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# Molecular Genetic Studies of Gene Identification for Osteoporosis: The 2009 Update

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**Osteoporosis is a complex human disease that results in increased susceptibility to fragility fractures. It can be phenotypically characterized using several traits, including bone mineral density, bone size, bone strength, and bone turnover markers. The identification of gene variants that contribute to osteoporosis phenotypes, or responses to therapy, can eventually help individualize the prognosis, treatment, and prevention of fractures and their adverse outcomes. Our previously published reviews have comprehensively summarized the progress of molecular genetic studies of gene identification for osteoporosis and have covered the data available to the end of September 2007. This review represents our continuing efforts to summarize the important and representative findings published between October 2007 and November 2009. The topics covered include genetic association and linkage studies in humans, transgenic and knockout mouse models, as well as gene-expression microarray and proteomics studies. Major results are tabulated for comparison and ease of reference. Comments are made on the notable findings and representative studies for their potential influence and implications on our present understanding of the genetics of osteoporosis. (*Endocrine Reviews* 31: 0000–0000, 2010)**

I. Introduction
II. Candidate Gene Association Studies
   A. Receptors for calciotropic hormones
   B. Cytokines and receptors
   C. Bone matrix proteins
   D. Limitations and improvements
III. Genome-Wide Linkage (GWL) Studies
   A. Univariate linkage analyses
   B. Bivariate linkage analyses
IV. Genome-Wide Association (GWA) Studies
   A. Single nucleotide polymorphism (SNP) analyses
   B. Copy number variation (CNV) analyses
V. Transgenic/Knockout Mouse Models
VI. Gene-Expression Microarray Studies
   A. Regulation of osteoblast and osteoclast activity
   B. Proliferation and differentiation of mesenchymal stem cells (MSCs)
   C. Gene expression in healthy *vs.* diseased tissues
   D. Effects of therapeutic agents on the healing of fractures
   E. Endocrine regulation of bone remodeling
VII. Proteomics Studies
   A. Studying cultured cells using *in vitro* systems
   B. Studying fresh cells, serum, or tissues
VIII. Future Directions
   A. Functional studies
   B. Epigenetic variation
   C. New phenotypes

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Abbreviations: 2-DE, Two dimensional electrophoresis; AAM, age at menarche; ALP, alkaline phosphatase; BFM, body fat mass; BMC, bone mineral content; BMD, bone mineral density; BR, buckling ratio; BS, bone size; CNV, copy number variation; COX-2, cyclooxygenase-2; CSI, compression strength index; CT, cortical thickness; ER, estrogen receptor; FGF, fibroblast growth factor; FN, femoral neck; GWA, genome-wide association; GWL, genome-wide linkage; HA, hydroxyapatite; LRP5, low-density lipoprotein receptor-related protein 5; LS, lumbar spine; miRNA, microRNA; MS, mass spectrometry; MSC, mesenchymal stem cell; OA, osteoarthritis; 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin $D_3$; OPG, osteoprotegerin; OPLL, ossification of the posterior longitudinal ligament; PBMD, peak BMD; QTLs, quantitative trait loci; RANK, receptor activator of nuclear factor $\kappa$-$\beta$; RANKL, RANK ligand; SNP, single nucleotide polymorphism; SRC, steroid receptor coactivator; TBLM, total body lean mass; TRAP, tartrate-resistant acid phosphatase; VDR, vitamin D receptor.



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IX. Future Prospects for the Application of Genetic Risk Assessment in Osteoporosis Prediction and Treatment
X. Summary

## I. Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mineral density (BMD) and microarchitectural deterioration of bone tissue, with a consequent increase in susceptibility to fracture. Molecular genetic studies have been extensively performed to search for genes underlying osteoporosis. Since 2002, we have regularly published reviews that summarize the progress in molecular genetic studies of gene identification for osteoporosis (1–3). In this review, we continue these reviews with an update that captures the important and representative findings published from October 2007 to November 2009.

Similar to our previous updates (1–3), this article systematically reviews publications relevant to osteogenesis and osteoporosis that involve genetic association and linkage studies or functional genomics (including gene-expression microarray and proteomics) in human populations and transgenic and knockout animal models. The data presented in this review were collected from PubMed using the searching key words "BMD," "osteoporosis," or "bone" in combination with "association," "polymorphisms," "linkage," "knock out," "transgenic," "microarray," or "proteomics." The results of important studies are incorporated in tables for clear comparison and ease of reference. Table 1 summarizes the major candidate genes subjected to association studies, classified by their functional relevance to bone and mineral metabolism. Table 2 reviews major results from approximately 120 reported candidate gene association studies. Genome-wide linkage (GWL) and genome-wide association (GWA) studies for osteoporosis-related phenotypes in humans are summarized in Tables 3 and 4, respectively. Table 5 highlights studies using transgenic and knockout mouse models relevant to osteoporosis, and Table 6 reviews approximately 110 gene-expression microarray studies on the pathogenesis of osteoporosis and other bone-related diseases. In this review, due to space limitations, we only comment on the most representative results that have had an immediate influence on our understanding and research of genetic mechanisms underlying osteoporosis.

## II. Candidate Gene Association Studies

Candidate gene association analysis has the advantages of higher statistical power and easier sample recruitment compared with the linkage approach. During the period from October 2007 to November 2009, a series of genes catalogued on the basis of biological functions in Table 1 have been recognized and tested as candidates to osteoporosis-related traits by association studies. Several classical candidate genes, such as vitamin D receptor (*VDR*), estrogen receptor (*ER*), and low-density lipoprotein receptor-related protein 5 (*LRP5*), have been most widely studied (Table 2).

### A. Receptors for calciotropic hormones

#### 1. VDR

1,25-Dihydroxyvitamin $D_3$ [1,25-$(OH)_2D_3$], the biologically active metabolite of vitamin D, is required for calcium and phosphorus homeostasis, for normal skeletal development, and for maintenance of skeletal architecture. The action of 1,25-$(OH)_2D_3$ is mediated by VDR, which is a member of the superfamily of steroid/thyroid hormone/retinoid receptors. *VDR* was the first candidate gene to be studied in relation to osteoporosis (4). Frequently studied markers of *VDR* include *Bsm*I, *Apa*I, *Taq*I, *Cdx2*, and *Fok*I. For example, *Cdx2* polymorphism was associated with femoral neck (FN) BMD in a study of 239 osteoporotic postmenopausal women carried out by Mencej-Bedrac *et al.* (5). In another study performed by Gentil *et al.* (6), *Cdx2* polymorphism did not influence BMD in postmenopausal women by itself, but actually affected the BMD response to physical activity. The interpretation of *VDR* polymorphisms is currently hindered by the fact that most studies were performed with relatively small sample sizes and investigated only limited polymorphisms (*e.g.*, *Bsm*I, *Apa*I, *Taq*I, *Cdx2*, and *Fok*I), which largely have unknown effects. Whole-gene analyses that exhaustively explore all potential sequence variations within/around the *VDR* gene in samples of larger size are critical for identifying potential functional variants.

#### 2. ER-α and ER-β

Estrogens play an important role in regulating bone homeostasis, bone turnover, and maintenance of bone mass. The effects of estrogens on skeletal structure are mediated through binding to two different ERs, which are encoded by the *ER-α* and *ER-β* genes. Both receptors are highly expressed in bone (7). Although some studies showed significant associations between the three major polymorphisms [*e.g.*, *Pvu*II (T>C) and *Xba*I (A>G) in intron 1, and the TA repeat in the promoter region] of *ER-α* and osteoporosis-related phenotypes, the sample sizes for most of these studies were relatively small (8–11). *Rs1801132*, another marker for *ER-α*, was found to be associated with osteoporotic

**TABLE 1.** Major candidate genes tested for association with osteoporosis-related phenotypes

| Candidate genes | Protein | Chromosome location |
|---|---|---|
| Calciotropic hormones and receptors | | |
| *AR* | Androgen receptor | Xq11.2-q12 |
| *CASR* | Calcium-sensing receptor | 3q13 |
| *CRHR1* | Corticotropin-releasing hormone receptor 1 | 17q12-q22 |
| *CTR* | Calcitonin receptor | 7q21.3 |
| *CYP17A1* | Steroid 17-$\alpha$-hydroxylase | 10q24.3 |
| *CYP19* | Cytochrome P450, family XIX | 10q26 |
| *CYP19A1* | Aromatase | 15q21.1 |
| *CYP1B1* | Aryl hydrocarbon hydroxylase | 2p21 |
| *DBP* | Vitamin D-binding protein | 4q12-q13 |
| *ER-a* | Estrogen receptor-a | 6q25.1 |
| *ER-b* | Estrogen receptor-b | 14q23.2 |
| *ESRRA* | Estrogen-related receptor $\alpha$ | 11q13 |
| *GR* | Glucocorticoid receptor | 5q31.3 |
| *LHB* | Luteinizing hormone $\beta$ polypeptide | 19q13.32 |
| *LHCGR* | Luteinizing hormone-choriogonadotropin receptor | 2p21 |
| *PRL* | Prolactin | 6p22.2-p21.3 |
| *PTH* | Parathyroid hormone | 11p15.3-p15.1 |
| *PTHLH* | Parathyroid hormone-like hormone | 12p12.1-p11.2 |
| *PTHR1* | Parathyroid hormone receptor 1 | 3p22-p21.1 |
| *PTHR2* | Parathyroid hormone receptor 2 | 2q33 |
| *SHBG* | Sex hormone-binding globulin | 17p13-p12 |
| *SRD5A2* | Steroid-5-$\alpha$-reductase, $\alpha$ polypeptide 2 | 2p23 |
| *TSHR* | Thyroid-stimulating hormone receptor | 14q31 |
| *VDR* | Vitamin-D receptor | 12q13.11 |
| Cytokines, growth factors, and receptors | | |
| *BMP2* | Bone morphogenetic protein 2 | 20p12 |
| *BMP7* | Bone morphogenetic protein 7 | 20q13 |
| *CD40* | Tumor necrosis factor receptor superfamily member 5 | 20q12-q13.2 |
| *CNR2* | Cannabinoid receptor 2 | 1p36.11 |
| *DKK2* | Dickkopf homolog 2 | 4q25 |
| *FGFR1* | Fibroblast growth factor receptor 1 | 8p11.2-p11.1 |
| *FGFR2* | Fibroblast growth factor receptor 2 | 10q26 |
| *GDF5* | Growth differentiation factor 5 | 20q11.2 |
| *GHRH* | Growth hormone-releasing hormone | 20q11.2 |
| *IGFBP2* | Insulin-like growth factor binding protein 2 | 2q33-q34 |
| *IL-23* | Interleukin 23 | 12q13.2 |
| *IL-23R* | Interleukin 23 receptor | 1p31.3 |
| *IL-6* | Interleukin 6 | 7p21 |
| *IL-6R* | Interleukin 6 receptor | 1q21 |
| *IL-15* | Interleukin 15 | 4q31 |
| *LEPR* | Leptin receptor | 1p31 |
| *LRP1* | Low-density lipoprotein receptor-related protein 1 | 12q13-q14 |
| *LRP5* | Low-density lipoprotein receptor-related protein 5 | 11q13.4 |
| *LRP6* | Low-density lipoprotein receptor-related protein 6 | 12p11-p13 |
| *LTBP2* | Latent transforming growth factor $\beta$ binding protein 2 | 14q24 |
| *MSTN* | Myostatin | 2q32.2 |
| *NPY* | Neuropeptide Y | 7p15.1 |
| *OPG* | Osteoprotegerin | 8q24 |
| *RANK* | Receptor activator of nuclear factor $\kappa$-$\beta$ | 18q22.1 |
| *RANKL* | Receptor activator of nuclear factor $\kappa$-$\beta$ ligand | 13q14 |
| *TGF-b1* | Transforming growth factor b-1 | 19q13.1 |
| *TNF-a* | Tumor necrosis factor a | 6p21.3 |
| *TNFRSF1B* | Tumor necrosis factor receptor superfamily, member 1B | 1p36.3-p36.2 |
| *TNF-$\beta$* | Lymphotoxin $\alpha$ | 6p21.3 |
| *VEGF* | Vascular endothelial growth factor A | 6p12 |
| Bone matrix proteins | | |
| *COL1A1* | Collagen type I $\alpha$-1 | 17q21.33 |
| *COL1A2* | Collagen type I $\alpha$-2 | 7q22.1 |
| *ITGA1* | Integrin, $\alpha$ 1 | 5q11.2 |
| *MMP2* | Matrix metallopeptidase 2 | 16q13-q21 |
| *OCIL* | Osteoclast inhibitory lectin | 12p13 |
| *SPARC* | Osteonectin | 5q31.3-q32 |

(*Continued*)

**TABLE 1.** Continued

| Candidate genes | Protein | Chromosome location |
|---|---|---|
| Miscellaneous | | |
| *ADCY10* | Adenylate cyclase 10 | 1q24 |
| *ALOX15* | Arachidonate 15-lipoxygenase | 17p13.3 |
| *ALOX5* | Arachidonate 5-lipoxygenase | 10q11.2 |
| *ALPL* | Alkaline phosphatase | 1p36.12 |
| *ANXA6* | Annexin A6 | 5q32-q34 |
| *APC* | Adenomatous polyposis coli | 5q21-q22 |
| *ARHGEF3* | Rho guanine nucleotide exchange factor 3 | 3p21-p13 |
| *BMPR1B* | Bone morphogenetic protein receptor, type IB | 4q22-q24 |
| *CA10* | Carbonic anhydrase X | 17q21.33 |
| *CA8* | Carbonic anhydrase VIII | 8q11-q12 |
| *CALM1* | Calmodulin 1 | 14q24-q31 |
| *CLCN7* | Chloride channel 7 | 16p13 |
| *COMT* | Catechol-O-methyltransferase | 22q11.21 |
| *CRTAP* | Cartilage-associated protein | 3p22.3 |
| *DMP1* | Dentin matrix acidic phosphoprotein 1 | 4q21 |
| *ENPP1* | Ectonucleotide pyrophosphatase/phosphodiesterase 1 | 6q22-q23 |
| *FABP3* | Fatty acid-binding protein 3 | 1p33-p32 |
| *FLNB* | Filamin $\beta$ | 3p14.3 |
| *FLT1* | Fms-related tyrosine kinase 1 | 13q12 |
| *FOXC2* | Forkhead box C2 | 16q22-q24 |
| *FZD1* | Frizzled homolog 1 | 7q21 |
| *FZD6* | Frizzled homolog 6 | 8q22.3-q23.1 |
| *HMGA2* | High mobility group AT-hook 2 | 12q15 |
| *HOXA* | Homeobox A cluster | 7p15-p14 |
| *HSD11B1* | Hydroxysteroid (11-$\beta$) dehydrogenase 1 | 1q32-q41 |
| *MDR1* | Multidrug resistance 1 | 7q21.1 |
| *MTHFR* | Methylenetetrahydrofolate reductase | 1p36.3 |
| *NFATC1* | Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 | 18q23 |
| *NOG* | Noggin | 17q21-q22 |
| *NOS3* | Endothelial nitric oxidase synthase | 7q36 |
| *NR1I3* | Constitutive androstane receptor | 1q23.3 |
| *P2X 7* | Purinergic receptor P2X, ligand-gated ion channel, 7 | 12q24 |
| *PBX1* | Pre-B-cell leukemia homeobox 1 | 1q23 |
| *PIR* | Pirin | Xp22.2 |
| *PLOD* | Procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase 1 | 1p36.22 |
| *PPAR-g* | Peroxisome proliferator-activated receptor $\gamma$ | 3p25 |
| *PTN* | Pleiotrophin | 7q33-q34 |
| *RIZ1* | Retinoblastoma protein-interacting zinc finger protein | 1p36.21 |
| *ROR2* | Receptor tyrosine kinase-like orphan receptor 2 | 9q22 |
| *RUNX2* | Runt-related transcription factor 2 | 6p21 |
| *SFRP1* | Secreted frizzled-related protein 1 | 8p12-p11.1 |
| *SFRP2* | Secreted frizzled-related protein 2 | 4q31.3 |
| *SOST* | Sclerostin | 17q11.2 |
| *SREBF1* | Sterol regulatory element binding transcription factor 1 | 17p11.2 |
| *THSD4* | Thrombospondin, type I, domain containing 4 | 15q23 |
| *THSD7* | Thrombospondin, type I, domain containing 7A | 7p21.3 |
| *TIMP2* | Tissue inhibitor of metalloproteinase 2 | 17q25 |
| *TWIST1* | Twist homolog 1 | 7p21.2 |
| *WISP3* | WNT1 inducible signaling pathway protein 3 | 6q21 |
| *WNT10B* | Wingless-type MMTV integration site family, member 10B | 12q13 |
| *WNT3A* | Wingless-type MMTV integration site family, member 3A | 1q42 |
| *WNT7B* | Wingless-type MMTV integration site family, member 7B | 22q13 |

Adapted from Y. Guo *et al*.: *Expert Rev Endocrinol Metab* 3:223–267, 2008 (3), with permission from Expert Reviews Ltd. MMTV, Mouse mammary tumor virus.

fractures in 6752 Caucasian women (12). Other positive results included associations between G2014A polymorphisms and osteoporosis (13) and between *rs3020314* and *rs1884051* and hip fractures (14). For *ER-*$\beta$, polymorphisms of *rs960070* were associated with susceptibility to fractures (14). The effects of individual polymorphisms of *ER-*$\alpha$ and *ER-*$\beta$ on osteoporosis warrant further confirmatory studies. These results are important and have the potential to impact therapy of osteoporosis through the development of estrogen replacement therapy and selective ER modulators that may be based on individual genetic makeup.

