

NOT TO BE MISSED

Clinical and Basic Research Papers – May 2010

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Clinical Studies and Drug Effects

◆ Bolland MJ, Grey AB, Gamble GD, Reid IR. Effect of osteoporosis treatment on mortality: a meta-analysis. *J Clin Endocrinol Metab.* 2010 Mar;95(3):1174-81. [\[Abstract\]](#)

In 8 eligible studies of four agents (risedronate, strontium ranelate, zoledronic acid, and denosumab), treatment was associated with an 11% reduction in mortality (relative risk (RR), 0.89; 95% CI, 0.80-0.99; P = 0.036). In the secondary analysis, the results were similar (RR, 0.90; 95% CI, 0.81-1.0; P = 0.044). Mortality reduction was greatest in trials conducted in populations with higher mortality rates. Treatments for osteoporosis with established vertebral and nonvertebral fracture efficacy reduce mortality in older, frailer individuals with osteoporosis who are at high risk of fracture. —ES

◆ Huntjens KM, Kosar S, van Geel TA, Geusens PP, Willems P, Kessels A, Winkens B, Brink P, van Helden S. Risk of subsequent fracture and mortality within 5 years after a non-vertebral fracture. *Osteoporos Int.* 2010 Feb 17. [Epub ahead of print] [\[Abstract\]](#)

In 1,921 consecutive patients 50+ years, the absolute risk (AR) for a subsequent non-vertebral fracture (NVF) was 17.6% (hazard ratio (HR) per decade, 1.44; 95% CI, 1.29-1.60). The AR for mortality was 32.3% and was related to age (HR per decade, 2.59; 95% CI, 2.37-2.84), male sex (HR, 1.74; 95% CI, 1.44-2.10), major fracture at baseline (HR, 5.56; 95% CI, 3.48-8.88; not constant over time) and subsequent fracture (HR, 1.65; 95% CI, 1.33-2.05). The highest risks were found within the first year (NVFs, 6.4%; mortality, 12.2%). Within 5 years after an initial NVF, about one in five patients sustained a subsequent NVF and one in three died. One-third of subsequent NVFs and mortality occurred within 1 year. —ES

◆ Melhus H, Snellman G, Gedeberg R, Byberg L, Berglund L, Mallmin H, Hellman P, Blomhoff R, Hagström E, Arnlöv J, Michaëlsson K. Plasma 25-hydroxyvitamin D levels and fracture risk in a community-based cohort of elderly men in Sweden. *J Clin Endocrinol Metab.* 2010 Mar 23. [Epub ahead of print] [\[Abstract\]](#)

In the Uppsala Longitudinal Study of Adult Men, a population-based cohort (mean age, 71 yrs., n = 1,194), during follow-up (median 11 yrs.), 309 participants (26%) sustained a fracture. 25(OH)D levels below 40 nmol/liter were associated with a hazard ratio of 1.65 (1.09-2.49). 3% of the fractures were attributable to low 25(OH)D levels. Vitamin D insufficiency is not a major cause of fractures in community-dwelling elderly men in Sweden; one in 20 had 25(OH)D levels below 40 nmol/liter, the threshold at which the risk for fracture started to increase. —ES

- ◆Undale A, Srinivasan B, Drake M, McCreedy L, Atkinson E, Peterson J, Riggs BL, Amin S, Moedder UI, Khosla S. Circulating osteogenic cells: Characterization and relationship to rates of bone loss in postmenopausal women. *Bone*. 2010 Mar 31. [Epub ahead of print] [\[Abstract\]](#)

Osteogenic cells are present in peripheral blood (PB). PB hematopoietic lineage negative (lin-)/AP+ cell gene expression from postmenopausal women undergoing rapid versus slow bone loss was studied. Relative to bone marrow (BM) cells, PB lin-/AP+ cells expressed similar levels of runx2, osterix, osteopontin, OPG, and periostin but lower levels of mRNAs for AP and type I collagen but higher levels of osteocalcin, osteonectin, and PTHR1 mRNAs, and RANKL and ICAM-1 mRNAs important in osteoclastogenesis. Compared to postmenopausal women undergoing slow bone loss, PB lin-/AP+ cells from those with rapid bone loss expressed lower levels of mRNAs for hydroxyprostaglandin dehydrogenase, interferon regulator factor 3, Wnt1-induced secreted protein 1, and TGFβ2, but higher levels of the Smad3 interacting protein, zinc finger DHHC-type containing 4 and col1α2. PB lin-/AP+ cells are a relatively quiescent population in terms of proliferation and matrix synthesis. Their higher expression of RANKL and ICAM-1 mRNAs suggests a role for PB lin-/AP+ cells in regulating osteoclastogenesis. —ES

