

## **NOT TO BE MISSED**

### **Clinical and Basic Research Papers – March 2009**

**Serge Ferrari, Editor-in-Chief**  
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**Hong-Wen Deng, Associate Editor**  
**David G. Little, Associate Editor**  
**Toshio Matsumoto, Associate Editor**

#### **Bone Modeling, Remodeling and Repair**

◆Pennypacker B, Shea M, Liu Q, Masarachia P, Saftig P, Rodan S, Rodan G, Kimmel D. Bone density, strength, and formation in adult cathepsin K (-/-) mice. *Bone*. 2009 Feb;44(2):199-207. [\[Abstract\]](#)

*Detailed analysis of the skeletal phenotype of CatK null mice reveals a 3- to 4-fold increase in osteoclast numbers on bone surfaces and elevated surface-based and total tissue-based bone formation rates (BFR), with a normal bone volume-based BFR. Hence decreased bone resorption in this model elicits some stimuli or prevents some inhibitory signals for OC proliferation and/or survival and does not reduce bone formation, thereby dissociating the cellular function of osteoclasts and matrix digestion from the coupling to osteoblasts. —SF*

#### **Clinical Studies and Drug Effects**

◆Cauley JA, Lui LY, Barnes D, Ensrud KE, Zmuda JM, Hillier TA, Hochberg MC, Schwartz AV, Yaffe K, Cummings SR, Newman AB; SOF Research Group. Successful skeletal aging: a marker of low fracture risk and longevity. The Study of Osteoporotic Fractures (SOF). *J Bone Miner Res*. 2009 Jan;24(1):134-43. [\[Abstract\]](#)

*This is an interesting study because it suggests there is a group of women who lose little or no bone with age. If this is the case, then the pathogenetic mechanisms need to be studied. Cauley et al. report that out of 8,224 subjects measured over 15 years, about 10% lost little or no BMD and these subjects exhibited a 20% lower risk of nonspine fracture and a 67% lower risk of hip fracture. Mortality risks were also lower. —ES*

◆Chlebowski RT, Kuller LH, Prentice RL, Stefanick ML, Manson JE, Gass M, Aragaki AK, Ockene JK, Lane DS, Sarto GE, Rajkovic A, Schenken R, Hendrix SL, Ravdin PM, Rohan TE, Yasmeen S, Anderson G; WHI Investigators. Breast cancer after use of estrogen plus progestin in postmenopausal women. *N Engl J Med*. 2009 Feb 5;360(6):573-87. [\[Abstract\]](#)

*Following the release of the 2002 report of the Women's Health Initiative (WHI) trial of estrogen plus progestin, the use of menopausal hormone therapy decreased substantially. The incidence of breast cancer also dropped thereafter, suggesting a cause-and-effect relationship between hormone treatment and breast cancer. However, the cause of this decrease remained controversial. This study demonstrates that the increased risk of breast cancer associated with the use of estrogen plus progestin declined markedly soon after discontinuation of combined hormone therapy and was unrelated to changes in frequency of mammography. —TM*

- ◆Gnant M, Mlineritsch B, Schippinger W, Luschin-Ebengreuth G, Pöstlberger S, Menzel C, Jakesz R, Seifert M, Hubalek M, Bjelic-Radisic V, Samonigg H, Tausch C, Eidtmann H, Steger G, Kwasny W, Dubsy P, Fridrik M, Fitzal F, Stierer M, Rücklinger E, Greil R; ABCSG-12 Trial Investigators, Marth C. Endocrine therapy plus zoledronic acid in premenopausal breast cancer. *N Engl J Med*. 2009 Feb 12;360(7):679-91. [\[Abstract\]](#)

*Zoledronate (4 mg every 6 months) in addition to ovarian suppression by goserelin plus tamoxifen or an aromatase inhibitor, anastrozole, given for 4 years to pre-menopausal women with ER+ breast cancer, significantly improved disease-free survival (RRR, 36%) and non-significantly reduced the risk of death compared to endocrine therapy alone. Arthralgias, bone pain and fever were more common in the zoledronate groups, but no ONJ or serious renal toxicity were confirmed. Is this the basis for a revival of bisphosphonates in cancer treatment strategies? —SF*