**TABLE 2.** Association studies for osteoporosis-related phenotypes in humans (published between October 2007 and November 2009)

| Gene | Marker locus | Population characteristics | Phenotype | *P* value | Ref. |
|---|---|---|---|---|---|
| *ADCY10* | 16 SNPs | 1,692 premenopausal Caucasian women and 715 Caucasian men | BMD at LS | <0.01 | 36 |
| *ALPL* | 3 SNPs | 360 postmenopausal Hungarian women | BMD at LS, total hip and distal radius, non-vertebral fracture | NS | 137 |
| *ALOX5* | rs7084793, rs378090, rs3802548 | 1,688 premenopausal European-American sisters, 512 premenopausal African-American sisters, and 715 European-American brothers | BMD at LS and FN | ≤0.05 | 138 |
| *ALOX15* | rs2619112, rs916055 | 942 southern Chinese women, with either low or high BMD | High BMD in premenopausal women and low BMD in postmenopausal women | <0.05 | 139 |
| | rs7220870 (G48924T) | 6,752 Caucasian women recruited at four U.S. clinical centers and enrolled into the Study of Osteoporotic Fractures | Hip fracture | <0.05 | 12 |
| *ANXA6* | rs9324679, rs9324677, rs10037814, rs11960458, and haplotypes | 443 Korean patients with osteonecrosis of the femoral head and 273 Korean control | Osteonecrosis of the femoral head | <0.05 | 140 |
| *APC* | rs459552, rs4705573, rs6594646 | 862 Caucasian men aged ≥65 yr | BMD at LS and FN | <0.05 | 141 |
| *AR* | CAG repeat | 299 Swedish men aged 41–76 yr, from the population register in the county of Uppsala | BMD at LS, FN, and total body modified by androgen levels | <0.05 | 142 |
| | | 2,693 European men aged 40–79 yr | Ultrasound BMD | 0.021 | 143 |
| *ARHGEF3* | rs7646054 | 769 Caucasian women from 335 families, recruited in Australia and the UK | BMD at LS, FN, and total hip | 0.0007–0.041 | 144 |
| *BMP2* | rs2273073 (Ser37Ala) | 192 Chinese including 57 OPLL patients and 135 controls | Occurrence of OPLL in the cervical spine | <0.05 | 145 |
| | rs235764 (A125611G) | 6,752 Caucasian women recruited at four U.S. clinical centers and enrolled into the Study of Osteoporotic Fractures | Vertebral fracture | <0.05 | 12 |
| *BMP7* | rs17404303 | 920 European-Americans from 374 Diabetes Heart Study families | Quantitative CT at thoracic spine and LS | 0.03, 0.02 | 146 |
| *BMPR1B* | rs1434536, rs3796443 | 862 Caucasian men aged ≥65 yr | BMD at LS | <0.05 | 141 |
| *CA8* | rs6984526 | 337 Japanese women with osteoporosis | BMD at LS and FN | 0.00017, 0.00029 | 147 |
| *CA10* | rs2106329 | 337 Japanese women with osteoporosis | BMD at FN | 0.00002 | 147 |
| *CALM1* | rs12885713 | 158 patients with idiopathic knee osteoarthritis and 193 controls in a Greek Caucasian population | Knee osteoarthritis | NS | 148 |
| *CAR* | rs2502815 | 548 healthy Japanese postmenopausal women | BMD at LS and total body | 0.0185, 0.0416 | 149 |
| *CASR* | rs1801725 (A986S) | 176 premenarche Chinese girls aged 9–11.5 yr | Increased BMD at LS and BMD at Ward's triangle | 0.022, 0.049 | 150 |
| *CD40* | rs1883832 | 602 postmenopausal Spanish women | BMD at LS and FN, osteopenia or osteoporosis at LS and FN | <0.05 | 151 |
| *CLCN7* | 6 SNPs and 1 VNTR | 1,692 healthy premenopausal white sisters aged 33.1 ± 7.2 yr and 715 healthy white brothers aged 33.6 ± 10.9 yr | BMD at LS and FN | NS | 152 |
| *CNR2* | 16 SNPs | 574 Caucasians from 126 two- to four-generation pedigrees | BMD at radiographic hand breaking bending resistance index | 0.007–0.008, 0.001–0.003 | 153 |
| | rs2501431 | 1,243 Chinese subjects | BMD at hip and trochanter | 0.02, 0.04 | 154 |
| *COL1A1* | rs1800012 (Sp1, +1245G /T) | 21 osteoporotic patients with proximal femur fracture and 21 controls | BMD at FN, Ward's triangle, trochanter and total body | NS | 155 |
| | rs2412298 (−1663indelT), rs1800012 (Sp1, +1245G /T) | 124 females aged 50 to 70 yr cases and 150 controls from Volga-Ural region | Traumatic fracture | <0.05 | 156 |

(*Continued*)

**TABLE 2.** Continued

| Gene | Marker locus | Population characteristics | Phenotype | *P* value | Ref. |
|---|---|---|---|---|---|
| | rs1107946, rs2412298 (−1663indelT), rs1800012 (Sp1, +1245G /T) | 98 consecutive patients including 75 women and 23 men who were admitted to Aberdeen Royal Infirmary with low-trauma hip fractures; 3275 perimenopausal women from the Aberdeen Prospective Osteoporosis Screening Study and 143 postmenopausal women from the same region | Material density of the bone core, yield strength, and toughness | <0.0001 | 23 |
| *COL1A2* | rs42524 | 2,004 elderly Swedish men | BMD at several skeletal sites | <0.05 | 24 |
| *COMT* | rs4680 (Val158met) | 2,822 Swedish men aged 75.4 ± 3.2 yr | Early fractures (≤50 yr of age) | <0.05 | 157 |
| *CRHR1* | rs1876828 | 157 men long-term survived of ALL at St. Jude Children's Research Hospital | BMD at LS | 0.02 | 158 |
| *CRTAP* | rs7623768, rs4076086-rs7623768 haplotype | 1080 Chinese females | BMD at FN | 0.009, 0.003 | 159 |
| *CTR* | AluI | 201 Korean men aged 51.6 ± 11.7 yr | QUS | <0.05 | 160 |
| | AluI | 301 healthy young Caucasian men aged 18–25 yr | BMD, BMC, and geometrical areas at specific skeletal sites of the forearm | NS | 161 |
| *CYP1B1* | rs1056836 (Val432Leu) | 468 Caucasian postmenopausal women (220 from St. Louis, MO, mean age 63.5 ± 0.53 yr; and 248 from Palermo, Italy, mean age 72.9 ± 0.44 yr) | BMD at LS and FN | 0.03, 0.03 | 162 |
| | rs1056836 (L432V) | 124 Japanese women, diagnosed with osteopenia or osteoporosis and taking hormone therapy for 12 months | Lumbar BMD and low-density lipoprotein cholesterol before and after hormone therapy | <0.05 | 163 |
| *CYP17A1* | rs743572, rs743575 | 2,693 European men aged 40–79 yr | Ultrasound BMD | 0.018, 0.025 | 143 |
| *CYP19* | $(TTTA)_{7\text{-}3}$/*ER-α* $(TA)_{19}$ combination, | 92 Croatian males aged 21–35 yr | BMD at LS | 0.02 | 11 |
| | $(TTTA)_{7\text{-}3}$/ER-α $(TA)_{21}$ combination | | BMD at LS, FN, and total hip | 0.02, 0.02, 0.008 | |
| *CYP19A1* | rs11575899 | 2,693 European men aged 40–79 yr | Ultrasound BMD | <0.01 | 143 |
| | rs1062033 | 1,163 postmenopausal Spanish women | BMD at hip | <0.05 | 164 |
| *DBP* | rs222029, rs222020 | 1,873 Caucasians from 405 nuclear families | CSI at FN | 0.0019, 0.0042 | 131 |
| *DKK2* | rs17037102, rs6827902, rs17037297 | 344 Australian women with extreme BMD, aged 55–80 and >5 yr postmenopausal | BMD, BMC at total hip | <0.05 | 18 |
| *DMP1* | rs1381632 | 862 Caucasian men aged ≥65 yr | BMD at FN | <0.05 | 141 |
| *ENPP1* | rs1974201 | 1,513 unrelated subjects from the Framingham Offspring cohort | FNW | $3.8 \times 10^{-7}$ | 165 |
| *ER-α* | rs2228480 (G2014A) | 640 Mexican natives including 70 osteoporotic women, 70 nonosteoporotic women, and 500 subjects from the Mexican population | Osteoporosis | <0.05 | 13 |
| | rs2234693 (*Pvu*II) | 228 premenarche Chinese girls aged 9–11.5 yr | BMD at total body and femoral intertrochanter | 0.010, 0.038 | 9 |
| | rs2234693 (*Pvu*II), rs9340799 (*Xba*I) | 158 healthy Chinese adolescent girls aged 12–14 yr | BMD and bone turnover markers | NS | 166 |
| | rs2234693 (*Pvu*II), rs9340799 (*Xba*I) | 146 Chinese boys aged 13–17 yr | BMD at total body, forearm and LS influenced by the first spermorrhea | <0.05 | 10 |
| | rs2234693 (*Pvu*II), rs9340799 (*Xba*I) | 42 Turkish including 21 osteoporotic patients with proximal femur fracture and 21 controls | BMD at FN, Ward's triangle, and trochanteric | NS | 155 |
| | rs2234693 (*Pvu*II) –rs9340799 (*Xba*I) haplotype | 691 postmenopausal Chinese women aged 45–65 yr | Decreased BMD at whole body, LS, and hip | <0.05 | 8 |
| | rs2234693 (*Pvu*II)- *IL-6* rs2010963 (634C/G) interaction | 228 premenarche Chinese girls aged 9–11.5 yr | BMD at total left hip and femoral intertrochanter | 0.009, 0.007 | 9 |
| | rs1801132, rs726282 | 2,693 European men aged 40–79 yr | Ultrasound BMD | 0.002, 0.019 | 143 |
| | rs3020314, rs1884051 | 350 Chinese with osteoporotic hip fractures and 350 Chinese controls | Hip fracture | 0.0004, 0.0004 | 14 |

(*Continued*)

**TABLE 2.** Continued

| Gene | Marker locus | Population characteristics | Phenotype | *P* value | Ref. |
|---|---|---|---|---|---|
| | rs1801132 | 6,752 Caucasian women recruited at four U.S. clinical centers and enrolled into the Study of Osteoporotic Fractures | Vertebral fracture | <0.05 | 12 |
| | $(TA)^{19}$ repeat | 92 unrelated healthy males aged 21–35 yr | BMD at LS, trochanter | 0.006, 0.02 | 11 |
| | $(TA)_{21}$ repeat | | BMD at LS, trochanter, and total hip | 0.04, 0.02, 0.03 | |
| | rs2234693, rs1884052, rs3778099 | 425 Chinese adults | BMD | NS | 71 |
| *ER-β* | CA repeat | 400 Chinese women including 78 FN PMO cases and 122 controls | Postmenopausal osteoporosis | 0.001 | 167 |
| | | 108 LS PMO cases and 92 controls | | 0.023 | |
| | rs1256063, rs1256049, rs1256031 | 2,693 European men aged 40–79 yr | Ultrasound BMD | NS | 143 |
| | rs960070 | 350 Chinese with osteoporotic hip fractures and 350 Chinese controls | Hip fracture | 0.0070 | 14 |
| *ESRRA* | 23 bp repeat polymorphism in promoter | 673 premenopausal women from the Toronto metropolitan area | BMD at LS | NS | 168 |
| *FABP3* | rs10914367 | 360 postmenopausal Hungarian women | BMD at total hip | 0.028 | 137 |
| *FGFR1* | rs6996321 | 360 postmenopausal Hungarian women | BMD at LS | 0.002 | 137 |
| *FGFR2* | rs7916940 | 862 Caucasian men aged ≥65 yr | BMD at FN | <0.05 | 141 |
| *FLNB* | rs7637505, rs9822918, rs2177153, rs2001972 | 1,085 UK female twins and 1,315 Australian women | BMD at LS and FN | <0.05 | 169 |
| | rs9828717 | 1,080 Chinese females | BMD at LS | <0.05 | 159 |
| *FLT1* | rs1408245 | 862 Caucasian men aged ≥65 yr | BMD at FN | <0.05 | 141 |
| *FOXC2* | rs3751797 | 862 Caucasian men aged ≥65 yr | BMD at LS | <0.05 | 141 |
| *FZD1* | rs2232157, rs2232158 | 1,084 African men | BMD at FN, bone size at the radius, strength-strain index | <0.05 | 170 |
| *FZD6* | rs3808553 (L345 M), rs12549394 (E664A) | 371 postmenopausal Korean women | BMD at LS and FN | NS | 171 |
| *GDF5* | rs143383 | 2,487 European cases and 2,018 age-matched controls from the UK and Spain | Osteoarthritis | <0.05 | 172 |
| | rs143383 | 6,365 Caucasian elderly subjects | Hip axis length and nonvertebral fractures | 0.0004, 0.02 | 173 |
| *GHRH* | rs4988492 | 498 men and 468 women aged 59–71 yr from the Hertfordshire Cohort Study | BMC and BMD at proximal femur and LS | <0.05 | 174 |
| *GR* | rs1866388 | 400 Chinese women and 400 Chinese men | Extreme age-adjusted BMD at hip | 0.028 | 175 |
| *HMGA2* | rs1042725 | 1,680 Afro-Caribbean men aged ≥40 yr and 1,548 Caucasian American men aged ≥69 yr | Trabecular volumetric BMD | 0.007 | 176 |
| | | | | 0.0007 | |
| *HOXA* | rs6951180, rs6964896 | 862 Caucasian men aged ≥65 yr | BMD at FN and LS | <0.05 | 141 |
| *HSD11B1* | 6 SNPs | 1,392 postmenopausal Korean women | BMD at FN and vertebral fracture | <0.05 | 177 |
| *IGFBP2* | rs10932669 | 862 Caucasian men aged ≥65 yr | BMD at LS | <0.05 | 141 |
| *IL-6* | rs603573 (−174G/C), CA repeat | 640 Mexican natives including 70 osteoporotic women, 70 nonosteoporotic women, and 500 subjects from the Mexican population | BMD at LS | < 0.0001 | 178 |
| | rs603573 (−174G/C) | 267 postmenopausal women of Wielkopolska region aged 58.5 ± 5.9 yr | BMD at LS | NS | 179 |
| | rs603573 (−174G/C) | 42 Turkish including 21 osteoporotic patients with proximal femur fracture and 21 controls | BMD at FN, Ward's triangle, and trochanteric | NS | 155 |
| | rs2010963 (−634C/G) | 228 premenarche Chinese girls aged 9–11.5 yr | Percentage accrual in BMD of total body and femoral trochanter | 0.032, 0.048 | 9 |
| | rs2010963 (−634C/G) | 176 premenarche Chinese girls aged 9–11.5 yr | Increased BMD at total body and femoral trochanter | 0.027, 0.028 | 150 |
| | rs2010963 (−634C/G) interaction with ER-α *Pvu*II | 228 premenarche Chinese girls aged 9–11.5 yr | BMD at total left hip and femoral intertrochanter | 0.009, 0.007 | 9 |

