

NOT TO BE MISSED

Clinical and Basic Research Papers – July 2010

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Clinical Studies and Drug Effects

◆Giusti A, Hamdy NA, Papapoulos SE. Atypical fractures of the femur and bisphosphonate therapy: A systematic review of case/case series studies. *Bone*. 2010 May 20. [Epub ahead of print] [\[Abstract\]](#)

This is the first and very laudable attempt to systematically review 196 published cases of atypical subtrochanteric fractures in relation to bisphosphonate (BP) therapy, of which 141 were non-duplicated reports. The authors also report 8 cases of their own experience. Nearly a third of the cases were related to BP treatment of glucocorticoid-induced osteoporosis. Association with use of proton pump inhibitors was also noted. No relationship between duration of BP treatment and the occurrence of cases was found, with a quarter of the cases being reported with less than 3 years of use. Due to incomplete reports in about half of the cases and/or an inappropriate time of sampling for bone biochemical markers, no relationship with suppression of bone turnover could be established. —SF

◆Kusamori K, Katsumi H, Abe M, Ueda A, Sakai R, Hayashi R, Hirai Y, Quan YS, Kamiyama F, Sakane T, Yamamoto A. Development of a novel transdermal patch of alendronate, a nitrogen-containing bisphosphonate, for the treatment of osteoporosis. *J Bone Miner Res*. 2010 Jun 7. [Epub ahead of print] [\[Abstract\]](#)

The authors developed a transdermal patch of alendronate. The maximum permeation fluxes of alendronate through rat and human skin after application of this patch were 1.9 and 0.3 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively. The bioavailability (BA) of alendronate in rats was approximately 8.3% by patch and 1.7% by oral administration. The plasma calcium level was effectively reduced after the application of the alendronate patch in 1α -hydroxyvitamin D_3 -induced hypercalcemia model rats. The patch also suppressed the decrease of bone mass in model rats with osteoporosis. —ES

◆Rikkonen T, Salovaara K, Sirola J, Kärkkäinen M, Tuppurainen M, Jurvelin J, Honkanen R, Alhava E, Kröger H. Physical activity slows femoral bone loss but promotes wrist fractures in postmenopausal women - a 15-year follow-up of osteoporosis study. *J Bone Miner Res*. 2010 Jun 7. [Epub ahead of print] [\[Abstract\]](#)

The association between physical activity (PA) and fracture risk was examined in 8560 women with a mean age of 52.2 (range 47-56) years during a 15-year follow-up. 2641 follow-up fractures were verified in 2073 (24.2%) women. Areal BMD (aBMD) at the proximal femur (N=2050) and lumbar spine (L2-L4) (N=1417) was followed at 5-year intervals. Weekly average time spent on leisure time PA was 0.4 h, 1.7 h, 3.3 h and 7.0 h from the least to the most active quartiles, respectively. The risk of wrist fracture was

higher in the active quartiles (II-IV) than in the most inactive quartile (I), HR 1.3 (95% CI, 1.05-1.57, p=0.014) for the second (II), 1.2 (CI, 1.01-1.51, p=0.045) for the third (III) and 1.4 (CI, 1.14-1.69, p=0.001) for the fourth (IV) quartiles, respectively. Overall, most of the fractures were reported as a result of a fall (69.0%), with a 2.1 times higher rate of wrist fractures during the winter. —ES

◆Rizzoli R, Laroche M, Krieg MA, Frieling I, Thomas T, Delmas P, Felsenberg D. Strontium ranelate and alendronate have differing effects on distal tibia bone microstructure in women with osteoporosis. *Rheumatol Int.* 2010 May 29. [Epub ahead of print] [\[Abstract\]](#)

◆Macdonald HM, Nishiyama KK, Hanley DA, Boyd SK. Changes in trabecular and cortical bone microarchitecture at peripheral sites associated with 18 months of teriparatide therapy in postmenopausal women with osteoporosis. *Osteoporos Int.* 2010 May 11. [Epub ahead of print] [\[Abstract\]](#)