- ◆Theman T, Collins M, Dempster D, Zhou H, Reynolds J, Brahim J, Roschger P, Klaushofer K, Winer K. PTH 1-34 replacement therapy in a child with hypoparathyroidism due to a sporadic calcium receptor mutation. *J Bone Miner Res*. 2008 Dec 8. [Epub ahead of print] [\[Abstract\]](#)

*A 20-year-old female with autosomal dominant hypocalcemia (ADH) treated with PTH 1-34 continuously since age 6 is presented. Hypercalciuria and hypermagnesuria persisted despite normal or subnormal serum calcium and magnesium levels. Nephrocalcinosis developed by age 19. Cancellous bone volume was dramatically elevated but a bone mineralization defect was not improved. Thus, 14 years of PTH replacement in a child with ADH increased bone mass with little impact on mineralization, improved serum mineral control, but did not prevent nephrocalcinosis. This report demonstrates that PTH 1-34 replacement cannot reverse all the abnormalities in ADH, and gives important clues for the role of the CaR in bone mineralization and renal calcium/magnesium handling. —TM*

- ◆Kostenuik P, Nguyen H, McCabe J, Warmington K, Kurahara C, Sun N, Chen C, Li L, Cattley R, Van G, Scully S, Elliott R, Grisanti M, Morony S, Tan H, Asuncion F, Li X, Ominsky M, Stolina M, Dwyer D, Dougall W, Hawkins N, Boyle W, Simonet W, Sullivan J. Denosumab, a fully human monoclonal antibody to RANKL, inhibits bone resorption and increases bone density in knock-in mice that express chimeric (murine/human) RANKL. *J Bone Miner Res*. 2009 Feb;24(2):182-95. [\[Abstract\]](#)

- ◆Gerstenfeld LC, Sacks DJ, Pelis M, Mason ZD, Graves DT, Barrero M, Ominsky MS, Kostenuik PJ, Morgan EF, Einhorn TA. Comparison of effects of the bisphosphonate alendronate versus the RANKL inhibitor denosumab on murine fracture healing. *J Bone Miner Res*. 2009 Feb;24(2):196-208. [\[Abstract\]](#)

*These two papers report the effects of denosumab, the human monoclonal antibody against RANKL, in mice expressing a chimeric human-mouse RANKL gene. Osteoclast and osteoblast surfaces are modestly decreased even before treatment in these mice compared to wild-type, pertaining to a modest reduction of chimeric RANKL activity. The first study shows complete suppression of osteoclasts and inhibition of bone remodeling by denosumab in intact mice, leading to improved trabecular and cortical bone density and microarchitecture. The second study that compares denosumab to alendronate on transverse femur fracture healing shows delayed callus remodeling with both antiresorptives, and a more significantly increased callus bone volume with denosumab. Both treatments improved mechanical properties after fracture repair. A commentary by D. Little that accompanies these two papers provides a detailed discussion of the implications of early anti-resorptives, particularly RANKL inhibitors, on callus biology and fracture repair. —SF*

## Genetics

- ◆ Novembre J, Johnson T, Bryc K, Kutalik Z, Boyko AR, Auton A, Indap A, King KS, Bergmann S, Nelson MR, Stephens M, Bustamante CD. Genes mirror geography within Europe. *Nature*. 2008 Nov 6;456(7218):98-101. [[Abstract](#)]

*Genome-wide association studies (GWAS) and other large-scale genetic association studies identify an increasing number of genes and SNPs associated with the risk of developing chronic diseases and their complications, including osteoporosis and fragility fractures. Now this study using a SNP chip (Affymetrix 500k) commonly used for GWAS examines the genetic structure of the European population, i.e., the relationship between the geographical origin of individuals (country/region of birth of their grandparents) and their genetic map. Those were closely correlated but started to markedly diverge between populations distant more than 500km (and sometimes less), and more so from North to South than East to West. Thus, in the case of a phenotype that is also strongly spatially structured, such as osteoporotic fractures, SNPs could appear to be associated with an increased risk of the disease not because of a true association between the two, but because both a few given SNPs and fractures are more prevalent in, say, Sweden than Portugal. Knowing that the SNP chip used here underrepresents rare alleles, which are even more likely to be both geographically segregated and prominently associated with disease, the risk of stratification may grow even further as new technologies will allow us to identify those rare SNPs more broadly. Hence, these results provide a strong caveat in the interpretation of GWAS based on geographically extended populations. —SF*