(*Continued*)

**TABLE 2.** Continued

| Gene | Marker locus | Population characteristics | Phenotype | *P* value | Ref. |
|---|---|---|---|---|---|
| *IL6R* | rs3887104, rs4845617, rs8192284 | 559 postmenopausal Spanish women | BMD at FN BMD at LS | 0.011, 0.025, 0.038 | 180 |
| *IL-15* | rs2857261, rs10519613, rs56245420, rs1057972 | 1,921 postmenopausal Korean women | BMD at LS and FN | <0.05 | 181 |
| *IL-23R* | rs4655686, rs1569922, rs7539625 | 443 patients with osteonecrosis of the femoral head and 273 control subjects enrolled in Korea | Osteonecrosis of the femoral head | 0.0198–0.0447 | 182 |
| *ITGA1* | 8 SNPs | 946 postmenopausal Korean women | BMD at proximal femur and LS | 0.009–0.05, 0.002–0.005 | 183 |
| *LEPR* | rs1137100 (Lys109Arg) | 145 premenopausal and 118 postmenopausal Korean women | BMD at total hip | 0.044 | 184 |
| *LHB* | rs2013040 | 2,693 European men aged 40–79 yr | Ultrasound BMD | NS | 143 |
| *LHCGR* | rs6545061 | 2,693 European men aged 40–79 yr | Ultrasound BMD | 0.023 | 143 |
| *LRP1* | rs4759044 and rs4759044-rs11172113 haplotype | 344 Australian women with extreme BMD, aged 55–80 and >5 yr postmenopausal | BMD, BMC at total hip and FN area | <0.05 | 18 |
| *LRP5* | rs3781590 | 344 Australian women with extreme BMD, aged 55–80 and >5 yr postmenopausal | BMD at total hip | 0.0006 | 18 |
| | rs3736228 (Ala1330Val) | 6,082 men from three cohorts (MrOS Sweden: n = 3,014, aged 69–81 yr; MrOs Hong Kong: n = 2,000, aged >65 yr; Swedish GOOD study: n = 1,068, aged 18–20 yr) | BMD at LS and FN | NS | 21 |
| | rs3736228 (Ala1330Val) | 739 postmenopausal Japanese women | BMD at total body | 0.0026 | 185 |
| | rs3736228 (Ala1330Val) | Meta-analysis | BMD at LS and FN | 0.55, 0.05 | 186 |
| | rs3736228 (Ala1330Val) | Meta-analysis | BMD at LS, FN, and trochanter | <0.001, <0.001, 0.053 | 187 |
| | rs4988321 (Val667Met) | 3,800 men from two cohorts (MrOS Sweden: n = 3,014, aged 69–81 yr; Swedish GOOD study: n = 1,068, aged 18–20 yr) | BMD at LS | <0.05 | 21 |
| | rs4988321 (Val667Met) | 673 premenopausal women from the Toronto metropolitan area | BMD at LS | 0.015 | 168 |
| | rs3736228 (Ala1330Val), rs4988321 (Val667Met) | 589 physically active Caucasian men aged 20–30 yr | BMD at LS and whole body BMD at FN | <0.02, 0.04 | 188 |
| | rs3736228 (Ala1330Val), rs4988321 (Val667Met) | 37,534 subjects from 18 participating teams in Europe and North America | BMD at LS and FN, prevalence of all fractures and vertebral fractures | $<10^{-7}$ | 20 |
| | rs312009, rs2508836, rs729635, rs643892 | 964 postmenopausal Spanish women | BMD at LS and FN, fracture | <0.05 | 189 |
| | rs682429, rs686921 | 286 young southern Chinese females, aged 22–44 yr with low BMD | BMD at LS and hip | <0.05 | 190 |
| | rs4988330, rs4988331, rs312786 | 608 with OA (348 undergoing hip replacement surgery and 260 knee surgery) and 520 with hip fractures | Osteoporosis and osteoarthritis | 0.008–0.03 | 191 |
| | rs41494349 (266A>G) | 800 healthy recently perimenopausal Danish women | Parameters of hip geometry | NS | 192 |
| | rs545382, rs2277268 | 126 prepubertal children (64 with premature adrenarche and 62 controls) | BMD at LS and FN | <0.05 | 193 |
| | rs3736228 | 652 Slovenian subjects | BMD at LS and FN | NS | 194 |
| *LRP6* | rs2075241, rs11054704 | 344 Australian women with extreme BMD, aged 55–80 and >5 yr postmenopausal | BMD and BMC at total hip | <0.05 | 18 |
| | rs2302685 (Ile1062Val) | 37,534 subjects from 18 participating teams in Europe and North America | BMD at LS and FN, prevalence of all fractures and vertebral fractures | NS | 20 |

(*Continued*)

**TABLE 2.** Continued

| Gene | Marker locus | Population characteristics | Phenotype | *P* value | Ref. |
|---|---|---|---|---|---|
| | rs1181334 | 608 with OA (348 undergoing hip replacement surgery and 260 knee surgery) and 520 with hip fractures | Osteoporosis and osteoarthritis | <0.05 | 191 |
| | rs2302685 | 652 Slovenian subjects | BMD at LS and FN | NS | 194 |
| *LTBP2* | 16 SNPs | 1,459 subjects in 306 southern Chinese pedigrees, 706 and 760 case-control subject pairs with extremely high and low trochanter and total hip BMD | BMD at total hip and fracture | 0.0004, 0.01 | 195 |
| *MDR1* | rs1045642 (C3435T) rs2032582 (G2677T) | 127 Chinese systemic lupus erythematosus patients | Development of osteonecrosis of femoral head observed by magnetic resonance imaging | 0.038 0.05 | 196 |
| *MMP2* | 5 SNPs | 360 postmenopausal Hungarian women | BMD at LS, total hip and distal radius, nonvertebral fracture | NS | 137 |
| | rs243865 (C595T) | 6,752 Caucasian women recruited at four U.S. clinical centers and enrolled into the Study of Osteoporotic Fractures | Vertebral fracture | <0.05 | 12 |
| *MSTN* | 3 SNPs | 1,260 Chinese from 401 nuclear families | BMD at proximal femur and LS | <0.05 | 197 |
| *MTHFR* | rs1801133 (677C>T) | 3,196 children aged 9.9 yr from the Avon Longitudinal Study of Parents and Children | BMD at LS | <0.001 | 198 |
| | rs1801133 (677C>T) | 1,243 Chinese subjects | BMD at FN, hip, and trochanter | 0.02–0.05 | 154 |
| | rs1801133 (677C>T) | 800 healthy recently perimenopausal Danish women | Parameters of hip geometry | NS | 192 |
| *NFATC1* | rs177820 | 862 Caucasian men aged ≥65 yr | BMD at LS | <0.05 | 141 |
| *NOG* | 7 SNPs | 2,060 Afro-Caribbean men age 40 and older | BMD at the proximal femur and LS | NS | 199 |
| *NOS3* | 18 SNPs and haplotypes | 1,451 subjects from Framingham Offspring Cohort | Bone density/ultrasound and geometry | NS | 200 |
| | rs2070744 [T(−786)C] | 167 Americans including 95 cases with femoral head necrosis and 72 controls | Osteonecrosis of femoral head | <0.05 | 201 |
| | rs2070744 [T(−786)C] | 305 postmenopausal Turkish females | BMD at femoral trochanter and LS | 0.046, 0.005 | 202 |
| *NPY* | rs16135, rs16123 | 1,113 randomly selected men of African ancestry | BMD at proximal femur | <0.05 | 203 |
| *OCIL* | rs16914640 (Asn19Lys) | 500 postmenopausal Spanish women | BMD at LS and FN | <0.05 | 204 |
| *OPG* | rs2073618 (Lys3Asn) | 69 premenopausal and 263 postmenopausal Spanish women | BMD at LS | <0.05 | 205 |
| | rs2073618 (Lys3Asn) | 6,695 Caucasian women aged 65 yr and older in the United States | BMD at calcaneus and LS, FN fracture | <0.05 | 16 |
| | rs237025 (A163G) | 87 postmenopausal Caucasian women | Calcaneal velocity of sound | 0.0102 | 206 |
| | G209A, T245G | 42 Turkish including 21 osteoporotic patients with proximal femur fracture and 21 controls | BMD at FN, Ward's triangle, and trochanter | NS | 155 |
| | rs2073618 (K3N) | 239 osteoporotic and 228 nonosteoporotic postmenopausal, 57 premenopausal women, and 117 elderly men | BMD at LS, FN, and total hip | 0.021, 0.041, 0.032 | 5 |
| | rs1032129 | 964 postmenopausal Spanish women | BMD at FN | 0.001 | 207 |
| *PBX1* | rs2800791, rs9661977 | 720 southern Chinese subjects from 231 families; another independent unrelated southern Chinese cohort with a total of 835 extremely high and extremely low BMD subjects; 1,268 case-control Japanese subjects with 703 osteoporotic subjects and 565 normal controls | BMD at LS | 0.004, 0.050 | 208 |
| *PIR* | rs5935970 | 4,000 Chinese subjects | BMD at LS | <0.05 | 209 |
| *PLOD* | rs7529452, rs2273291, rs7514577 | 1,243 Chinese subjects | BMD | <0.05 | 154 |
| *PPAR-γ* | rs1801282 rs1801282-rs3856806 haplotype | 239 Korean women with mean age 51 yr | Serum OPG level | 0.035 0.010 | 210 |

(*Continued*)

**TABLE 2.** Continued

| Gene | Marker locus | Population characteristics | Phenotype | *P* value | Ref. |
|---|---|---|---|---|---|
| | rs12486170 (−796A>G), rs1801282 (+34C>G), rs3856806 (+82466C>T) | 448 patients with osteonecrosis of the femoral head and 336 control subjects | Osteonecrosis of the femoral head | NS | 211 |
| *P2X7* | rs3751143 (Glu496Ala), rs1653624 (Ile568Asn) | 800 healthy recently perimenopausal Danish women | Parameters of hip geometry | NS | 192 |
| *PRL* | rs7739889 (T228C) | 6,752 Caucasian women recruited at four U.S. clinical centers and enrolled into the Study of Osteoporotic Fractures | Vertebral and hip fracture | <0.05 | 12 |
| *PTH* | 3 haplotypes | 1,044 women aged 75 yr from the Malmo Osteoporosis Prospective Risk Assessment study | Fractures | 0.038 | 212 |
| *PTHRLH* | rs805512, rs10492364, rs1268693 | 1,044 women aged 75 yr from the Malmo Osteoporosis Prospective Risk Assessment study | BMD at LS and FN, fracture | NS | 212 |
| | VNTR | 234 young Finnish males | BMD, BUA, and SOS at LS | <0.0034 | 213 |
| *PTHR1* | rs6442037, rs724449, rs7652849 | 1,044 women aged 75 yr from the Malmo Osteoporosis Prospective Risk Assessment study | BMD at LS and FN, fracture | NS | 212 |
| | rs724448-rs2242116 haplotype | 1,080 Chinese females | BMD at LS and FN | 0.02, 0.044 | 159 |
| *PTHR2* | rs9288393, rs10497900, rs897083 | 1,044 women aged 75 yr from the Malmo Osteoporosis Prospective Risk Assessment study | BMD at LS and FN, fracture | NS | 212 |
| *PTN* | rs322297 | 862 Caucasian men aged ≥65 yr | BMD at FN | <0.05 | 141 |
| *RANK* | +35966insdelC | 467 postmenopausal Slovenian women | BMD at LS, FN, and hip | 0.020, 0.034, 0.024 | 214 |
| *RANKL* | −290C>T, −643C>T, −693G>C<br>CCG, TTC haplotypes | 404 postmenopausal Slovenian women aged 42–91 yr | BMD at LS | 0.001, 0.041, 0.013<br>0.005, 0.007 | 17 |
| | −290C>T, | 239 osteoporotic and 228 nonosteoporotic postmenopausal, 57 premenopausal women, and 117 elderly men | BMD at LS | 0.017 | 5 |
| | −290C>T-K3N combination | | BMD at FN and total hip | 0.029, 0.041 | |
| | rs12585014, rs7988338, rs2148073 | 1,873 Caucasians from 405 nuclear families | CSI at FN | 0.0007, 0.0007, 0.0005 | 130 |
| *RIZ1* | Pro704 ins/del | 2,424 men and 3,517 women from the Rotterdam study | BMD at LS and FN, fracture | NS | 215 |
| *ROR2* | 19 SNPs | 705 Caucasians from 212 nuclear families | Bone length and BMD for hand bones, proximal phalanges and metacarpal bones | <0.05 | 216 |
| *Runx2* | 4 SNPs and haplotypes | 743 Caucasians from 212 nuclear families | Hand bone length and BMD | <0.05 | 217 |
| | rs7771980 | 729 postmenopausal Korean women | BMD at LS, trochanter and total femur | 0.02, 0.05, 0.04 | 218 |
| *SFRP1* | rs921142, rs4736965, and their haplotype | 344 Australian women with extreme BMD, aged 55–80 and >5 yr postmenopausal | BMD and BMC at total hip | <0.005 | 18 |
| | rs16890444, rs3242 | 931 Japanese women | BMD at LS, FN, and hip | 0.037, 0.025, 0.027 | 219 |
| *SFRP2* | 4 SNPs | 344 Australian women with extreme BMD, aged 55–80 and >5 yr postmenopausal | BMD, BMC at total hip and FN | NS | 18 |
| *SHBG* | rs1799941, rs6259, TAAAA repeat in promoter | 2,693 European men aged 40–79 yr | Ultrasound BMD | NS | 143 |
| | rs1799941, rs6259 | 213 healthy postmenopausal Caucasian women | BMD at the proximal femur sites | <0.05 | 220 |

(*Continued*)

**TABLE 2.** Continued

| Gene | Marker locus | Population characteristics | Phenotype | *P* value | Ref. |
|---|---|---|---|---|---|
| *Smad6* | rs755451 | 721 postmenopausal Japanese women | BMD at total body and LS | 0.0004, 0.005 | 221 |
| *SOST* | rs851054, rs851056 | 344 Australian women with extreme BMD, aged 55–80 and >5 yr postmenopausal | BMD and BMC at total hip | 0.004–0.008 | 18 |
| | rs1230399 | 1,243 Chinese subjects with low BMD and high BMD | BMD at LS, FN, trochanter and, total hip, osteoporosis | 0.004–0.03 0.005 | 222 |
| | rs1534401, rs1877632 rs851054, rs851056 | 862 Caucasian men aged ≥65 yr | BMD at LS | <0.05 | 141 |
| | rs10534024 | 652 Slovenian subjects | BMD at LS and FN | NS | 194 |
| *SPARC* | 1046C-1599C-1970T, 1046C-1599G-1970T haplotypes | 115 middle-aged Caucasian men with idiopathic, low turnover osteoporosis (n = 56) and matched controls (n = 59) | BMD at LS, hip, and radius | <0.05 | 223 |
| *SRD5A2* | rs632148, rs523349, rs765138, rs7571644, TA repeat in exon 5 | 2,693 European men aged 40–79 yr | Ultrasound BMD | NS | 143 |
| *SREBF1* | rs12601420 (IVS6-48 C>T), rs9925115 (IVS7+117A>G) | 423 patients with osteonecrosis of the femoral head and 348 controls | Osteonecrosis of the femoral head | <0.0001 | 224 |
| *TGFB1* | rs1800469 (1348 C>T), rs1800472 (788 C>T) | 28,924 subjects from 10 European research studies | BMD at LS, vertebral fracture | <0.05 | 225 |
| *THSD4* | rs10851839 | Japanese women with osteoporosis aged 72.7 ± 7.3 yr | BMD at LS and FN | 0.0092, 0.0046 | 226 |
| *THSD7A* | rs12673692 | 337 Japanese women with osteoporosis aged 72.7 ± 7.3 yr | BMD at LS and FN | 0.00017, 0.036 | 226 |
| *TIMP2* | 7 SNPs | 360 postmenopausal Hungarian women | Nonvertebral fracture | 0.0187 | 137 |
| *TNF-α* | rs1800629 (−308G/A) | 267 postmenopausal women of Wielkopolska region aged 58.5 ± 5.9 yr | BMD at LS | NS | 179 |
| | rs1800629 (−308 G/A) | 159 Portuguese | BMD at LS and hip | NS | 227 |
| | rs1799964 (−1031 T>C) | 377 postmenopausal Korean women | Osteoporosis | <0.05 | 228 |
| *TNF-β* | rs909253 (A252G) | 377 postmenopausal Korean women | Osteoporosis | <0.05 | 228 |
| *TNFRII* | rs1061622 (T676G), rs3397 (1690T>C), A1663G | 377 postmenopausal Korean women | Osteoporosis, bone turnover markers | <0.05 | 228 |
| *TNFRSF1B* | rs976881 | 1,243 Chinese subjects | BMD | 0.04–0.08 | 154 |
| *TSHR* | rs1991517 (Asp727Glu) | 4,934 elderly Caucasian men and women of the Rotterdam Study | BMD at FN | <0.05 | 229 |
| *TWIST1* | BD0027 (+1871A>G) | 729 postmenopausal Korean women | Osteoporosis, BMD at FN | 0.02, 0.039 | 230 |
| *VEGF* | rs2010963 (−634G>C) | 814 Korean including 317 osteonecrosis of the femoral head cases and 497 controls | Osteonecrosis of the femoral head cases | 0.015 | 231 |
| | rs2010963 (−634 G>C), rs3025039 (+936 C>T) | 252 postmenopausal Caucasian women aged 46–80 yr | BMD at LS | 0.017 | 232 |
| | | | | 0.05 | |
| *VDR* | rs11568820 (Cdx-2) | 190 postmenopausal Brazilian women | BMD at LS, FN, great trochanter, and Ward's triangle with different physical activity level | <0.05 | 6 |
| | rs10735810 (*Fok*I) | 192 postmenopausal active women | BMD at FN and Ward's triangle | <0.05 | 233 |
| | rs1544410, rs7975232, rs731236 (*Bsm*I, *Apa*I, *Taq*I) | 246 postmenopausal Turkey women including 100 osteoporosis and 146 controls | Osteoporosis | NS | 234 |
| | rs1544410, rs7975232, rs731236 (*Bsm*I, *Apa*I, *Taq*I) | 47 Caucasian patients with ulcerative colitis and 47 control subjects matched for age and gender | BMD at LS | <0.05 | 235 |
| | rs1544410, rs7975232, rs731236 (haplotype BBAAtt) | 147 postmenopausic Venezuelan women including 71 cases with osteoporosis and 76 controls | Osteoporosis | <0.05 | 236 |