Genetics

- ◆Barr R, Macdonald H, Stewart A, McGuigan F, Rogers A, Eastell R, Felsenberg D, Glüer C, Roux C, Reid DM. Association between vitamin D receptor gene polymorphisms, falls, balance and muscle power: results from two independent studies (APOSS and OPUS). *Osteoporosis Int*. 2010 Mar;21(3):457-66. [\[Abstract\]](#)

Falls are a major factor in the etiology of osteoporotic fractures. The vitamin D receptor (VDR) is an obvious candidate for risk of fracture since it may be associated with components of fracture other than bone mass and shape. Therefore, five polymorphisms in the VDR gene were analyzed for associations with falls, muscle power and balance in the Aberdeen Prospective Osteoporosis Screening Study (APOSS); the associations were then replicated in another cohort, the Osteoporosis and Ultrasound (OPUS) study. Though these association results are not strikingly significant, they are an important step forward in studying the genetic component of the risk of falling, since they may help explain some of the predisposition to fracture. —DK

- ◆Duncan EL, Brown MA. Genetic determinants of bone density and fracture risk--state of the art and future directions. *J Clin Endocrinol Metab*. 2010 Apr 7. [Epub ahead of print] [\[Abstract\]](#)

As its title proclaims, this perspective article deals with the progress and state of the art of genome-wide association (GWA) studies in osteoporosis. In a sense, it is a continuation of the authors' [BoneKEy Commentary from December 2007](#), in which they proposed criteria for GWA study validity and raised expectations for skeletal genetics. In this detailed JCEM review, the authors organize and provide quality assessment for GWA studies in the field. They point out that most genes involved in osteoporosis identified to date belong to known pathways involved in bone synthesis or resorption, and therefore testify for the biological validity of the "agnostic" approach of mining the human genome. They optimistically predict that, as the field progresses, new pathways will be identified. —DK

- ◆Seo IY, Kang IH, Chae SC, Park SC, Lee YJ, Yang YS, Ryu SB, Rim JS. Vitamin D receptor gene Alw I, Fok I, Apa I, and Taq I polymorphisms in patients with urinary stone. *Urology*. 2010 Apr;75(4):923-7. [\[Abstract\]](#)

- ◆Aksoy H, Aksoy Y, Ozturk N, Aydin HR, Yildirim AK, Akçay F. Fetuin-A gene polymorphism in patients with calcium oxalate stone disease. *Urology*. 2010 Apr;75(4):928-32. [\[Abstract\]](#)

These two back-to-back papers published in Urology are dedicated to discovering candidate genes associated with urinary stones. In the paper from Seo et al., VDR gene polymorphisms were explored in Korean patients; no statistically significant differences were found between the 278 patients with any (including calcium) stones and 535 healthy controls. However, in 103 Turkish patients with renal calcium stones and 73 healthy controls, Aksoy et al. found that a polymorphism in the fetuin-A gene was associated with calcium oxalate nephrolithiasis. Fetuin-A is a major contributor to the calcification inhibitory capacity of human plasma. Circulating fetuin-A levels have been linked with cardiovascular mortality, and therefore the gene's candidacy for a role in calcium disbalance is well-grounded. Studies of fetuin-A in vascular calcification and in mineral homeostasis are warranted. —DK

Bone Modeling, Remodeling, and Repair

- ◆Gourion-Arsiquaud S, Allen MR, Burr DB, Vashishth D, Tang SY, Boskey AL. Bisphosphonate treatment modifies canine bone mineral and matrix properties and their heterogeneity. *Bone*. 2010 Mar;46(3):666-72. [\[Abstract\]](#)

Distal tibiae from 30 normal beagles treated daily for 1 year with oral vehicle, alendronate at 0.2 or 1 mg/kg, and risedronate at 0.1 or 0.5 mg/kg were analyzed by Fourier Transform Infrared Imaging (FTIRI). Both drugs increased mineral content and collagen maturity in cancellous bone and at the endocortical surface. No significant differences were observed between doses or drugs. These positive effects are associated with a loss of bone heterogeneity increasing brittleness and micro-crack accumulation. —ES