- ◆ Timpson NJ, Tobias JH, Richards JB, Soranzo N, Duncan EL, Sims AM, Whittaker P, Kumanduri V, Zhai G, Glaser B, Eisman J, Jones G, Nicholson G, Prince R, Seeman E, Spector T, Brown MA, Peltonen L, Smith GD, Deloukas P, Evans DM. Common variants in the region around Osterix are associated with bone mineral density and growth in childhood. *Hum Mol Genet*. 2009 Jan 30. [Epub ahead of print] [[Abstract](#)]

*This study reports a genome-wide association study on bone mineral density (BMD) in children. The Osterix (SP7) locus showed significant association with BMD. The authors conclude that genetic variants in the region of Osterix are associated with BMD in children and adults probably through primary effects on growth. —HWD*

## Molecular and Cell Biology

- ◆ Aitken SJ, Landao-Bassonga E, Ralston SH, Idris AI. Beta2-adrenoreceptor ligands regulate osteoclast differentiation in vitro by direct and indirect mechanisms. *Arch Biochem Biophys*. 2009 Feb;482(1-2):96-103. [[Abstract](#)]

*$\beta$ -adrenoceptor ligands stimulate bone resorption by enhancing expression of RANKL.  $\beta$ 2-adrenoceptor is expressed by bone cells. Noradrenaline and the selective agonists isoprenaline and salmeterol stimulated osteoclast formation and resorption in osteoblast co-cultures and increased expression of RANKL by osteoblasts. All three ligands enhanced RANKL-induced osteoclast formation and multinuclearity. There was no significant effect of noradrenaline or isoprenaline on osteoblast growth, differentiation or function. —ES*

- ◆ Kim S, Takahashi H, Lin WW, Descargues P, Grivennikov S, Kim Y, Luo JL, Karin M. Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. *Nature*. 2009 Jan 1;457(7225):102-6. [[Abstract](#)]

*Secretion of the extracellular matrix proteoglycan versican is upregulated in many human tumors including lung carcinoma. Versican directly activates the TLR2 receptor and its co-receptors TLR6 and CD14 on macrophages, which in turn promotes tumor metastasis by producing TNF- $\alpha$ . Thus, cancer cells utilize signaling pathways of the innate immune system to generate an inflammatory microenvironment suitable for metastatic growth.*  
—TM

## Reviews, Perspectives and Editorials

◆Pinzone JJ, Hall BM, Thudi NK, Vonau M, Qiang YW, Rosol TJ, Shaughnessy JD Jr. The role of Dickkopf-1 in bone development, homeostasis, and disease. *Blood*. 2009 Jan 15;113(3):517-25. [\[Abstract\]](#)

## Other Studies of Potential Interest

◆Andersson T, Södersten E, Duckworth JK, Cascante A, Fritz N, Sacchetti P, Cervenka I, Bryja V, Hermanson O. CXXC5 is a novel BMP4-regulated modulator of Wnt signaling in neural stem cells. *J Biol Chem*. 2009 Feb 6;284(6):3672-81. [\[Abstract\]](#) [\[Full Text\]](#)

◆Cao G, Gu Z, Ren Y, Shu L, Tao C, Karaplis A, Goltzman D, Miao D. Parathyroid hormone contributes to regulating milk calcium content and modulates neonatal bone formation cooperatively with calcium. *Endocrinology*. 2009 Feb;150(2):561-9. [\[Abstract\]](#) [\[Full Text\]](#)

◆Heckel T, Czupalla C, Expirto Santo AI, Anitei M, Arantzazu Sanchez-Fernandez M, Mosch K, Krause E, Hoflack B. Src-dependent repression of ARF6 is required to maintain podosome-rich sealing zones in bone-digesting osteoclasts. *Proc Natl Acad Sci U S A*. 2009 Feb 3;106(5):1451-6. [\[Abstract\]](#) [\[Full Text\]](#)