(*Continued*)

**TABLE 2.** Continued

| Gene | Marker locus | Population characteristics | Phenotype | *P* value | Ref. |
|---|---|---|---|---|---|
| | rs1544410, rs7975232, rs731236, rs10735810 (*Bsm*I, *Apa*I, *Taq*I, *Fok*I) | 238 Polish women including 75 premenopausal cases with Graves' disease and 163 controls | BMD at LS and FN | NS | 237 |
| | rs1544410, rs7975232, rs731236, rs10735810 (*Bsm*I, *Apa*I, *Taq*I, *Fok*I) | 126 Chilean elderly women aged 65–94 yr including 67 hip fractures and 59 controls | Hip fracture | NS | 238 |
| | rs1544410 (*BsmI*) | 301 healthy young Caucasian men aged 18–25 yr | BMD, BMC and geometrical areas at specific skeletal sites of the forearm | NS | 161 |
| | rs1544410 (*BsmI*) | 335 Korean women older than 65 yr with low calcium intake | BUA of calcaneus | 0.013 | 239 |
| | rs1544410 (*BsmI*) | 42 Turkish including 21 osteoporotic patients with proximal femur fracture and 21 controls | BMD at FN, Ward's triangle, and trochanteric | NS | 155 |
| | rs1544410 (*BsmI*) | 68 healthy, 54 osteopenic, and 64 osteoporotic Argentine postmenopausal women | BMD at LS and FN | <0.05 | 240 |
| | rs1544410 (*BsmI*) interaction with ER-α *Pvu*II and *Xba*I | 68 healthy, 54 osteopenic, and 64 osteoporotic Argentine postmenopausal women | BMD at FN | <0.05 | 240 |
| | rs7975232, rs731236 (*Apa*I, *Taq*I ) | 40 Lactating Brazilian adolescents aged 15–18 yr | BMD at LS and BMC at total body | <0.05 | 241 |
| | *Bsm*I, *Fok*I, *Cdx*2 and combination of K3N(OPG)-Cdx2 | 239 osteoporotic and 228 nonosteoporotic postmenopausal, 57 premenopausal women, and 117 elderly men | BMD at FN, LS, and total hip | <0.05 | 5 |
| | rs7975232 (*Apa*I) | 136 postmenopausal women | BMD at LS and hip | <0.05 | 242 |
| *WISP3* | 4 SNPs | 344 Australian women with extreme BMD, aged 55–80 and >5 yr postmenopausal | BMD, BMC at total hip and FN area | NS | 18 |
| *WNT3a* | rs708114, rs4653533, rs752107 | 344 Australian women with extreme BMD, aged 55–80 and >5 yr postmenopausal | BMD at total hip | <0.05 | 18 |
| *WNT7b* | 8 SNPs | 344 Australian women with extreme BMD, aged 55–80 and >5 yr postmenopausal | BMD at total hip | <0.05 | 18 |
| *WNT10b* | rs1051886, rs3741627 | 1,035 Afro-Caribbean men aged ≥40 yr and 980 Afro-Caribbean men | Cross-sectional area, periosteal circumference, BMD at hip and BMC at the radius and cortical | <0.05 | 243 |
| | 7 SNPs | 1,438 Caucasian postmenopausal women | BMD at LS and hip | NS | 244 |

Adapted from Y. Guo *et al.*: *Expert Rev Endocrinol Metab* 3:223–267, 2008 (3), with permission from Expert Reviews Ltd. ALL, Acute lymphoblastic leukemia; BMC, bone mineral content; BUA, broadband ultrasound attenuation; CSI, compression strength index; FNW, FN width; GOOD study, Gothenburg Osteoporosis and Obesity Determinants study; NS, not significant; PMO, postmenopausal osteoporosis; QUS, quantitative ultrasound; SOS, speed of sound; VNTR, variable number tandem repeat.

**B. Cytokines and receptors**

***1. OPG-RANKL system***

Two cytokines, osteoprotegerin (OPG) and receptor activator of nuclear factor κ-β ligand (RANKL), have been identified as important mediators in the pathogenesis of osteoporosis. OPG is a decoy receptor for RANKL. Its binding to RANKL blocks interaction of the latter with the receptor activator of nuclear factor κ-β (RANK) on the osteoclast surface, thereby inhibiting bone resorption (15). Moffett *et al.* (16) investigated the association between the Lys3Asn polymorphism (G-to-C polymorphism at codon 3 in exon 1) in the *OPG* gene and both BMD and the risk of fractures in 6695 white women. Women homozygous for the G (Lys) allele had significantly lower BMD at the intertrochanter, distal radius, and lumbar spine (LS) than those with the C (Asn) allele. Additionally, compared with women with the G/G (Lys-Lys) genotype, those with the C/C (Asn-Asn) genotype had a 26% increased risk of hip fractures (95% confidence interval, 1.02–1.54) and 51% increased risk of FN fractures (95% confidence interval, 1.13–2.02) (16). In postmenopausal women, the promoter polymorphisms of *RANKL*, including −290C>T, −643C>T, −693G>C, and two common haplotypes, CCG and TTC, showed association with LS BMD (17). Interestingly, the −290C>T (*RANKL*)-K3N (*OPG*) combination was associated with total hip BMD and FN BMD in 239 osteoporotic and 228 non-osteoporotic postmenopausal women, respectively (5), suggesting the presence of gene-gene interactions between *RANKL* and *OPG* on BMD. Further confirmation of their combined influence in larger cohorts is needed.

#### *2. LRP5*

LRP5 functions as a cell-membrane coreceptor for Wnt proteins in the canonical Wnt signaling pathway (18). Several lines of evidence suggest that LRP5 may be a key determinant of bone mass (19). Val667Met and Ala1330Val polymorphisms of the *LRP5* gene have been widely studied, and both of these polymorphisms were consistently associated with LS BMD, FN BMD, and fracture risk across different Caucasian populations (20). Grundberg *et al.* (21) also showed an association between the Val667Met polymorphism and LS BMD by a meta-analysis in 3800 young and elderly men from Swedish cohorts. The approach of meta-analyses, by combining results across studies, is helpful in resolving problems of underpowered studies, revealing unexpected sources of heterogeneity, and resolving discrepancies in genetic studies (22).

### C. Bone matrix proteins

#### *1. COL1A1 and COL1A2*

Collagen type I is the most abundant protein in connective tissue and is essential for normal bone function. The collagen I triple helix consists of two $\alpha$1(I) chains and one $\alpha$2(I) chain encoded by the collagen $\alpha$1(I) (*COL1A1*) and collagen $\alpha$2(I) (*COL1A2*) genes, respectively. Genetic variants of these two genes may cause normal variation in BMD and bone strength. A common noncoding polymorphism lying in the transcription factor Specificity protein 1 (Sp1) binding site, named Sp1 polymorphism, is the most widely studied polymorphism in the *COL1A1* gene. Jin *et al.* (23) found that Sp1 was associated with biomechanical properties of bone and reduced bone quality. This polymorphism is one of the few polymorphisms that has been repeatedly associated with fractures and BMD (24). Genotypes of a common coding polymorphism in the *COL1A2* gene, *rs42524*, were tested for associations with bone phenotypes in 2004 elderly Swedish men (24). Associations were observed between *rs42524* genotype and BMD at several skeletal sites, and elderly Swedish men heterozygous for *rs42524* were found to have lower BMD than homozygous subjects (24).

### D. Limitations and improvements

Candidate gene association studies have substantial limitations for detecting the genetic basis of osteoporosis because this approach relies on selection of the correct genes for association studies based on either a biological hypothesis or the location of a particular gene in implicated linkage regions. In addition, most current candidate gene association studies in the bone field have generated inconsistent/inconclusive results. This limitation has been well addressed in our previous review (1). Several steps can be taken to increase the probability of discovering genes important to the pathogenesis of osteoporosis using candidate gene association studies, including: 1) controlling population stratification and enlarging sample size to increase statistical power; 2) using more efficient and reasonable methods, such as the single nucleotide polymorphism (SNP) spectral decomposition method, to correct for multiple testing by estimating the effective test number (25); 3) performing whole-gene analyses, rather than analyses for limited polymorphisms in the target genes, to explore all potential sequence variations within and around the gene of interest; and 4) taking into account the influence of gene-gene epistasis and gene-environment interactions on osteoporosis.

## III. GWL Studies

Generally, family-based GWL studies are conducted to examine whether any markers from panels of microsatellite markers spaced about uniformly throughout the entire human genome cosegregate with phenotypic traits. In contrast to candidate gene association studies, the GWL approach is robust with respect to population admixture/stratification. Because GWL studies do not rely on linkage disequilibrium among genes or markers in adjacent genomic regions, it is a promising approach for identifying genomic regions contributing to relatively large variations in complex traits, without any prior knowledge about the potentially important function of specific genomic regions. Both univariate linkage analyses and bivariate linkage analyses were used extensively in GWL studies between October 2007 and November 2009. Specific details regarding these two types of GWL studies are outlined in Table 3.

### A. Univariate linkage analyses

Most of the univariate GWL studies focused on BMD variations. Quantitative trait loci (QTLs) were identified on chromosomes 1q42-43, 11q12-13, 12q23-24, 17q21-23, 21q22, 22q11 (26), 1q36 (27) and 15q13 (28) for LS BMD; on chromosomes 5q31-33, 13q12-14 (26) for FN BMD; on chromosomes 12p12 and 15q26 for hip peak BMD (PBMD); and on chromosomes 2p13 and Xq27 for wrist PBMD (29). To identify genetic factors influencing bone loss, Shaffer *et al.* (30) measured the 5-yr change in BMD in 300 Mexican-Americans and found that chromosomes 6q and 3p were linked to BMD changes at the hip and the distal third of the ulna, respectively. Another frequently studied phenotype is bone size (BS). Preliminary evidence shows that spine length is linked to chromosome 5 (31). It is important to recognize that the univariate linkage analyses discussed above have limited statistical power and the linkage regions identified are relatively broad.

### B. Bivariate linkage analyses

Bivariate linkage analyses may improve statistical power considerably and facilitate the identification of

**TABLE 3.** GWL scans for osteoporosis-related phenotypes in humans (published between October 2007 and November 2009)

| Study subjects | No. of markers | Phenotype | Results | Candidate gene | Ref. |
|---|---|---|---|---|---|
| 300 Mexican-Americans (>45 yr of age) from the San Antonio Family Osteoporosis Study | 460 | ΔBMD at 33% ulna | 3p (81 cM), LOD = 1.9 | | 30 |
| | | ΔBMD at hip | 6q (103 cM), LOD = 1.75 | | |
| 103 Caucasian pedigrees (Network in Europe on Male Osteoporosis Family Study) ascertained through a male relative with low (Z-score ≤ −2) BMD values at either LS or FN | 441 | BMD at LS | 1q42-43, LOD = 1.75 | | 26 |
| | | | 11q12-13, LOD = 2.64 | *LRP5, TCIRG1* | |
| | | | 12q23-24, LOD = 1.65 | | |
| | | | 17q21-23, LOD = 3.63 | *COL1A1, SOST* | |
| | | | 21q22, LOD = 2.05 | *COL6, COL6A2* | |
| | | | 22q11, LOD = 2.74 | | |
| | | BMD at FN | 5q31-33, LOD = 1.53 | *RIL* | |
| | | | 3q12-14, LOD = 2.71 | *RANKL* | |
| 879 Caucasians of AFOS (mean age ± SD = 49.8 ± 16.1 yr, range 18–91 yr) from large multigenerational families | 731 | NN_ID | 1p35.5, LOD = 2.57; 2p25, LOD = 2.37 | *ALKPL* (1p35.5) | 245 |
| | | NN_Z | 1p36, LOD = 2.36; 14q23, LOD = 2.5 | *BMND3, MTHFR* (1p36) | |
| | | S_BR | 1q23-24, LOD = 2.65; 12q24.2, LOD = 2.63 | *SAC, Hypercalciuria* (1q23-24) | |
| | | S_Z | 4p11, LOD = 1.94 | | |
| | | S_ID | 5q24-30, LOD = 2.59 | *LOX* | |
| | | S_CSA | 11q22.1, LOD = 2.3 | | |
| | | NN_CSA | 2p11.2, LOD = 1.92; 6q22-23, LOD = 2.46 | *COL10A1, ENPP-1, WISP3* (6q22-23) | |
| | | | 17q11.2-12, LOD = 2.16 | *SOST* | |
| 2522 Caucasian females from 414 pedigrees | 410 | BMD at FN/AAM | 22q13, LOD = 3.33; 3q13, LOD = 2.31 | *EP300* (22q13) | 35 |
| | | BMD at LS/AAM | 22q13, LOD = 3.30; 15q13, LOD = 2.97 | | |
| | | | 3p25, LOD = 2.36; Xp22, LOD = 2.20 | *PPARG* (3p25) | |
| | | | Xq13, LOD = 2.18; Xq23, LOD = 2.53 | | |
| | | | Xq27, LOD = 2.34 | | |
| | | BMD at UD/AAM | 22q13, LOD = 3.12; 7p15, LOD = 2.44 | | |
| 2200 Caucasians aged 20–50 yr from 207 pedigrees | 410 | PBMD at wrist in total sample | 2p13, LOD = 2.04 | | 29 |
| | | | 10p14, LOD = 2.31 | | |
| | | | 14q23, LOD = 2.07 | *ER-β, BMP-4* | |
| | | | Xq27, LOD = 2.64 | | |
| | | PBMD at hip in total sample | 2p12, LOD = 2.79 | *MGP* | |
| | | | 22q13, LOD = 2.16 | *MCHR1* | |
| | | PBMD at wrist in female | 2p13, LOD = 2.64 | | |
| | | | 6q24, LOD = 1.91 | *ER-α* | |
| | | | 11q13, LOD = 1.97 | *FRA-1, LRP5, TCIRG1* | |
| | | | 18q21, LOD = 2.29 | | |
| | | PBMD at hip in male | 15q26, LOD = 2.93 | | |
| | | | 7p21, LOD = 2.10 | *IL-6* | |
| 4126 Caucasians for a composite osteoporosis phenotype that combines OF and low BMD | 393 | A composite osteoporosis phenotype that combines OF and low BMD in total sample | 14q32, LOD = 2.61; 7p14, LOD = 2.42; 11q25, LOD = 2.09; 9p21, LOD = 1.26 | *BMP4* | 246 |
| | | A composite osteoporosis phenotype that combines OF and low BMD in female | 14q22, LOD = 3.53; 9p21, LOD = 2.29; 7p14, LOD = 3.07 | *GLI3* | |
| | | Imprinting analyses by assigning weights to allele sharing specific to parental origins among affected sibling pairs | 1q42, LODFA = 2.12; 9q34, LODFA = 1.88; 7q22, LODMO = 1.67 | *COL1A2* | |