- ◆Kobayashi Y, Hiraga T, Ueda A, Wang L, Matsumoto-Nakano M, Hata K, Yatani H, Yoneda T. Zoledronic acid delays wound healing of the tooth extraction socket, inhibits oral epithelial cell migration, and promotes proliferation and adhesion to hydroxyapatite of oral bacteria, without causing osteonecrosis of the jaw, in mice. *J Bone Miner Metab*. 2010 Mar;28(2):165-75. [\[Abstract\]](#)

*Zoledronic acid (ZOL) impairs wound healing as assessed using a tooth extraction socket mouse model. The amount of new bone and the numbers of blood vessels in the socket were decreased in ZOL-treated mice. ZOL inhibited angiogenesis induced by vascular endothelial growth factor in vivo and the proliferation of endothelial cells in culture in a dose-dependent manner. Etidronate, a non-nitrogen-containing bisphosphonate, showed no effects on osteogenesis and angiogenesis in the socket. ZOL also suppressed the migration of oral epithelial cells, a crucial step for tooth socket closure. ZOL promoted the adherence of *Streptococcus mutans* to hydroxyapatite and the proliferation of oral bacteria obtained from healthy individuals. —ES*

- ◆Lotinun S, Pearsall RS, Davies MV, Marvell TH, Monnell TE, Ucran J, Fajardo RJ, Kumar R, Underwood KW, Seehra J, Bouxsein ML, Baron R. A soluble activin receptor type IIA fusion protein (ACE-011) increases bone mass via a dual anabolic-antiresorptive effect in Cynomolgus monkeys. *Bone*. 2010 Apr;46(4):1082-8. [\[Abstract\]](#)

Activin A belongs to the TGF- β superfamily. A soluble form of the extracellular domain of the activin receptor type IIA (ActRIIA) fused to the Fc domain of murine IgG, an

activin antagonist, is anabolic in intact and ovariectomized mice. ActRIIA-IgG1-Fc (ACE-011) given to adult female Cynomolgus monkeys increased cancellous bone volume (+93%), bone formation rate (+166%) and osteoblast surface (+196%), and decreased osteoclast surface and number at the distal femur, but not at the femur midshaft. An increase in bone formation rate and osteoblast surface with a decrease in osteoclast surface was observed in thoracic vertebrae. ACE-011 is a dual anabolic-antiresorptive compound. —ES

Molecular and Cell Biology

◆ Jones DC, Schweitzer MN, Wein M, Sigrist K, Takagi T, Ishii S, Glimcher LH. Uncoupling of growth plate maturation and bone formation in mice lacking both Schnurri-2 and Schnurri-3. *Proc Natl Acad Sci U S A*. 2010 May 4;107(18):8254-8. [\[Abstract\]](#) [\[Full Text\]](#)

The authors report that mice lacking Schnurri 2 (Shn2) and Schnurri 3 (Shn3), members of the Schnurri family of large zinc finger proteins, develop a chondrodysplastic phenotype, resulting from massively elevated trabecular bone formation in the face of impaired growth plate maturation during endochondral ossification. Such phenotypes are not observed in mice lacking only one of these genes. These findings demonstrate that growth plate maturation and bone formation can be uncoupled under certain circumstances, and that Schnurri proteins have both unique and redundant functions for proper skeletal patterning and remodeling. Because there is a compensatory increase in Shn1 expression, overexpressed Shn1 may at least in part contribute to the skeletal phenotypes of the compound mutant mice. —TM

◆ Miyauchi Y, Ninomiya K, Miyamoto H, Sakamoto A, Iwasaki R, Hoshi H, Miyamoto K, Hao W, Yoshida S, Morioka H, Chiba K, Kato S, Tokuhisa T, Saitou M, Toyama Y, Suda T, Miyamoto T. The Blimp1-Bcl6 axis is critical to regulate osteoclast differentiation and bone homeostasis. *J Exp Med*. 2010 Apr 12;207(4):751-62. [\[Abstract\]](#)

Controlling osteoclastogenesis is critical to maintain physiological bone homeostasis and prevent skeletal disorders. Although signaling activating nuclear factor of activated T cells 1 (NFATc1), a transcription factor essential for osteoclastogenesis, has been intensively investigated, factors antagonistic to NFATc1 in osteoclasts have not been characterized. This report describes a novel pathway that maintains bone homeostasis by inhibiting NFATc1 signaling via two transcriptional repressors, B cell lymphoma 6 (Bcl6) and B lymphocyte-induced maturation protein-1 (Blimp1). Overexpression of Bcl6 inhibits osteoclastogenesis in vitro, whereas Bcl6-deficient mice show accelerated osteoclast differentiation and severe osteoporosis. Blimp1 directly suppresses Bcl6 expression, and mice lacking Blimp1 in osteoclasts exhibit osteopetrosis caused by impaired osteoclastogenesis resulting from Bcl6 up-regulation. These results demonstrate that the Blimp1-Bcl6 axis negatively regulates osteoclastogenesis, and suggest that this axis may become a new therapeutic target. —TM