High-resolution pQCT provides new opportunities to re-assess the effects of bone drugs on volumetric BMD (vBMD) and microstructure at the distal radius and tibia. A recent study by Seeman et al. thus showed that anti-resorptives such as alendronate (ALN) and denosumab improved trabecular vBMD compared to placebo and actually increased cortical vBMD and thickness over baseline (see recent BoneKEy Commentary by S. Ferrari [here](#)). These two studies now examine on one side the one-year effects of strontium ranelate (SR) vs. ALN in a RCT of postmenopausal women with osteoporosis, and on another side the 18-month effects of teriparatide (TPT) in an open-label observation in postmenopausal osteoporotic women with prior bisphosphonates. The study of SR shows a significantly greater gain of cortical thickness, and to a lesser extent of trabecular BV/TV, at the distal tibia compared to ALN (distal radius was not reported). In contrast to the study by Seeman et al. (ALN vs. denosumab), this study does not show any significant change of cortical thickness over baseline with ALN. Meanwhile, the study of TPT shows a decrease in cortical vBMD, trabecular BV/TV and thickness, but a trend towards increased cortical thickness. Altogether these studies suggest that apparent changes in bone, particularly cortical, vBMD and microstructure, as evaluated by in vivo micro-CT, are influenced prominently by drug-induced changes in bone mineralization and porosity. —SF

Genetics

◆Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, Jacobs EJ, Ascherio A, Helzlsouer K, Jacobs KB, Li Q, Weinstein SJ, Purdue M, Virtamo J, Horst R, Wheeler W, Chanock S, Hunter DJ, Hayes RB, Kraft P, Albanes D. Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet.* 2010 Jul 1;19(13):2739-45. [\[Abstract\]](#)

◆Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Järvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hyppönen E, Spector TD. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet.* 2010 Jun 9. [Epub ahead of print] [\[Abstract\]](#)

These two articles report GWAS of circulating vitamin D levels in individuals of

European ancestry. 25-hydroxyvitamin D [25(OH)D] is associated with diseases of the musculoskeletal system, including rickets, osteoporosis, and probably sarcopenia, and seem to be heritable. In the first study, 4501 subjects from five cohorts were used for discovery, and 2221 additional individuals for replication. The investigators identified significantly-associated or suggestive SNPs in several genes involved in vitamin D synthesis or activation: a vitamin D binding protein (or GC), on chr. 4q12-13; nicotinamide adenine dinucleotide synthetase (NADSYN1) and 7-dehydrocholesterol reductase (DHCR7); and cytochrome P450, family 2, subfamily R, polypeptide 1 (CYP2R1).

In the second study – by an independent group – GWAS of 33,996 individuals from 15 cohorts confirmed associations with GC (actually, the same SNP), NADSYN1/DHCR7, and CYP2R1, and also identified an additional locus (CYP24A1 on 20q13). This study went further, by attempting to predict vitamin D insufficiency based on these genetic variants. Indeed, participants with a genotype score (combining the three confirmed variants) in the highest quartile had a 2.5 times higher risk of having 25(OH)D concentrations lower than 75 nmol/L, compared with those in the lowest quartile. These 2 studies are good examples of the robustness of the GWAS approach and its possible value for clinical prediction. —SF

- ◆Fröhlich LF, Mrakovcic M, Steinborn R, Chung UI, Bastepe M, Jüppner H. Targeted deletion of the Nesp55 DMR defines another Gnas imprinting control region and provides a mouse model of autosomal dominant PHP-1b. *Proc Natl Acad Sci U S A*. 2010 May 18;107(20):9275-80. [\[Abstract\]](#) [\[Full Text\]](#)

This brilliant work reports a mouse model for autosomal dominant pseudohypoparathyroidism type 1b, obtained by deleting a region of the GNAS allele (coding for Gs α) that corresponds to the human microdeletion, preventing methylation/imprinting of the maternal allele causing the disease. —SF

- ◆Liu JM, Zhang MJ, Zhao L, Cui B, Li ZB, Zhao HY, Sun LH, Tao B, Li M, Ning G. Analysis of recently identified osteoporosis susceptibility genes in Han Chinese women. *J Clin Endocrinol Metab*. 2010 Jun 16. [Epub ahead of print] [\[Abstract\]](#)