(*Continued*)

**TABLE 3.** Continued

| Study subjects | No. of markers | Phenotype | Results | Candidate gene | Ref. |
|---|---|---|---|---|---|
| 1473 Caucasians aged 31–96 yr in 323 pedigrees from the Framingham Osteoporosis Study | 636 | S_CSA/height | Chr. 2, LOD = 1.56; Chr. 4, LOD < 1.0<br>Chr. 6, LOD = 2.34; Chr. 9, LOD = 1.73 | *CALM2, FSHR, TGF α* (Chr. 2); *IL2* (Chr. 4) | 247 |
| | | NN_CSA/height | Chr. 9, LOD = 1.04 | | |
| | | FNL/height | Chr. 15, LOD = 1.57 | | |
| | | NN_Z/height | Chr. 4, LOD = 2.05 | | |
| | | IT_OD/height | Chr. 6, LOD = 2.13 | *ESR1, IGF2R* | |
| | | S_Z/height | Chr. 6, LOD = 2.08; Chr. 21, LOD = 2.45 | | |
| | | S_OD/height | Chr. X, LOD = 3.28 | | |
| 3899 Caucasians from 451 pedigrees | 410 | PC phenotype of BS at LS, hip, and wrist in total sample | 7q34, LOD = 2.85 | *IRF 5, Leptin* | 248 |
| | | PC phenotype of BS at LS, hip, and wrist in male | | | |
| | | PC phenotype of BS at LS, hip, and wrist in total female | 5q23, LOD = 2.39<br>11p11, LOD = 2.82<br>7q34, LOD = 1.79; 8q24, LOD = 2.30<br>21q21, LOD = 1.85 | <br>*EXT2* | |
| 34 Caucasians in a single extended family | 380 | BMD at LS | 1q36.3, LOD = 3.07 | *WDR8, EGFL3* | 27 |
| 4126 Caucasians from 451 pedigrees | 410 | aBMD/ABS at FN | 20q11, LOD = 3.11; Xp11, LOD = 2.46 | *GDF5* (20q11); *BMP15* (Xp11) | 32 |
| | | aBMD/ABS at LS | Xq27, LOD = 4.30; 12p11, LOD = 2.86<br>17q21, LOD = 2.44 | *BGN, IRAK1* (Xq27); *LRP6* (12p11)<br>*COL1A1, CHAD, HOXB, SOST* | |
| | | aBMD/ABS at UD forearm | 5q23, LOD = 3.01; Xq27, LOD = 2.43<br>7p15, LOD = 2.92; 12p11, LOD = 2.42 | *IL4, LOX* (5q23)<br>*IL6, NPY, HOXA@, HOXB@* (7p15) | |
| 2582 Caucasian females from 451 pedigrees including 1486 premenopausal and 1096 postmenopausal women | 410 | BMD at LS in total sample | 15q13, LOD = 3.67; 3p25, LOD = 3.06<br>16p13, LOD = 1.94; Xq25, LOD = 2.95 | | 28 |
| | | BMD at LS in postmenopausal women | 15q13, LOD = 2.49 | *HERC2* | |
| | | BMD at LS in premenopausal women | 15q13, LOD = 1.52 | | |
| | | BMD at hip in total sample | Xp11.4, LOD = 1.95 | | |
| 4126 Caucasians from 451 pedigrees | 410 | BMD at LS/TBLM in total sample | 7p22, LOD = 2.53; Xq25, LOD = 5.26<br>Xp22.3, LOD = 5.20; Xq13.3, LOD = 4.31<br>Xp11.4-Xq11.1, LOD = 4.54 | *TWIST, IL6* | 33 |
| | | BMD at hip/TBLM in female | Xq23-Xq24, LOD = 3.78; Xq27.1, LOD = 3.68 | | |
| | | BMD at LS/TBLM in female | 7q32, LOD = 2.67<br>15q13, LOD = 4.86; 7q32, LOD = 2.44<br>Xq13.3, LOD = 3.94; Xp22.3, LOD = 3.92 | *LEP*<br>*GREM1* (15q13) | |
| | | BMD at hip/TBLM in male | Xq22.33, LOD = 3.92 | | |
| | | BMD at LS/TBLM in male | 7q21, LOD = 2.52<br>13p11, LOD = 3.25; Xq11.1, LOD = 3.78 | | |

(*Continued*)

**TABLE 3.** Continued

| Study subjects | No. of markers | Phenotype | Results | Candidate gene | Ref. |
|---|---|---|---|---|---|
| 4126 Caucasians from 451 pedigrees | 410 | BMD at LS/BFM in total sample | 7p22-p21, LOD = 2.69 | *IL6, RAC1* | 34 |
| | | | 6q27, LOD = 2.42 | *ESR1* | |
| | | BMD at hip/BFM in total sample | 6q27, LOD = 2.30 | | |
| | | | 2q32, LOD = 2.29 | *GDF8, STAT1* | |
| | | BMD at wrist/BFM in total sample | 11q13, LOD = 2.64 | *GAB2, FRA-1, LRP5* | |
| | | BMD at hip, LS/BFM in total sample | 6p21, LOD = 2.32 | *TNF α, RUNX2, HLA-A* | |
| | | BMD at LS/BFM in female | 6q27, LOD = 2.34 | | |
| | | BMD at wrist/BFM in female | 15q13, LOD = 3.32 | | |
| | | BMD at LS/BFM in male | 6p25-24, LOD = 3.15 | *BMP6* | |
| | | BMD at hip/BFM in male | 13q12, LOD = 3.23 | *KL* | |
| | | | 7q21, LOD = 2.59 | *CTR, SERPINE1, PON1* | |
| 2584 Caucasian females from 414 pedigrees | 410 | BMD at LS/AAM | 1q44, LOD = 4.28; 2q37, LOD = 3.39 | | 36 |
| | | | 8q24, LOD = 4.59; 13q33, LOD = 3.31 | *EXT1* (8q24) | |
| | | | 15q13, LOD = 4.80; 22q13, LOD = 4.60 | | |
| | | BMD at FN/AAM | 8q11, LOD = 3.36; 22q13, LOD = 4.88 | | |
| | | BMD at UD/AAM | 15q24, LOD = 5.25; 22q13, LOD = 4.79 | | |
| 4498 Caucasians from 451 pedigrees | 410 | BR/TBFM | 20q12, LOD = 3.23 | | 249 |
| | | CSA/TBFM | 20p11, LOD = 2.47 | | |
| | | CT/TBFM | 6q27, LOD = 3.19 | | |
| | | W/TBFM | 20p12, LOD = 1.68 | | |
| | | Z/TBFM | 7q11, LOD = 2.47 | | |
| 327 Mexican-Americans (aged 25–45 yr) from 32 extended pedigrees | 460 | ΔBMD at FNK BMD in total sample | 1q23, LOD = 3.6 | *BGLAP, osteocalcin, IL6R, SLC39A1* | 250 |
| | | ΔBMD at 33% ulna | 11p14-15, LOD = 2.5 | *CALCA, CALCB, CALCP* | |
| 1346 Caucasians from 327 extended families of the Framingham Study | 636 | S_CSA / LLM | 12p12.3-12p13.2, LOD = 3.49 | | 251 |
| | | NSA/LLM | 14q21.3-22.1, LOD = 3.77 | | |
| 3782 Caucasian females aged 18–80 yr | 400 | Length of spine | 5q15-5q23.1, LOD = 3.00 | *IRX* | 31 |
| | | Length of femur | 5q15-5q23.1, LOD = 2.19 | | |
| 348 Caucasian dizygotic healthy female twin volunteers | 737 | Lumbar degenerative disc disease | Chr. 1 (285 cM), LOD = 6.05 | | 252 |
| | | | Chr. 5 (175 cM), LOD = 6.05 | | |
| | | | Chr. 19 (80 cM), LOD = 4.06 | | |
| 210 individuals from 39 families of predominantly British descent without mutations of SQSTM1 | 382 | Paget's disease of bone | 10p13, LOD = 4.08 | | 253 |
| 405 Caucasian pairs and 110 American black pairs of brothers aged 18–61 yr | 402 | aBMD at LS | 7q34, LOD = 5.26 | | 254 |
| | | aBMD at hip | 4q21, LOD = 4.14; 14q32, LOD = 4.19 | *ESRβ* (14q32) | |
| | | | 19p13, LOD = 5.77; 21q21, LOD = 4.63 | | |
| | | | 22q13, LOD = 4.71 | | |

Adapted from Y. Guo *et al.*: *Expert Rev Endocrinol Metab* 3:223–267, 2008 (3), with permission from Expert Reviews Ltd. Z, Section modulus; NN, narrowest section of the femoral neck; AFOS, Amish Family Osteoporosis Study; TR, trochanter; OF, osteoporotic fracture; IT, intertrochanteric; S, femoral shaft; NSA, neck-shaft angle; CSA, cross-sectional area; FNL, femoral neck length; PC, Principal component; aBMD, areal BMD; ABS, areal bone size; TBFM, total body fat mass; LLM, leg lean mass; ID, inner diameter; UD, ultradistal; W, sub-periosteal diameter; LOD, likelihood; Chr., chromosome; OD, outer diameter; BR, buckling ratio. The two traits for bivariate analysis are separated by a slash (/). Bivariate LOD scores listed here were converted into one degree of freedom, equivalent to univariate LOD score level.

QTLs whose effects are too small to be detected by univariate linkage analyses for genetically correlated traits. Efforts have been made to explore the correlations between BMD and BS, total body lean mass (TBLM), body fat mass (BFM), and age at menarche (AAM). Significant linkage was observed on chromosome Xq27 for BMD and BS (32) and on chromosomes Xq25 and 15q13 for spine BMD and TBLM in 4126 Caucasian individuals from 451 pedigrees (33, 34). A strong linkage signal influencing both BMD and AAM

was found on chromosome 22q13 in 2522 females from 414 Caucasian pedigrees (35).

In total, more than 60 QTLs have been identified, and they have been found on all chromosomes, with the exception of chromosome Y. A number of QTLs have been replicated by at least two studies, such as 7p21-22 (29, 33, 34), 11q12-13 (26, 29, 34), 15q13 (28, 33–36), and Xq27 (29, 32, 35). 7p21-22 and 11q13 are of particular interest because these regions harbor two strong candidate genes, *IL6* in 7p21-22 and *LRP5* in 11q13. However, many of the QTLs have not been replicated in independent studies. A significant limiting factor in replicating these linkages is genetic heterogeneity, especially when the sample size is relatively small or participants from various ethnic origins are included. In addition, other factors such as magnitude of the genetic effect, density of markers, definition and assessment of phenotypes, and statistical approaches might contribute to difficulties in replicating the findings of GWL scans. Despite these concerns and limitations, significant progress has been made in the last 2 yr. As the number of GWL studies continues to grow and more new GWA studies start to emerge, it is anticipated that some previously identified genomic regions will be replicated for linkages and that some genomic regions will eventually be proven to be falsely positive.

## IV. GWA Studies

In contrast to candidate gene studies that select genes for study based on known or suspected disease mechanisms, GWA study is a non-hypothesis-driven approach in which a large number of subjects are genotyped for dense genetic markers covering the genome, usually in the form of SNPs and copy number variations (CNVs), in an unbiased fashion. Consequently, GWA studies have the potential to identify totally novel genes/genomic loci with modest effects on human complex diseases/traits (37). However, GWA studies have their own issues that involve multiple considerations in study design and data analyses, such as sample size, level of statistical significance, correction for multiple testing, population stratification, marker density, and replication of results by independent studies. Shortly after the initial GWA study on osteoporosis (38), results of 12 more GWA studies were published (Table 4). Nine of these were published in the *New England Journal of Medicine* (NEJM) (39), *The Lancet* (40), *Nature Genetics* (41–44), *Annals of Internal Medicine* (45), and *The American Journal of Human Genetics* (AJHG) (46, 47).

### A. Single nucleotide polymorphism (SNP) analyses

In the NEJM paper, sequence variants in five genomic regions were significantly associated with spine BMD in the discovery set (5861 Icelandic subjects) and were confirmed in the replication sets (3750 Icelandic, Danish, and Australia subjects) (39). Three of these five regions are close to, or within, candidate genes (*RANKL*, *OPG*, *ESR1*) for osteoporosis that were previously implicated by candidate gene association studies. The other two regions are close to the zinc finger and BTB (Bric-a-brac, Tramtrack, Broad-complex) domain containing 40 (*ZBTB40*) gene (1p36) and the major histocompatibility complex region (6p21). The *ZBTB40* gene, of unknown function, is expressed in bone (UniGene accession no., Hs.418966), indicating a potentially unidentified role in bone biology. By enlarging the discovery sample size from 5861 to 6865 Icelanders and the follow-up replication sample size from 3750 to 5375 subjects of European descent, two new genome-wide significant loci for hip BMD were found near the *SOST* and *MAPK3* genes (42). The *SOST* gene encodes sclerostin, which interferes with the Wnt pathway by disrupting Wnt-induced frizzled-Lrp complex formation. The *MAPK3* gene encodes a protein kinase that phosphorylates microtubule-associated proteins and plays a role in determining cell polarity. However, 20p12 and BMP2, which the authors found to be associated with osteoporosis in their previous study of the same Icelandic population, were not confirmed in their current GWA papers. In the *Lancet* paper, GWAs with hip BMD and LS BMD were found for two SNPs: *rs4355801* on chromosome 8, close to the *OPG* gene, and *rs3736228* on chromosome 11 in the *LRP5* gene (40). Further analyses in the replication cohort (6463 people from three other cohorts in Western Europe) corroborated the findings in discovery subjects (2094 women from the Twins UK cohort). The relationships of these two genes with bone metabolism have been well established in previous candidate gene association studies. In the *Nature Genetics* paper (41), *rs7776725* within the *FAM3C* gene and *rs1721400* mapping close to the *SFRP4* gene showed association with BMD at the radius, tibia, and heel in a sample of 8842 Korean subjects from population-based cohorts and were replicated in an independent sample of 7861 Korean subjects. Previous evidence that the *FAM3C* gene is related to bone is rare, suggesting that this gene might be a novel candidate gene for osteoporosis. SFRP4 belongs to the SFRP family, members of which are well known for their involvement in bone formation and resorption and act as soluble modulators of the Wnt pathway (48).