◆Ishii M, Egen JG, Klauschen F, Meier-Schellersheim M, Saeki Y, Vacher J, Proia RL, Germain RN. Sphingosine-1-phosphate mobilizes osteoclast precursors and regulates bone homeostasis. *Nature*. 2009 Feb 8. [Epub ahead of print] [\[Abstract\]](#)

◆Jilka RL, O'Brien CA, Ali AA, Roberson PK, Weinstein RS, Manolagas SC. Intermittent PTH stimulates periosteal bone formation by actions on post-mitotic preosteoblasts. *Bone*. 2009 Feb;44(2):275-86. [\[Abstract\]](#)

◆Josephsen K, Praetorius J, Frische S, Gawenis LR, Kwon TH, Agre P, Nielsen S, Fejerskov O. Targeted disruption of the Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> exchanger Ae2 results in osteopetrosis in mice. *Proc Natl Acad Sci U S A*. 2009 Feb 3;106(5):1638-41. [\[Abstract\]](#) [\[Full Text\]](#)

◆Karagiosis SA, Chrisler WB, Bollinger N, Karin NJ. Lysophosphatidic acid-induced ERK activation and chemotaxis in MC3T3-E1 preosteoblasts are independent of EGF receptor transactivation. *J Cell Physiol*. 2009 Feb 2. [Epub ahead of print] [\[Abstract\]](#)

◆Kärner E, Unger C, Cerny R, Ahrlund-Richter L, Ganss B, Dilber MS, Wendel M. Differentiation of human embryonic stem cells into osteogenic or hematopoietic lineages: a dose-dependent effect of osterix over-expression. *J Cell Physiol*. 2009 Feb;218(2):323-33. [\[Abstract\]](#)

◆Kashima TG, Nishiyama T, Shimazu K, Shimazaki M, Kii I, Grigoriadis AE, Fukayama M, Kudo A. Periostin, a novel marker of intramembranous ossification, is expressed in fibrous dysplasia and in c-Fos-overexpressing bone lesions. *Hum Pathol*. 2009 Feb;40(2):226-37. [\[Abstract\]](#)

- ◆ Kim HJ, Zhang K, Zhang L, Ross FP, Teitelbaum SL, Faccio R. The Src family kinase, Lyn, suppresses osteoclastogenesis in vitro and in vivo. *Proc Natl Acad Sci U S A*. 2009 Feb 17;106(7):2325-30. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆ Macrae VE, Horvat S, Pells SC, Dale H, Collinson RS, Pitsillides AA, Ahmed SF, Farquharson C. Increased bone mass, altered trabecular architecture and modified growth plate organization in the growing skeleton of SOCS2 deficient mice. *J Cell Physiol*. 2009 Feb;218(2):276-84. [\[Abstract\]](#)
- ◆ McCarthy HS, Williams JH, Davie MW, Marshall MJ. Platelet-derived growth factor stimulates osteoprotegerin production in osteoblastic cells. *J Cell Physiol*. 2009 Feb;218(2):350-4. [\[Abstract\]](#)
- ◆ Pettit AR, Chang MK, Hume DA, Raggatt LJ. Osteal macrophages: a new twist on coupling during bone dynamics. *Bone*. 2008 Dec;43(6):976-82. [\[Abstract\]](#)
- ◆ Robling AG, Childress P, Yu J, Cotte J, Heller A, Philip BK, Bidwell JP. Nmp4/CIZ suppresses parathyroid hormone-induced increases in trabecular bone. *J Cell Physiol*. 2009 Feb 2. [Epub ahead of print] [\[Abstract\]](#)
- ◆ Wilson SR, Peters C, Saftig P, Brömme D. Cathepsin K activity-dependent regulation of osteoclast actin ring formation and bone resorption. *J Biol Chem*. 2009 Jan 23;284(4):2584-92. [\[Abstract\]](#) [\[Full Text\]](#)

**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. Deng report no conflicts of interest.