Genome-wide association study (GWAS) results from cohorts of European ancestry need to be replicated in other ethnic samples, to make a claim that these results are generalizable. In this study, 21 single nucleotide polymorphisms (SNPs) in 11 candidate genes were tested in a sample of 1012 Han Chinese women. Five SNPs in four genes, ZBTB40, ESR1, OPG, and RANK, were found to be associated with lumbar spine BMD. Seven SNPs in five genes, ZBTB40, OPG, RANK, LRP5, and SOST, were associated with total hip BMD. Finally, SPTBN1 and SOST were associated with osteoporotic fracture mostly in post-menopausal Chinese women. —DK

- ◆Varanasi SS, Olstad OK, Swan DC, Sanderson P, Gautvik VT, Reppe S, Francis RM, Gautvik KM, Datta HK. Skeletal site-related variation in human trabecular bone transcriptome and signaling. *PLoS One*. 2010 May 18;5(5):e10692. [\[Abstract\]](#)

- ◆Kalogeropoulos M, Varanasi SS, Olstad OK, Sanderson P, Gautvik VT, Reppe S, Francis RM, Gautvik KM, Birch MA, Datta HK. Zic1 transcription factor in bone: neural developmental protein regulates mechanotransduction in osteocytes. *FASEB J*. 2010 Apr 6. [Epub ahead of print] [\[Abstract\]](#)

These two collaborative works by groups from the United Kingdom and Norway

compare gene expression in human bone biopsy samples taken from the lumbar spinal lamina and iliac crest of 13 men. Despite a small sample of participants, the investigators were able to identify differentially-expressed genes, since these sites experience high and low levels of applied mechanical stress, respectively. Thus in the PLoS One paper, in the lumbar spine, compared to the iliac crest, the majority of the markedly up-regulated genes that showed significantly increased levels of expression happened to be markers of osteocyte-, as well as osteoblast- and osteoclast-related genes. (Not to forget, though, that trans-iliac bone biopsies capture tissue from both cortical and trabecular compartments as well as from bone marrow, and therefore the proportion of bone cells is lower than in the vertebral lamina).

More strikingly, there were a number of genes involved in muscle action among the up-regulated genes. A number of novel signaling molecules, which have not been known to be expressed in bone cells, were identified; for example, a member of the zinc finger protein of cerebellum family – transcription factor Zic1. In the FASEB J paper, the authors suggest that Zic1, a neural developmental transcription factor, may act as a link between mechanosensing and Wnt signaling. Zic1 seems to play an important role in shear flow mechanotransduction in osteocytes. —DK

Bone Modeling, Remodeling, and Repair

◆Edwards JR, Nyman JS, Lwin ST, Moore MM, Esparza J, O'Quinn EC, Hart AJ, Biswas S, Patil CA, Lonning S, Mahadevan-Jansen A, Mundy GR. Inhibition of TGF-beta signaling by 1D11 antibody treatment increases bone mass and quality in vivo. *J Bone Miner Res.* 2010 May 17. [Epub ahead of print] [[Abstract](#)]

TGF-β produced by bone cells and present in the bone matrix represents a major coupling factor between osteoblasts and osteoclasts. Whether its effects are predominantly catabolic or anti-catabolic, however, remains uncertain. Intraperitoneal injections (3x/week) of an antibody against the 3 isoforms of TGF-β (Genzyme) in young, intact mice, after 4 weeks resulted in a marked improvement of trabecular BV/TV, with reduced osteoclast and increased osteoblast number and surfaces. The quality of the bone material as well as the bending strength of cortices were also improved. These results suggest that TGF-β inhibition may favorably uncouple bone formation and resorption and thereby increase bone strength. Whether similar results would be found in estrogen-deprived animals – in which a decrease in TGF-β levels in bone marrow has been associated with bone loss – remains to be established. —SF