In each of the GWA studies mentioned above, the tested subjects had similar ancestry, *e.g.*, in the NEJM paper, all the participants were whites of European descent. Thus, the effects of the identified candidate genes are of interest to be investigated in individuals from other an-

**TABLE 4.** GWA study for osteoporosis-related phenotypes in humans (published between October 2007 and November 2009)

| Genotyping methods | Total markers | Significant markers | Discovery subjects | Replication subjects | Phenotype | *P* value | Candidate gene | Ref. |
|---|---|---|---|---|---|---|---|---|
| Infinium HumanHap300; HumanCNV370 SNP chip; Centaurus platform (Nanogen) | 301,019 SNPs with genotypic call rates ≥ 97%, HWE $P \geq 10^{-7}$ | rs9594759, rs9594738 rs6469804, rs6993813 rs7524102, rs6696981 rs3130340, rs3018362 rs9479055, rs4870044 rs1038304, rs6929137 rs1999805 | 5,861 Icelandic persons, 87% of whom are women | 4,165 other Icelandic persons, 74% of whom are women; 2,269 postmenopausal Danish women; 1,491 persons from the Australian Dubbo Osteoporosis Epidemiology Study cohort, 61% of whom are women | BMD at LS and hip, osteoporotic fracture | $1.2 \times 10^{-7}$ to $2.0 \times 10^{-21}$ | *RANKL, OPG, ZBTB4, MHC, RANK, ESR1* | 39 |
| | | rs2306033, rs7935346 | | | | | *LRP4* | |
| | | rs11898505 | | | | | *SPTBN1* | |
| Infinium assay; HumanHap 550 v3.0 array; Taqman system | 314,075 SNPs with genotypic call rates ≥ 90%, HWE $P \geq 0.0001$, and MAF ≥ 1% | rs4355801 | 2,094 women from the Twins UK cohort | 6,463 people from three other cohorts in Western Europe (4081 from Rotterdam cohort, 1,692 from Twins UK replication cohort, and 690 from Chingford cohort) | BMD at LS and FN, osteoporosis | $<5 \times 10^{-8}$ | *TNFRSF11B* | 40 |
| | | rs3736228 | | | | | *LRP5* | |
| IlluminaHap300K | 186 SNPs | rs1513670, rs7220711 | 6,865 Icelanders, 5,934 women, and 931 men | 3,015 Icelanders, 3,884 Danish postmenopausal women, and 1,491 from the Australian cohort | BMD at hip or spine | $1.8 \times 10^{-9}$ to $1.3 \times 10^{-7}$ | *SOST* | 42 |
| | | rs1107748 rs2010281, rs10876432 | | | | | *MARK3, SP7* | |
| | | rs3018362 | | | | | *TNFRSF11A* | |
| Affymetrix Human Mapping 500K Array Set | 727 CNV regions | CNV 4q13.2 | 700 elderly Chinese individuals including 350 cases with homogeneous hip osteoporotic fractures and 350 controls | Chinese, 399 cases with hip osteoporotic fracture and 400 controls; 689 Chinese unrelated subjects, and 1,000 white unrelated subjects; 236 young Chinese males | Osteoporotic fracture, hip BMD and FN bone geometry, concentrations of serum testosterone and estradiol | $2.0 \times 10^{-4}$ | *UGT2B17* | 47 |
| Affymetrix Mapping 250 k Nsp and Affymetrix Mapping 250 k Sty arrays; Illumina Infinium assay; Affymetrix Human Mapping 500 K array set | 379,319 SNPs with genotypic call rates ≥ 95%, HWE $P \geq 0.001$ and MAF ≥ 1% | rs7595412 | 1,000 homogeneous unrelated Caucasian subjects, including 501 females and 499 males | 1,216 women of Caucasian European ancestry from the UK; a Chinese sample containing 403 female subjects including 226 with low trauma osteoporotic hip fracture, and 177 controls | Hip bone size | $3.72 \times 10^{-7}$ | *PLCL1* | 51 |
| | | rs892515 | | | | $8.62 \times 10^{-3}$ | | |
| | | rs9789480 | | | | $2.44 \times 10^{-3}$ | | |
| | | rs3771362 | | | | $7.66 \times 10^{-3}$ | | |

*(Continued)*

**TABLE 4.** Continued

| Genotyping methods | Total markers | Significant markers | Discovery subjects | Replication subjects | Phenotype | *P* value | Candidate gene | Ref. |
|---|---|---|---|---|---|---|---|---|
| Affymetrix Gene Chip Human Mapping 500K Array Set; Affymetrix 50K supplemental array | 379,319 SNPs with genotypic call rates $\geq$ 95%, HWE $P \geq 0.001$ and MAF $\geq$ 1% | rs11864477, rs11860781, rs16945612, rs11859065 | 1,000 homogeneous unrelated Caucasian subjects, including 501 females and 499 males | 593 white U.S. families (1,972 subjects), a Chinese hip fracture sample (350 cases, 350 controls), a Chinese BMD sample (2,955 subjects), and a Tobago cohort of African ancestry (908 males) | BMD and hip fracture | $2.56 \times 10^{-5}$ to $2.13 \times 10^{-8}$ (meta-analyses) | *ADAMTS18* | 46 |
| | | rs17131547 | | | | | *TGFBR3* | |
| Illumina HumanHap317K SNP chip | 317,504 SNPs with missing genotypes $\leq$5%, HWE $P \geq 10^{-7}$ and MAF $\geq$ 1% | rs4759021 | 1,518 children from the Avon Longitudinal Study of Parents and Children | 3,692 children from the Avon Longitudinal Study of Parents and Children | BMD | $5.8 \times 10^{-4}$ | *Osterix* | 255 |
| | | | | | BMC | $1.7 \times 10^{-3}$ | | |
| | | | | | Bone area | $3.8 \times 10^{-3}$ | | |
| Affymetrix Genome-Wide Human SNP array 5.0 | 352,228 SNPs with missing genotypes $\leq$5%, HWE $P \geq 10^{-6}$, and MAF $\geq$ 1% | rs7776725 | 8,842 Korea samples from population-based cohorts | 7,861 independent Korean samples | BMD at radius, tibia, and heel | $1.0 \times 10^{-11}$ to $1.6 \times 10^{-6}$ | *FAM3C* | 41 |
| | | rs1721400 | | | | $1.4 \times 10^{-7}$ to $2.2 \times 10^{-3}$ | *SFRP4* | |
| Illumina HumanHap300; HumanHapCNV370 | 303,120 SNPs with missing genotypes $\leq$5%, HWE $P \geq 0.001$, and MAF $\geq$ 1% | rs219780 | 3,773 kidney stone cases and 42,510 controls from Iceland and The Netherlands | 1,520 Icelandic kidney stone cases and 4,726 controls, 746 kidney stone cases and 3,751 controls from The Netherlands | BMD at hip and spine | 0.00039; 0.0077 | *CLDN14* | 43 |
| Affymetrix Gene Chip Human Mapping 500K Array Set | 342,854 SNPs with HWE $P \geq 0.0001$ and MAF $\geq$ 5% | rs9630182, rs2036417, rs7125774; rs8057551, rs8061992, rs7199138 | 983 unrelated Caucasian subjects | A family-based sample of 2,557 Caucasian subjects | BMD at FN | $3.98 \times 10^{-7}$ to $6.74 \times 10^{-3}$ | *PTH, IL21R* | 256 |
| Meta-analysis | 36,016 SNPs in 150 candidate genes | 241 SNPs | BMD data were obtained from 19,195 participants from 5 populations of European origin; data on fractures were obtained from a prospective cohort (n = 5,974) from The Netherlands | | BMD at LS and FN fracture risk | $P < 2.39 \times 10^{-6}$ | *ESR1, LRP4, ITGA1, LRP5, SOST, SPP1, TNFRSF11A, TNFRSF11B, TNFSF11* | 45 |

*(Continued)*

**TABLE 4.** Continued

| Genotyping methods | Total markers | Significant markers | Discovery subjects | Replication subjects | Phenotype | *P* value | Candidate gene | Ref. |
|---|---|---|---|---|---|---|---|---|
| Meta-analysis | 2,543,686 SNPs | 467 SNPs | 19,195 participants from five populations of European origin, *e.g.*, the Rotterdam Study (n = 4,987), Erasmus Rucphen Family Study (n = 1,228), Twins UK Study (n = 2,734), deCODE Genetics Study (n = 6,743), and Framingham Osteoporosis Study (n = 3,503) | | BMD at LS and FN | $P < 5 \times 10^{-8}$ | *GPR177, MEPE, SPTBN1, CTNNB1, MEF2C, ZBTB40, STARD3NL, FLJ42280, LRP4, ARHGAP1, F2, DCDC5, SOX6, ESR1, FOXL1, HDAC5, CRHR1, TNFRSF11B LRP5, SP7, TNFSF11, TNFRSF11A* | 44 |

BMC, Bone mineral content; HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency.

cestries. In the AJHG paper (46), two genes, *ADAMTS18* and *TGFBR3*, were associated with BMD in Caucasian U.S. subjects. Importantly, this association was replicated in Chinese samples and a Tabogo cohort of African ancestry (46). Furthermore, National Center for Biotechnology Information (NCBI) Gene Expression Omnibus expression profiles showed that decreased expression levels of *ADAMTS18* and increased expression levels of *TGFBR3 in vivo* may potentially contribute to the nonhealing of skeletal fractures (46). Results of previous studies have suggested that the genetic basis for osteoporosis may be gender specific (49, 50), so testing sex differences in GWA studies is encouraged. In the first GWA study of hip BS, Liu *et al.* (51) identified a novel gene, *PLCL1*, that had four SNPs associated with hip BS in female subjects. The importance of this gene to hip BS was replicated in an independent UK cohort, and the relevance to hip fractures was also observed in a Chinese sample (51). In male subjects from the Caucasian sample, two SNPs in *SOX6* gene were identified to be associated with both hip BMD and body mass index by a bivariate GWA study (52), the approach of which has the potential to find pleiotropic genes underlying two genetically correlated diseases, like osteoporosis and obesity.

Due to the relatively small sample size in each dataset alone, the power to identify variants with small effects on osteoporosis is insufficient. Meta-analysis involves multidatasets, enlarges the sample size, and is designed to improve the power to detect more associations and investigate the consistency of associations across diverse study populations (53). Rivadeneira *et al.* (44) conducted a large-scale collaborative meta-analysis involving 19,195 participants from five study populations of European origin. A total of 20 loci showed associations with BMD at the genome-wide significance level, of which 13 were new for BMD and the remaining seven have been reported previously. For osteoporosis, it is important to further investigate whether the candidate genes associated with BMD are also associated with fractures. In a meta-analysis involving 19,195 participants with BMD data and 5,974 subjects with fracture data (45), of the nine candidate genes associated with BMD, four (*LRP5*, *SOST*, *SPP1*, and *TNFRSF11A*) were also significantly associated with risk for fractures. Potential noise may be introduced by including multiple populations that may be heterogeneous in environments and genetics factors and their interaction in influencing the study traits. The usefulness of meta-analyses may partially depend on the noise introduced relative to the genetic signal enhanced in larger samples. Therefore, findings from even meta-analyses should be replicated in various individual populations for their robustness.

Although the GWA studies reviewed above have brought us new insights into the genetic mechanisms of osteoporosis, the clinical value of these GWA studies is not immediately obvious due to the small variation (namely, ~1–4%) in BMD that is accounted for by each of the implicated SNPs. It is likely that many other sequence variants imparting smaller effects on osteoporosis will be uncovered, but the primary value of these studies is to identify targets for future functional studies that enhance our knowledge of the molecular pathogenesis of osteoporosis, which will, in turn, facilitate the development of novel therapeutic modalities targeting these molecular pathways.

Approximately 30,000 genes are coded by the human genome, and currently, GenBank contains only about 16,000 known genes. Thus, SNPs associated with osteoporosis in future GWA studies may fall into genomic regions with known and predicted genes or into genomic regions that are more poorly characterized. For SNPs that are located within genes or within regulatory regions close to known genes, public databases (*e.g.*, HapMap, NCBI, University of California Santa Cruz Genome Browser, and Ensembl) or private databases (*e.g.*, Applied Biosystems SNPbrowser, Celera, and Perlegen) can be used to characterize the linkage disequilibrium block structure around those positive SNPs and then to identify the genes annotated on or near the linkage disequilibrium blocks. The strategy for identifying unknown genes in these genomic regions is to blast the Expression Sequence Tag databases and then perform computational gene prediction using gene prediction software (Genscan, GrailEXP, Michael Zhang's Exon Finder, GeneMark) and Vista analysis in combination with experimental confirmation by real-time RT-PCR in bone-related cells and tissues. Due to false-positives and false-negatives in gene prediction using current software, new and better gene prediction tools are being developed and are likely to become available. In addition, databases containing information on known and predicted genes will be continuously updated. The new tools for gene prediction and enhanced information about existing genes will enhance our capacity to identify genes that reside around positive association signals, and RT-PCR can be used to confirm any unknown candidates in major bone cells and bone-related tissues.

#### B. Copy number variation (CNV) analyses

CNVs are newly appreciated structural genomic variations that include duplication or deletion of genomic segments. In contrast to SNPs, which only represent a single nucleotide, CNVs represent a relatively large segment of DNA, with variations in size ranging from 1 kilobase to several megabases. Using the Affymetrix 500K Array Set, Yang *et al.* (47) conducted case-controlled genome-wide CNV analyses in elderly Chinese individuals. A variant of *UGT2B17* in CNV 4q13.2 showed association with osteoporotic fractures that was confirmed in replication cohorts. Because the *UGT2B17* gene encodes a key enzyme catabolizing steroid hormones, the relationship between serum levels of sex hormones and *UGT2B17* gene copy number was further assessed. Subjects with homozygous deletions of *UGT2B17* were found to have significantly higher concentrations of total testosterone and estradiol than subjects with one or two copies of this gene. Compared with the well-developed resources available for SNP association studies, we are still in the early phases of incorporating structural genetic variation in GWA studies. GWA CNV analysis generates a distinct set of statistical and technical challenges, and the list of CNVs available for detection and analysis is undoubtedly incomplete (54). Over the next few years, however, a much more global understanding of the extent and precise location of CNVs will likely be achieved as new platforms and novel technical, statistical methods become available to accurately capture CNV information. These advances will undoubtedly benefit the complex phenotype studies, providing a more comprehensive understanding of the role of CNVs in the pathogenesis of osteoporosis. Despite the many obstacles yet to be overcome, we expect analyses of CNVs to continue to gain importance in osteoporosis studies.

The variants themselves found in candidate gene association and GWA studies may not directly cause higher risk to osteoporosis. They may just be closely linked to the actual causal variants. Therefore, further analyses, such as sequencing DNA base pairs in the implicated genome, are needed to identify the exact genetic change responsible for the development of osteoporosis.

## V. Transgenic/Knockout Mouse Models

Transgenic mice contain artificially introduced inducible or tissue-specific transgenes that have been added to the genome to study the function and regulation of those genes. These transgenes often confer a gain of function by producing a new protein, increasing expression levels of an existing protein, or presenting the protein in a different type of cells. However, if the transgene disturbs the expression of an existing gene, it will cause loss of function, which is a strategy that has been used to generate knockout mice. Knocking out the activity of a specific gene can offer valuable clues about the function of that gene. Because humans share many genes with mice, detailed study of transgenic/knockout mice has been used to provide a better understanding of how similar genes may contribute to similar complex traits in humans. Knockout and transgenic mouse models have been widely used to confirm the

contribution of many well-known or novel genes to abnormal bone phenotypes. From October 2007 to November 2009, approximately 70 new studies (summarized in Table 5) using transgenic and knockout mouse models were reported in the osteoporosis field. For instance, in a study using knockout and overexpression manipulation, bone mass in Cthrc1-null mice was significantly lower than in Cthrc1 transgenic mice (55). Using overexpression manipulation, Ding *et al.* (56) observed increased osteoblastic activity in glucose-dependent insulinotropic peptide transgenic mice. Transgenic animal models also help isolate and highlight the functions of a particular gene, providing further evidence to support the gene's relevance to osteoporosis. For example, to determine the role of steroid receptor coactivator (SRC)-1 in mediating the maintenance effects of estrogen on bone, Mödder *et al.* (57) used SRC-1 knockout mice with gonadectomy and estrogen replacement (10 μg/kg/d) to show that the skeletal response to estrogen was impaired in female, but not in male, SRC-1 knockout mice. It is important to note that there are also some important limitations to the use of knockout mouse technology, as reviewed by the National Institutes of Health (http://www.genome.gov/12514551). Generally, approximately 15% of the genes that have been knocked out are developmentally lethal, so these genes cannot be studied using a knockout model. Additionally, despite the genetic similarities of mice and humans, there are still important differences, so knocking out a gene in mice may have effects that are not indicative that genes function in humans. Despite these limitations, knockout mice give us one of the most powerful methods now available for studying gene functions *in vivo*.

## VI. Gene-Expression Microarray Studies

Because osteoporosis is largely determined genetically, the etiology of osteoporosis cannot be explicitly and comprehensively revealed unless the mechanisms of gene action underlying osteoporosis are established. This problem has been partially addressed by gene expression studies of osteoporosis. Gene expression studies explore relationships between diseases and certain genes at the mRNA level rather than at the DNA level. This approach can shed light on gene function and can also be used to elucidate some of the intermediate biochemical processes leading to a disease. In contrast, genetic epidemiology studies at the DNA level obviate the study of biological processes. Gene-expression microarray has proven to be a powerful tool for assessing gene expression for tens of thousands of genes simultaneously. Approximately 110 gene-expression microarray studies of osteoporosis and other related traits have been published since our 2007 review. These studies have focused primarily on a few key topics such as regulation of osteoblast and osteoclast activity, differentiation of mesenchymal stem cells (MSCs), gene expression comparing healthy and diseased tissues, effects of therapeutic agents on the healing of fractures, and endocrine regulation of bone remodeling. The results from these studies are summarized in Table 6.