◆ Ochiai E, Kitagawa H, Takada I, Fujiyama S, Sawatsubashi S, Kim MS, Mezaki Y, Tshushima Y, Takagi KI, Azuma Y, Takeyama KI, Yamaoka K, Kato S, Kamimura T. CDP/cut is an osteoblastic coactivator of the vitamin D receptor (VDR). *J Bone Miner Res*. 2010 May;25(5):1157-66. [\[Abstract\]](#)

In order to clarify the precise role of the nuclear vitamin D receptor (VDR) in osteoblast differentiation, the authors purified an osteoblast-specific coregulator of the VDR, using a GST-fused VDR ligand-binding domain as bait. Among the interactants identified by mass fingerprinting, CCAAT displacement protein (CDP) was found as a novel VDR

interactant specifically expressed in osteoblastic cells. CDP forms a complex with VDR in a ligand-dependent manner, is recruited to VDR target gene promoters, and acts as a coactivator of the VDR. Modulation of CDP expression in osteoblastic SaM-1 cells affects vitamin D-dependent osteoblast differentiation. These findings demonstrate that CDP is a novel VDR coactivator that specifically regulates osteoblast differentiation.
—TM

Other Studies of Potential Interest

- ◆ Bassett JH, Boyde A, Howell PG, Bassett RH, Galliford TM, Archanco M, Evans H, Lawson MA, Croucher P, St Germain DL, Galton VA, Williams GR. Optimal bone strength and mineralization requires the type 2 iodothyronine deiodinase in osteoblasts. *Proc Natl Acad Sci U S A*. 2010 Apr 20;107(16):7604-9. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆ Kim MS, Yang YM, Son A, Tian YS, Lee SI, Kang SW, Muallem S, Shin DM. RANKL-mediated reactive oxygen species pathway that induces long lasting Ca²⁺ oscillations essential for osteoclastogenesis. *J Biol Chem*. 2010 Mar 5;285(10):6913-21. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆ Kramer I, Keller H, Leupin O, Kneissel M. Does osteocytic SOST suppression mediate PTH bone anabolism? *Trends Endocrinol Metab*. 2010 Apr;21(4):237-44. [\[Abstract\]](#)
- ◆ Krause U, Harris S, Green A, Ylostalo J, Zeitouni S, Lee N, Gregory CA. Pharmaceutical modulation of canonical Wnt signaling in multipotent stromal cells for improved osteoinductive therapy. *Proc Natl Acad Sci U S A*. 2010 Mar 2;107(9):4147-52. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆ Maruhashi T, Kii I, Saito M, Kudo A. Interaction between periostin and BMP-1 promotes proteolytic activation of lysyl oxidase. *J Biol Chem*. 2010 Apr 23;285(17):13294-303. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆ McMichael BK, Cheney RE, Lee BS. Myosin X regulates sealing zone patterning in osteoclasts through linkage of podosomes and microtubules. *J Biol Chem*. 2010 Mar 26;285(13):9506-15. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆ Shimizu E, Selvamurugan N, Westendorf JJ, Olson EN, Partridge NC. HDAC4 represses matrix metalloproteinase-13 transcription in osteoblastic cells, and parathyroid hormone controls this repression. *J Biol Chem*. 2010 Mar 26;285(13):9616-26. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆ Sunters A, Armstrong VJ, Zaman G, Kypta RM, Kawano Y, Lanyon LE, Price JS. Mechano-transduction in osteoblastic cells involves strain-regulated estrogen receptor alpha-mediated control of insulin-like growth factor (IGF) I receptor sensitivity to ambient IGF, leading to phosphatidylinositol 3-kinase/AKT-dependent Wnt/LRP5 receptor-independent activation of beta-catenin signaling. *J Biol Chem*. 2010 Mar 19;285(12):8743-58. [\[Abstract\]](#) [\[Full Text\]](#)

Conflict of Interest: Dr. Ferrari reports that he receives research support from Amgen and Merck Sharp & Dohme, and is an advisory committee member and lectures occasionally at conference symposia for the Alliance for Better Bone Health (sanofi aventis/P&G), Amgen, Merck Sharp & Dohme, Eli Lilly, Servier, and Novartis. 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.