◆Guo J, Liu M, Yang D, Bouxsein ML, Thomas CC, Schipani E, Bringhurst FR, Kronenberg HM. Phospholipase C signaling via the parathyroid hormone (PTH)/PTH-related peptide receptor is essential for normal bone responses to PTH. *Endocrinology.* 2010 May 25. [Epub ahead of print] [[Abstract](#)]

PTH effects on bone formation and resorption are primarily mediated by cAMP signaling (see recent BoneKEy Perspective by Ferrari and Bouxsein [HERE](#)). In addition to cAMP, PTH elicits other signaling pathways including phospholipase C (PLC)-calcium/IP3, mitogen-activated protein kinases (MAPKs), etc. This group previously reported that knock-in mice expressing a mutant PTH/PTHrP receptor (so-called DSEL) that retains its ability to signal through cAMP but does not elicit PLC activation had delayed chondrocyte differentiation. This study now shows that DSEL mice have somewhat decreased trabecular, but not cortical, bone microarchitecture. Moreover, in response to a low calcium diet (secondary hyperparathyroidism) or PTH infusion, these mice exhibit increased serum phosphate levels (contrary to wild type

animals) – suggesting low PTH activity – poor bone formation in vivo, and a low proliferative response to PTH in vitro. Interestingly, low calcium induced cortical thinning in both wild type and DSEL mice, while BV/TV was increased in wild type but not mutant mice. Hence PLC signaling appears to contribute to PTH anabolic, but not catabolic, effects on the skeleton. —SF

- ◆Jilka RL, O'Brien CA, Bartell SM, Weinstein RS, Manolagas SC. Continuous elevation of PTH increases the number of osteoblasts via both osteoclast-dependent and -independent mechanisms. *J Bone Miner Res.* 2010 Jun 7. [Epub ahead of print] [\[Abstract\]](#)

6 month-old Swiss-Webster mice were infused for 5 days with 470 ng/h PTH(1-84) or 525 ng/h soluble RANKL (sRANKL). Both agents increased osteoclasts and osteoblasts in vertebral cancellous bone, but the ratio of osteoblasts to osteoclasts and the increase in bone formation were greater in PTH-treated mice. Cancellous bone mass was maintained in mice receiving PTH, but lost in mice receiving sRANKL, indicating that maintenance of balanced remodeling requires osteoblastogenic effects beyond those mediated by osteoclasts. PTH, but not sRANKL, decreased sclerostin, and increased the expression of the Wnt target genes Nkd2, Wisp1 and Twist1. PTH, but not sRANKL, increased the number of blood vessels in the marrow. Weekly injections of the RANKL antagonist OPG at 10 µg/g for 2 weeks prior to PTH infusion eliminated osteoclasts and osteoblasts, and prevented the PTH-induced increase in osteoclasts, osteoblasts and blood vessels. —ES

- ◆Kramer I, Halleux C, Keller H, Pegurri M, Gooi JH, Weber PB, Feng JQ, Bonewald LF, Kneissel M. Osteocyte Wnt/beta-catenin signaling is required for normal bone homeostasis. *Mol Cell Biol.* 2010 Jun;30(12):3071-85. [\[Abstract\]](#)

Previous work from Gerard Karsenty's lab had shown that β-catenin deletion from differentiated osteoblasts leads to osteopenia largely because of increased bone resorption. This elegant study confirms that Wnt/β-catenin canonical signaling regulates osteoprotegerin (OPG) expression and thereby osteoclastogenesis post-natally, but in this case in osteocytes, where β-catenin was specifically deleted. This is consistent with the concept that osteocytes do not only control bone formation at the surface (through Sost), but bone remodeling at endo- and probably intra-cortical surfaces (through OPG). It also establishes that osteocyte-regulated bone resorption is a potential target for drugs modulating Wnt/β-catenin signaling. —SF

Pathophysiology

- ◆Grigorieva IV, Mirczuk S, Gaynor KU, Nesbit MA, Grigorieva EF, Wei Q, Ali A, Fairclough RJ, Stacey JM, Stechman MJ, Mihai R, Kurek D, Fraser WD, Hough T, Condie BG, Manley N, Grosveld F, Thakker RV. Gata3-deficient mice develop parathyroid abnormalities due to dysregulation of the parathyroid-specific transcription factor Gcm2. *J Clin Invest.* 2010 Jun 1;120(6):2144-55. [\[Abstract\]](#)