### A. Regulation of osteoblast and osteoclast activity

Regulation of osteoblast and osteoclast activity has been a major focus of microarray experiments in the past 2 yr and has provided novel insights into the mechanisms of bone formation and resorption. The activity of osteoblasts and osteoclasts is regulated by a variety of specific biomaterials, transcription factors, growth differentiation factors, and cytokines.

Several important biomaterials have been identified that promote bone formation by regulating the activity of osteoblasts. For example, Palmieri *et al.* (58) used microRNA (miRNA) microarray techniques to investigate translation regulation in human MG63 cells cultured with PerioGlas, an alloplastic material that is effective as an adjunct to conventional surgery for the treatment of intrabony defects. There were 10 up-regulated and two down-regulated miRNAs that in turn regulate the expression of several genes by either enhancing catabolism or repressing translation of their mRNA targets. The vast majority of these genes are down-regulated by miRNA, some of which are homeobox genes like *NOG*, *EN1*, and *CHRD*. Other down-regulated genes include receptors like *GHRHR* and extracellular matrix proteins like *COMP*. Among the genes that were down-regulated by PerioGlas, *NOG*, and *COMP* were up-regulated by Bio-Oss, a deproteinized sterilized bovine bone material containing calcium-deficient carbonate apatite (59). Palmieri *et al.* (60–65) also explored osteoblast regulation by several other biomaterials including zirconium oxide, calcium sulfate, porous polyethylene, titanium, and anatase. These materials were found to alter expression of several genes related to osteogenesis and bone remodeling, such as *SHOX*, *IGF-I*, *GHRHR*, *BMP1*, and *FGFR1*. Additional biomaterials like hydroxyapatite (HA), bioactive glass, stainless steel, polymethylmethacrylate (66), and demineralized freeze-dried bone allografts (67) were also used to study early osteogenesis. Results of the gene expression studies reviewed above have enhanced our understanding of the molecular mechanism underlying osteoblast function in bone regenerative procedures. This new knowledge can aid in the development of surgical techniques and biomaterials that are more suitable for use in the treatment of fractures in osteoporotic patients.

The recent discoveries of transcription factors and signal transduction pathways critical for osteoblast differen-

**TABLE 5.** Studies using transgenic and knockout mouse models relevant to osteoporosis (published between October 2007 and November 2009)

| Candidate gene | Protein | Manipulation | Phenotype | Ref. |
|---|---|---|---|---|
| *Adipoq* | Adiponectin | Overexpression | Decreased BMC at femur and lumbar vertebra | 257 |
| *AR* | Androgen receptor | Knockout | Decreased trabecular bone mass in male mice | 258 |
| | | AR and ER-α double-knockout | Additionally reduced cortical bone and muscle mass | 258 |
| *Agtr2* | Angiotensin II receptor, type 2 | Treatment with AT2 receptor blocker | Enhanced levels of bone mass | 259 |
| *BSP* | Bone sialoprotein | Overexpression | Osteopenia and mild dwarfism | 260 |
| *CB1* | Type 1 cannabinoid receptor | CB1−/− mice | Increased peak bone mass due to reduced bone resorption, age-related osteoporosis with reduced bone formation, and accumulation of adipocytes in the bone marrow space | 261 |
| *CCR2* | C-C chemokine receptor-2 | Knockout | High bone mass | 262 |
| *CD200* | CD 200 molecule | CD200−/− mice, produced by homologous recombination | Fewer osteoclasts and more bone | 263 |
| *Cftr* | Cystic fibrosis transmembrane conductance regulator | Knockout | Increased trabecular bone volume | 264 |
| *COL1A2* | Collagen, type I, α 2 | Knock-in | Reduced body mass, areal BMD, and bone strength | 265 |
| *Col6a1* | Collagen, type VI, α 1 | Knockout | Accelerated development of OA joint degeneration; delayed secondary ossification and reduced BMD | 266 |
| *Col9a1* | Short collagen IX | Col9a1−/− and col9a1+/− mice | Trabecular bone loss in young adult female col9a1 (−/−) and col9a1 (+/−) mice; trabecular bone architecture deterioration in both male and female heterozygous col9a1 (+/−) mice while aging | 267 |
| *Cthrc1* | Collagen triple helix repeat containing-1 | Knockout and overexpression | Low bone mass in Cthrc1-null mice and high bone mass in Cthrc1 transgenic mice | 55 |
| *Cxcr4* | Chemokine (C-X-C motif) receptor 4 | Overexpression | Increased cell trafficking to bone in ovariectomy mice | 268 |
| *CYLD* | Deubiquitinating enzyme | Knockout | Osteoporosis | 269 |
| *CYP191A1* | Aromatase | Osteoblast-specific overexpress aromatase | Increased total body BMD, trabecular BMD, cortical BMD, and cortical thickness associated with elevated osteoprotegerin mRNA levels and reduced number of osteoclasts | 270 |
| *DKK1* | Dickkopf-1 | Mice with hypomorphic Dkk1d (doubleridge) alleles, express low amounts of Dkk1 | Increased trabecular and cortical bone mass | 271 |
| *Dlx5* | Distal-less homeobox 5 | Dlx5+/− mice | Lower BMD and reduction in cortical thickness of femoral midshafts | 272 |
| *ER-α/ER-β* | Estrogen receptor | Knockout/knockdown | ERKs are not activated by stretching in osteocytic and osteoblastic cells | 273 |
| *Frzb* | Frizzled-related protein | Knockout | Increased cartilage proteoglycan loss | 274 |
| *GIP* | Glucose-dependent insulinotropic peptide | Overexpression | Increased osteoblastic activity | 56 |
| *Gpnmb* | Osteoactivin | Mutation leading to the generation of a truncated osteoactivin protein | Reduced alkaline phosphatase activity and calcium deposition | 275 |
| *GPR30* | G protein-coupled receptor 30 | GPR30−/− mice | Reduced bone growth | 276 |
| *Gpr48* | G-protein-coupled receptor 48 | Gpr48−/− mice | Dramatic delay in osteoblast differentiation and mineralization | 277 |
| *HIF-1α* | Hypoxia inducible factor-1 α | Knockout | Reduced bone formation ability of osteoblasts | 278 |
| | | Knockout | Reduced values of bone histomorphometry and BMD | 279 |
| *HIP/RPL29* | Ribosomal modulator of protein synthesis rate | HIP/RPL29-deficient mice | Increased bone fragility | 280 |
| *HSD17B2TG* | Hydroxysteroid (17β) dehydrogenase 2 | Express human hydroxysteroid (17β) dehydrogenase 2 | Decreased bone formation rate at prepubertal age in male mice | 281 |

(*Continued*)

**TABLE 5.** Continued

| Candidate gene | Protein | Manipulation | Phenotype | Ref. |
|---|---|---|---|---|
| *IGF-I* | Insulin-like growth factor 1 | Knockout | Lower femur BMD | 282 |
| | | Knockout | Reduced body length, areal BMD, and BMC | 283 |
| | | Crossbred IGF-I transgenic mice with homozygous weaver mice | Improved BMD and BMC | 284 |
| *IL-7* | Interleukin 7 | Overexpression | Age-related loss of trabecular bone in both axial and long bones | 285 |
| *Irf8* | Interferon regulatory factor-8 | Deficient in Irf8 | Osteoporosis | 286 |
| *JunD* | An activator protein-1 component | Knockout | Increased bone volume and sustained high bone mass even after estrogen depletion | 287 |
| *Mecp2* | Methyl CpG binding protein 2 | Knockout | Decreased osteoblast activity | 288 |
| *Nfatc1* | Nuclear factor of activated T cells c1 | Knockout | Osteopetrosis and inhibition of osteoclastogenesis | 289 |
| *Nmp4* | Nuclear matrix protein 4 | Knockout | Greater PTH-induced acquisition of femoral trabecular bone | 290 |
| *NMU* | Neuromedin U | Knockout | Increased bone mass | 291 |
| *OT* | Oxytocin | Deletion of OT or the OT receptor (Oxtr) in mice | Osteoporosis | 292 |
| *PAI-1* | Plasminogen activator inhibitor-1 | Overexpression | Increased mineralization and biomechanical properties in 32-wk females | 293 |
| *PAPP-A* | Pregnancy-associated plasma protein-A | Knockout | Insufficiency in mass, density, architecture, and strength | 294 |
| *PECAM-1* | Platelet/endothelial cell adhesion molecule 1 | Knockout | Reduced trabecular bone volume and number of trabeculae in femoral and tibial long bones | 295 |
| *PPAR-γ* | Peroxisome proliferator-activated receptor-γ | PPAR-γ is deleted in osteoclasts but not in osteoblasts | Increased bone mass, reduced medullary cavity space, and extramedullary hematopoiesis in the spleen | 296 |
| *PTHr1* | Parathyroid hormone receptor 1 | Expressing a constitutively active PTH receptor | Increased bone mass and elevated bone remodeling | 297 |
| *Rac1* | Rho-GTPase | Nf1+/−, Rac1−/− mice | Normalized Erk activation compared with *Nf1*+/− osteoclasts | 298 |
| *Rac2* | A member of the Rho family of small GTPases | Knockout | Increased bone mass and anabolic response to PTH | 299 |
| *RANKL* | Receptor activator of nuclear factor-κ B ligand | Deletion of the distal control region, located 76 kb upstream from the transcription start site | Increased bone mass and strength | 300 |
| *Runx2* | Runt related transcription factor 2 | Runx2+/− mice | Decreased BMD and trabecular bone volume and delayed bone formation | 301 |
| *Sca-1* | Stem cell antigen 1 | Knockout | Decreased BMD at femoral and whole body | 302 |
| *Sfrp1* | Secreted frizzled-related protein 1 | Knockout | Reduction of the cartilage callous, increased intramembranous bone formation | 303 |
| *Sfrp4* | Secreted frizzled-related protein 4 | Overexpressing in osteoblasts | Reduction of trabecular bone mass | 304 |
| *SOST* | Sclerostin | Knockout | High bone mass phenotype characterized by marked increases in BMD, bone volume, bone formation, and bone strength | 59 |
| | | Short treatment with an antibody to sclerostin | Halted bone loss | 305 |
| *Sp7* | Osterix | Inactivated in all osteoblasts | Decreased BMD and bone-forming rate in lumbar vertebra, thinner cortical bone, and more porous with reduced bone length | 306 |
| *Sparc* | Osteonectin | Knockout | Low-turnover osteopenia | 307 |
| *Spp1* | Osteopontin | Lacking expression of the osteopontin | Lower resorptive capacity of osteoclast | 308 |
| *SRC-1* | Steroid receptor coactivator 1 | Knockout | Decrease in trabecular volumetric BMD and impaired skeletal response to estrogen in female mice | 57 |
| *SRC-2* | Steroid receptor coactivator 2 | Knockout | Increased bone mass, decrease in bone marrow adipocytes | 309 |

(*Continued*)

**TABLE 5.** Continued

| Candidate gene | Protein | Manipulation | Phenotype | Ref. |
|---|---|---|---|---|
| *Spp24* | Secreted phosphoprotein 24 kDa | Constitutive expression of fl bovine spp24 (1-203) | Inhibition of ectopic bone formation in male mice and adverse affects on BMD and histological parameters related to bone mass and formation in female mice | 310 |
| *Terc* | Telomerase | Knockout | Osteoporosis | 311 |
| *TIMP-1* | Matrix metalloproteinases | Overexpression | Decrease in bone loss induced by estrogen deficiency | 312 |
| *TNFr* | Tumor necrosis factor receptor | Knockout | Reduced adverse effects of magnesium deficiency on bone | 313 |
| *TNFR2* | Tumor necrosis factor type 2 receptor | Knockout | Decrease by lipopolysaccharide on the BMD of tibiae, femurs, and lumber vertebrae | 314 |
| *TRα1* | Thyroid hormone receptor α1 | TRα 1(+/m)β(+/−) mice, express a mutant thyroid hormone receptor α1 with dominant-negative properties due to reduced ligand-binding affinity | Osteosclerosis in adults and delayed ossification in juveniles | 315 |
| *TRPV4* | Transient receptor potential vanilloid 4 | Knockout | Suppressed unloading-induced bone loss | 316 |
| *uPAR* | Urokinase receptor | Knockout | Increased BMD and osteogenic potential of osteoblasts; decreased osteoclast formation; altered cytoskeletal reorganization in mature osteoclasts | 317 |
| *VDR* | Vitamin D receptor | VDR−/− mice | Shorter latency to fall from the rotarod, smaller fall angle in the tilting box test, and aberrant poor swimming | 318 |
| *Wnt10b* | Wnt10b protein | Transgenic mice, in which mouse Wnt10b is expressed from the human osteocalcin promoter | Increased mandibular bone and impaired eruption of incisors during postnatal development, higher BMD, bone volume fraction, and trabecular number at femoral distal metaphyses | 319 |
| *Zmpste24* | Zinc metallopeptidase, STE24 homolog | Zmpste24-null progeroid mice | Age-related bone loss including lower osteoblast and osteocyte numbers and higher levels of marrow adipogenesis | 320 |
| *5-HT(2B)R* | Monoamine serotonin (2B) receptor | Knockout | Osteopenia that worsens with age in female mice | 321 |

Adapted from Y. Guo *et al.*: *Expert Rev Endocrinol Metab* 3:223–267, 2008 (3), with permission from Expert Reviews Ltd. BMC, Bone mineral content.

tiation have opened up new approaches to understanding the pathogenesis of osteoporosis. The identification of the critical role for Osterix/Sp7, a member of the Sp1 transcription factor family, in bone formation and osteoblastogenesis is of particular interest. Using Affymetrix GeneChip arrays with collagen tripeptide-treated human osteoblastic hFOB1.19 cells, Tsuruoka *et al.* (68) identified 169 genes that were up-regulated more than 2.12-fold. Among them, Sp7 transcription factor showed the greatest change in expression. Sp7 was found to be regulated by BMP2 signaling through Msx2 and Runx2 during osteoblast differentiation (69). A novel inhibitory pathway of osteoblast function, namely the cyclooxygenase-2 (COX-2) pathway, was described by Silvestris *et al.* (70). Their study supports the role of E4BP4 as a negative osteoblast transcriptional regulator of the COX-2 pathway, by negatively regulating the suppressive COX-2 pathway, thus up-regulating the expression of both Runx2 and Sp7. In view of these findings, it will be quite interesting to determine whether polymorphisms of these transcription factors are associated with osteoporosis.

