced the losses of BMD in the femur from 18.8 to 10.1%, and in the tibia from 16.7 to 7.1%. WBV had no effect on the lumbar spine. In the recovery period following HLU, there were no significant effects of WBV on BMD. —DGL

Molecular and Cell Biology

- ◆Grigoriadis AE, Kennedy M, Bozec A, Brunton F, Stenbeck G, Park IH, Wagner EF, Keller GM. Directed differentiation of hematopoietic precursors and functional osteoclasts from human ES and iPS cells. *Blood*. 2010 Jan 11. [Epub ahead of print]

This paper reports the step-wise generation of bone-resorbing osteoclasts from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). Generation of a primitive streak-like population in embryoid bodies, followed by specification to hematopoiesis and myelopoiesis by VEGF and hematopoietic cytokines in serum-free media, yielded a precursor population enriched for cells expressing monocyte-macrophage lineage markers. These precursors formed large, multinucleated osteoclasts in monolayer cultures with M-CSF and RANKL. Generation of hematopoietic and osteoclast populations from hESCs and iPSCs will become a valuable tool in understanding bone development and coping with bone diseases.

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- ◆ Qiu T, Wu X, Zhang F, Clemens TL, Wan M, Cao X. TGF-beta type II receptor phosphorylates PTH receptor to integrate bone remodelling signalling. *Nat Cell Biol.* 2010 Feb 7. [Epub ahead of print] [[Abstract](#)]

PTH-induced activation of the PTH receptor induces receptor autophosphorylation and endocytosis, thereby regulating PTH signaling (see [BoneKEy Perspective, Dec. 2009](#)). The cross-talk of PTH receptor-mediated signaling with other receptor pathways, including tyrosine receptor kinases and LRP5, is being increasingly recognized. Here it is shown that TGF- β 2 receptor activation transphosphorylates the PTH receptor and the receptors are co-internalized. Disruption of TGF- β in osteoblasts in mice increased the cell-surface expression of PTH1R, increasing trabecular and decreasing cortical bone mass, which was prevented in double KO mice. This could be one of the mechanisms by which bone formation and resorption – which releases TGF- β from bone matrix – are coupled. —SF

Reviews, Perspectives and Editorials

- ◆ Compston J. Management of glucocorticoid-induced osteoporosis. *Nat Rev Rheumatol.* 2010 Feb;6(2):82-8. [[Abstract](#)]

Other Studies of Potential Interest

- ◆ Binkley N, Buehring B. Beyond FRAX: it's time to consider "sarco-osteopenia". *J Clin Densitom.* 2009 Oct-Dec;12(4):413-6. [[Abstract](#)]

- ◆ Klenk C, Schulz S, Calebiro D, Lohse MJ. Agonist-regulated cleavage of the extracellular domain of parathyroid hormone receptor type 1. *J Biol Chem.* 2010 Jan 15. [Epub ahead of print]

- ◆ Piters E, Balemans W, Nielsen TL, Andersen M, Boudin E, Brixen K, Van Hul W. Common genetic variation in the DKK1 gene is associated with hip axis length but not with bone mineral density and bone turnover markers in young adult men: results from the Odense Androgen Study. *Calcif Tissue Int.* 2010 Jan 26. [Epub ahead of print] [[Abstract](#)]

- ◆ Richards JB, Waterworth D, O'Rahilly S, Hivert MF, Loos RJ, Perry JR, Tanaka T, Timpson NJ, Semple RK, Soranzo N, Song K, Rocha N, Grundberg E, Dupuis J, Florez JC, Langenberg C, Prokopenko I, Saxena R, Sladek R, Aulchenko Y, Evans D, Waeber G, Erdmann J, Burnett MS, Sattar N, Devaney J, Willenborg C, Hingorani A, Witteman JC, Vollenweider P, Glaser B, Hengstenberg C, Ferrucci L, Melzer D, Stark K, Deanfield J, Winogradow J, Grassl M, Hall AS, Egan JM, Thompson JR, Ricketts SL, König IR, Reinhard W, Grundy S, Wichmann HE, Barter P, Mahley R, Kesaniemi YA, Rader DJ, Reilly MP, Epstein SE, Stewart AF, Van Duijn CM, Schunkert H, Burling K, Deloukas P, Pastinen T, Samani NJ, McPherson R, Davey Smith G, Frayling TM, Wareham NJ, Meigs JB, Mooser V, Spector TD; GIANT Consortium. A genome-

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wide association study reveals variants in ARL15 that influence adiponectin levels. *PLoS Genet.* 2009 Dec;5(12):e1000768. [\[Abstract\]](#)

Conflict of Interest: Dr. Ferrari reports that he receives research support from Amgen and is an advisory committee member and lectures occasionally at conference symposia for Merck Sharp & Dohme, the Alliance for Better Bone Health (sanofi aventis/P&G), Amgen, Eli Lilly (Switzerland), Servier (Switzerland), and Novartis (Switzerland). Dr. Little reports that he receives royalties, research funds and consultancy fees from Novartis Pharma, as well as research support from Stryker Biotech. Dr. Seeman reports that he is an advisory committee member for sanofi-aventis, Eli Lilly, Merck Sharp & Dohme, Novartis, and Servier, and that he lectures occasionally at conference symposia for those companies. Dr. Matsumoto and Dr. Karasik report no conflicts of interest.