GATA3 belongs to a family of dual zinc-finger transcription factors, haploinsufficiency of which results in the congenital hypoparathyroidism-deafness-renal dysplasia (HDR) syndrome. Gata3(+/-) mice are viable and fertile, but Gata3(-/-) embryos die by E12.5. This study shows that while regular chow, which is enriched in calcium and vitamin D, maintains normal serum calcium levels and survival in Gata3(+/-) mice, a low calcium diet induces mild hypocalcemia (in the absence of secondary hyperparathyroidism) and lethality. The study proceeds by analyzing the role of Gata3 in parathyroid gland development and its target GCMB promoter region. —SF

Other Studies of Potential Interest

- ◆ Ashley EA, Butte AJ, Wheeler MT, Chen R, Klein TE, Dewey FE, Dudley JT, Ormond KE, Pavlovic A, Morgan AA, Pushkarev D, Neff NF, Hudgins L, Gong L, Hodges LM, Berlin DS, Thorn CF, Sangkuhl K, Hebert JM, Woon M, Sagreiya H, Whaley R, Knowles JW, Chou MF, Thakuria JV, Rosenbaum AM, Zaranek AW, Church GM, Greely HT, Quake SR, Altman RB. Clinical assessment incorporating a personal genome. *Lancet*. 2010 May 1;375(9725):1525-35. [\[Abstract\]](#)
- ◆ Darwech I, Otero JE, Alhawagri MA, Abu-Amer Y. Tyrosine phosphorylation is required for IkkappaB kinase-beta (IKKbeta) activation and function in osteoclastogenesis. *J Biol Chem*. 2010 Jun 9. [Epub ahead of print] [\[Abstract\]](#)
- ◆ Eash KJ, Greenbaum AM, Gopalan PK, Link DC. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. *J Clin Invest*. 2010 Jun 1. [Epub ahead of print] [\[Abstract\]](#)
- ◆ Farber CR. Identification of a gene module associated with BMD through the integration of network analysis and genome-wide association data. *J Bone Miner Res*. 2010 May 17. [Epub ahead of print] [\[Abstract\]](#)
- ◆ Ito K, Elkin EB, Girotra M, Morris MJ. Cost-effectiveness of fracture prevention in men who receive androgen deprivation therapy for localized prostate cancer. *Ann Intern Med*. 2010 May 18;152(10):621-9. [\[Abstract\]](#)
- ◆ Iwamoto T, Nakamura T, Doyle A, Ishikawa M, de Vega S, Fukumoto S, Yamada Y. Pannexin 3 regulates intracellular ATP/cAMP levels and promotes chondrocyte differentiation. *J Biol Chem*. 2010 Jun 11;285(24):18948-58. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆ Miedlich SU, Zalutskaya A, Zhu ED, Demay MB. Phosphate-induced apoptosis of hypertrophic chondrocytes is associated with a decrease in mitochondrial membrane potential and is dependent upon Erk1/2 phosphorylation. *J Biol Chem*. 2010 Jun 11;285(24):18270-5. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆ Rath AL, Bonewald LF, Ling J, Jiang JX, Van Dyke ME, Nicolella DP. Correlation of cell strain in single osteocytes with intracellular calcium, but not intracellular nitric oxide, in response to fluid flow. *J Biomech*. 2010 May 28;43(8):1560-4. [\[Abstract\]](#)
- ◆ Wu JJ, Weis MA, Kim LS, Eyre DR. Type III collagen, a fibril network modifier in articular cartilage. *J Biol Chem*. 2010 Jun 11;285(24):18537-44. [\[Abstract\]](#) [\[Full Text\]](#)
- ◆ Yang S, Kim J, Ryu JH, Oh H, Chun CH, Kim BJ, Min BH, Chun JS. Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. *Nat Med*. 2010 Jun;16(6):687-93. [\[Abstract\]](#)

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.