Gene expression studies of osteoclastogenesis have also provided important new insights into the maintenance of bone homeostasis. Circulating monocytes, which serve as early progenitors of osteoclasts, were collected from human premenopausal subjects with extremely low *vs.* high peak bone mass, and the expression of three genes (*GBP1*, *CXCL10*, and *STAT1*) was significantly different in these two populations (71). SDF1 and CXCL7 were identified as osteoclast enhancers in primary mouse bone marrow cells treated with IGF-II (72). Battaglino *et al.* (73) identified a novel gene, *NHA-oc/NHA2*, that was strongly up-regulated during RANKL-induced osteoclast differentiation *in vitro* and *in vivo*. NHA-oc/NHA2 is a new member of the cation-proton antiporter and is the first mitochondrial NHA characterized to date. In addition, Kominsky *et al.* (74) discovered a novel osteoclast stimulating factor, macrophage inflammatory protein-1 δ,

**TABLE 6.** A summary of the DNA microarray studies on osteogenesis and bone-related diseases (published between October 2007 and November 2009)

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Human trabecular bone | Trabecular bone from the IT region of the proximal femur from individuals with OP, OA, and control | Comparing the gene expression profiles of OA, control, and OP bone samples to identify altered gene expression in OP fracture bone | 150 | | | *TREM2, ANXA2, CD14, CCR1, ADAM9, ST14, CTSB, FST, PRRX1, IL10, CTGF, KLF10, COL4A1, CCL2, SPP1, ANGPTL4, PEA15, MARCO, AEBP1* | | *SPARC, DOK4, SLC14A1, ADM, TAZ* | 82 |
| | Trabecular bone from the IT region of the proximal femur from individuals with OP, OA, and control | Comparing the gene expression profiles of OA, control and OP bone samples to identify altered gene expression in OA bone | 150 | Targets component or modulating genes of either the WNT signaling pathway or the TGF-$\beta$/BMP signaling pathway | | *MMP25, S100A4, SMAD3, WNT5B* | | *IBSP, TWIST1, TIMP4, ADAMTS4, ADM, GADD45B, MEPE, COL4A1* | 81 |
| Human MG63 cells | MG63 cell lines stably expressing pcDNA3-Flag-VP16C-SXR or the empty vector Flag-tagged pcDNA3 | Vehicle (0.1% ethanol) or MK-4 (10 $\mu$M) treatment | 85 | | 85 | *GDF15, STC2, TRIB3, GDF8,MGC4504, ASNS, SLC7A11, COL15A1, TNFAIP6, DDIT4, PCK2, FGF2, PSAT1, HMOX1* | | | 322 |
| | Human MG63 cells | Bio-Oss and PerioGlas | | | | *NOG, CALCR, COL11A2, COMP, TNFRSF11B, CASR, HOXA13, DLX5* | | | 59 |
| | Human MG63 cells | Titanium | | | | | | *FGFR3, IGF1, MSX1, GDF10, CALCA, BMP1, BMP7, COL1A1, PHEX, CASR, COL11A1* | 60 |
| | MG63 osteoblast cells | 1.035 MHz pulsed ultrasound with three different acoustic pressures | 377 | Genes involved with cellular membranes, regulation of transcription | | *LRP5, BMP1, COL1A1, CD151, PTRF, HOXB8* | | *EZH2, BMP2K* | 323 |
| | Human MG63 cells | PerioGlas and P-15 | | | | *NOG, COL11A2, IGF1, COL11A1, CLO2A1, CHRD, HOXD13, ALPL, BMP1, COMP, CALCR* | | *GDF10, ANXA2,* | 324 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | Human MG63 cells | Zirconium oxide | | | | *EGR2, FKHL18, SEP1, PRDM2, DLX2, FGF5, SP110, TOP3A, PKD2, PTPRG, PTPN6, KCNK4, LEPR, NR4A1, PDCD8, DUSP16, CD79B* | | *GABRA6, RORB, GRCA, CLTCL1, PCBP4, WDR33, CDKN3, F11R, AAMP, VPS18, SEC23B, ILF2* | 325 |
| | Human MG63 cells | Mixed a type I collagen solution with human osteoblast-like MG-63 cells and intermittent compression, gels loaded in wells without mechanical stimulation were used as controls | 43 | | 30 | *COX-2, MMP-3, ODC* | 13 | *MIL-1RAcP* | 326 |
| | Human MG63 cells | PerioGlas | | Genes involved in bone formation and skeletal and cartilage development | | *ZNF547* | | *NOG, EN1, CHRD, GHRHR, COMP* | 58 |
| | Human MG63 cells | High magneto-gravitational environment | | Adducing 3, WIPF1, coactosin-like 1, filamin A, SORBS3, CDC42BPB, talin 1, paxillin, WASF2, tropomodulin 3, SPTBN1, supervillin, plectin 1 | | | | | 327 |
| | Human MG63 cells | Cultured with porous polyethylene | | | | | | *CHRD, EN1, NOG, ADAMTS4, GHRHR, OSTF1, MGP, PTH, LECT1* | 63 |
| | Human MG63 cells | Anatase coating | | | | *PRDX1, COL9A2, ADAMTS4, SHOX ALPL, AMBN, TUFT1* | | *PHEX, FBN1, IGFBP4, CALCA PTH, TFIP11* | 61 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | Human MG63 cells | Peptide-15 | | | | *TFIP11* | | *NOG, HOXD13, AEBP1, SHOX, EN1, COMP, IGF1, MATN1, SUFU* | 328 |
| | Human MG63 cells | Zirconium oxide | | *NOG, SHOX, IGF1, BMP1, FGFR1* | | *TRAPPC2* | | *COMP, CHRD, BMP1, SHOX, IGF1, FGFR1, GHRHR, PMF1, AHSG, AMBN* | 65 |
| | Human MG63 cells | Titanium and ZDCs | | | | *BMP7* | | *NOG, FGFR1, COMP, MSX1* | 62 |
| | Human MG63 cells | Demineralized freeze-dried bone allograft | | Genes involved in cell cycle regulation, immunity, vesicular transport, bone remodeling, production of cytoskeletal elements | | | | | 67 |
| | Human MG63 cells | Calcium sulfate | | | | *BMP1, BMP7, PTH, FGFR1, CALCA, SHOX, EXT2* | | *MSX1, EBP, EN1, INHBA, CMKLR1, COMP, NOG* | 64 |
| | Human MG63 cells | Anatase coating | | | | *ERBB3, TSPAN-2, CRK, HYAL3, WDR10, MYOC, ANK3, LILRB4* | | *CCR2, MYH11, CLTB, RTN4, MECP2, GLI2* | 329 |
| | Human MG63 cells | Danggui Buxue Tang | 883 | *CCL-2, CCL-7, CCL-8, galectin-9* | | | | | 330 |
| | Human MG63 cell and sub-line model | Cisplatin, doxorubicin, and etoposide | | *ABCG2, ADD3, NMT2, WNTSa, PTN* | | | | | 331 |
| Human osteoblast | Human osteoblast | Osteoblasts cocultured with U-266 myeloma | 38 | Genes involved in bone metabolism and other cell functions | 7 | *E4BP4, UNC5C, VCAM1, UGP2, PRKAR2A, Follistatin precursor, enzyme similar to tissue plasminogen activator* | 31 | *BMPs, Runx2, COX-2, Coronin, Osterix, osteocalcin, MTRR, PMS2L9, NRIF3, CYP3A43, LIC2, Type XVIII collagen* | 70 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | Human osteoblasts | Cyclosporin A | | Wnt signaling molecules and the cytoskeletal and focal adhesion cascade | | | | | 332 |
| | Human osteoblasts isolated from trabecular bone tissue of FN and proximal femur | Comparing the gene expression profiles of osteoblasts from osteoporotic *vs.* nonosteoporotic bone tissues | 1606 | | 144 | *IBSP, CXCL2* | 208 | *PTN, COL15A1* | 333 |
| | Human osteoblasts isolated from cancellous bone fragments | Shock wave | 94 | | | *PTHrP, prostaglandin E2-receptor EP3, chordin, BMP-2 inducible kinase* | | *Matrilin 3, cartilage oligomeric matrix protein* | 334 |
| | Human osteoblasts derived from alveolar bone | TAK-778 | | | | *ALP, osteocalcin, Msh homeobox 2, RANKL adhesion molecule 1* | | | 335 |
| | ODHPSCs and normal osteoblasts derived from human dental pulpar and alveolar bone fragments | Identify genes that are differently regulated in ODHPSC in comparison to normal osteoblasts | | Genes involved in cell differentiation, developmental maturation, cell adhesion, and production of cytoskeleton elements | | *SYNE1, LAMA1, MMP2, PPP1R9B NEO1, CDH1, AGT, MAP4K4, CDC2L5, GPC3, FYN, GNE, ATP2A2, TTN* | | *MARK2, BAIAP2, EPB42, SDK1, PGM5, LRRN5, CSF3R, CNTN2, CXCL12, MSN, DNM1, ACTA2, SNTA1, MMP24, COL6A1, PLOD1, ARMCX3, PRDM2* | 336 |
| | Human MC3T3-E1 preosteoblastic cells | Hydroxyapatite | | | 11 | *SOX9, GUGA1B, HAPLN 2, RRAGA* | 6 | *Calpain 9, PI3KS, AGTR 2, OGFR, TSLRP* | 337 |
| | Human nonunion osteoblasts | Evaluated global gene expression in human osteoblasts and human nonunion osteoblasts | 281 | | 200 | *HBEGF, VEGFB* | 81 | *IGF-2, TGF-β2, FGF-1, FGF-R2, BMP-4, PDGF, WISP2, WISP3* | 338 |
| | Primary cultures of osteoblastic cells differentiated from the human bone marrow | Polymethylmethacrylate, hydroxyapatite, bioactive glass 45S5, titanium and stainless steel | | | | *IGF binding protein 4* | | *BMP5, osteocalcin* | 66 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | Osteoblasts differentiated from human MSCs | siRNAs generated from PDE7 and PDE8 PCR products | | | 473 | *β-catenin, osteocalcin, caspase-8, CREB-5, BMP-5* | 156 | *BMP-1, osteoflycin, 1,25-dihydroxyvitamin D3 receptor* | 80 |
| | Tumor chemonaive osteoblastic populations | Comparing the expression profiles of osteoblasts from osteosarcoma patients *vs.* controls | | | | *EBF2* | | | 339 |
| Human MSCs | Human MSCs | Treated with lactate for different time periods | | Genes involved in wound healing | | *Interleukin-6, heat shock protein, hypoxia-inducible factor-1 α* | | *Superoxide dismutase 2, BCL2- associated X protein* | 340 |
| | Human MSCs | Two osteogenic nanoscale topographies (pitted surface *vs.* raised islands) are compared with cells treated with dexamethasone | | | | *Integrin αM, TGFR1, MMP8, collagens, ALPL, integrinα1* | | | 341 |
| | Human MSCs | Cell culture | | | | | | *Osteogenic and adipogenic differentiation capacity of hMSCs in late stage of the culture* | 75 |
| | Human MSCs | Growth medium and osteoblastogenesis induction medium | | Interferon γ-inducible genes | | *ALP, OCN, TGFBRII, PDGFR, BSPII, OPN, LRP5, Runx2* | | | 342 |
| | Human MSCs | Extracellular matrix substrates | | | | | | *MCAM, angiopoietin-1* | 343 |
| | Human MSCs | Dexamethasone and 1,25-$(OH)_2D_3$ | 2095 | | 961 | *c-Myc* | | | 344 |
| | Human MSCs and ASCs | Osteogenic differentiation medium | 41 | Extracellular matrix-related genes | | | | *COL1A2, COL3A1, COL4A1, COL5A2, COL15A1, PHR, COL2A1, COL6A1, COL9A1, INT-β 3, INT-β 1, osteopontin, osteonectin* | 345 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | Human MSCs and osteoblasts | Comparing gene expression in osteoblasts *vs.* MSCs from the same donor | 170 | | 120 | *COL15A1, CDKNIA, DST, GADD45A, MACF1, COMP, COL8A2, STAT1, TNFRSF10B* | | *MK167, PARP3, RAD54L, ECM1, NRP1, GSTP1* | 346 |
| Human unrestricted somatic stem cells | Human unrestricted somatic stem cells | Has-mir-135b | | | | | | *IBSP, osterix* | 347 |
| Human monocytes | Monocytes from premenopausal women with extremely low *vs.* high PBMD | | 49 | *GBP1, STAT1, CXCL10* | | | | | 71 |
| | Monocytes from premenopausal women with extremely low *vs.* high BMD | | 13 | | 6 | *STAT1, IFI44L, CXCL10, IFI44, GPB1, GPB2* | | | 348 |
| Human hFOB1.19 cells | Immortalized human osteoblast cells hFOB1.19 | Two-stage cell transformation using MNNG and TPA treatments | 10 | *H19, MKRN3, NDN, CDKN1C, PHLDA2, MEST, CD81, GRB10, SLC22A18, SLC22A3* | 6 | *H19, PHLDA2, SLC22A18* | 4 | *CD18* | 349 |
| | Human osteoblastic hFOB1.19 cells | Oral administration of collagen tripeptide | | | 169 | *Osterix* | | | 68 |
| Human STRO-$1^+$ skeletal stem cell | STRO-$1^+$ cell from bone marrow of hip | Cells cultured in basal, osteogenic, chondrogenic and adipogenic lineage-specifying culture conditions | 38 | | 16 | *COL111A1, FKBP5, LOC388610, MT1E, MT1F, MT1X, LBP, MT1H/MT1P2, XTLT1, C1Oorf10, CXCL6, NPY2R, C22orf16, PTGFR, FAM89A, STC2* | 22 | *GATA6, LDB2, GREM1,QSOX1, CLDN11, KCTD12, RBMS3, ZFP36L1, RBM39, ROCK1* | 350 |
| Human HeLa and NIH3T3 cells | Human HeLa and NIH3T3 cells | Transfected with 5–10 μg siRNA vector | | | 2 | *OPG, VEGF-A* | | | 351 |
| Human myeloma cell lines | KMS-26 cell line | Enzastaurin | 94 | | 62 | *CXCL12, CXCR4, CTSB, TRAF5, BCL2L1, IGF1, GADD45A, CDC20* | 32 | *MYC, IRF4, MX11* | 352 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Human MNCs | MNCs from bone marrow and umbilical cord blood | Chondrogenic differentiation | | Receptors for fibronectin, vitronectin, osteopontin, and collagens | | *Ilk, CD47, Integrin a5b1, components of the vitronectin/osteopontin-receptors (avb5), collagens (a1b1, a2b1, a3b1)* | | | 353 |
| Human chondrogenic cell line OUMS27 | Human OUMS27 cells | Compared mRNA levels in OUMS27 cells adenovirally transfected with KLF5 and the control empty vector | | | | *MMMp9* | | | 354 |
| Human aortic valve interstitial cells | Human aortic valve interstitial cells | LPS stimulation or BGP treatment; media alone was used as positive control | | | | *BMP2, PDGFA, FGF2, MMP1* | | | 355 |
| Human OA cases and controls | Serum samples | Human OA cases compared to controls | 16 | | | *MMP-7, IL-15, sVAP-1* | | *PAI-1, ICAM-1* | 121 |
| | Bone samples | Patients suffering from hip OA compared to younger donors undergoing spinal arthrodesis | 83 | CCL2, FOS, OMD, PRSS11, DVL2, AKT1, CA2, BMP6, MMP2, TGFBR3, FLT1, TNFRS11B, BMP1 | | | | | 96 |
| Human cartilage | Chondrocytes from human knee cartilage | IL-1$\beta$ | | | | *CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL8, CCL2, CCL3, CCL4, CCL5, CCL8, CCL20, CCL3L1, CX3CL1, LIF, IL-6, GCSF3, ELAM1, MMP-13, BMP-2* | | *COL2A1, aggrecan* | 356 |
| | Human OA cartilage | Notch signaling was blocked by DAPT treatment | | | | *IGFiBP7, TNFSF15, IGF2, TNFRSF10c, FGFR2, CALCR1* | | *BMP2, IL8* | 95 |
| Human bone tissue | Human femoral bone tissue from non-osteoporosis controls and cases with osteoporosis | | | IGSF4, FABP3, FABP4, FKBP2, TIMP2, TRIB, TMSB4X | | | | | 357 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Human OPLL cells and non-OPLL cells | Human OPLL cells and non-OPLL cells | Osteogenic induction and siRNA-mediated knockdown of Runx2 | 47 | | 24 | *Stathmin-like 3, Sestrin 2* | 23 | *COMP, CTGF, angiopoietin-1* | 93 |
| Human RBM cell line | Human RBM cell line | Compared gene expression between two human RBM tissues and patient-matched primary RCC tissues | | | | *MIP-1δ* | | | 74 |
| Human dental pulp cells and human MSCs | Human dental pulp cells and human MSCs | Inorganic polyphosphate | | | | *MMP1, OPN, OC, osteoprotegerin* | | | 79 |
| Human periodontal ligament cells | Human periodontal ligament cells | Static tensional forces for 2 h | | | 2856 | *IL-8, MMP2, PDGF-A* | 2574 | *ALP, COL1A2* | 358 |
| Human osteosarcoma cell line Saos-2 | Human RANK-positive Saos-2 osteosarcoma cells | RANKL | 69 | | 21 | *ROCK1, EMA3A* | 48 | *GDF15* | 89 |
| Human sarcoma cells | Human sarcoma cells from osteosarcoma patients | Comparing gene expression profiles of sarcoma cells *vs.* human MSCs | | | | *ROR2* | | | 359 |
| Human MDNSCs | Human adult MDNSCs | Investigated the expression profile of cancer-related genes in MDNSCs by comparing with that in fresh normal human adult bone marrow depleted of red blood cells | | | 63 | *MYC, MMP2, Notch2, STC1, ITGA3, Wnt1, STAT5b, RhoC* | | | 360 |
| Human chondrocytes | Human chondrocytes in osteoarthritic cartilage | Compared gene expression profiles of the divided 3 zones of the cartilage | 198 | | | | | | 94 |
| Human cultured discs and cells | Human discs and anulus cells cultured in 3-dimensional | | | | | *IGFBP-2, IGFBP-4, IGFBP-5* | | | 361 |
| Mouse RAW 264.7 cell line | Mouse RAW264.7 cell line | RANKL | | | | *MMP-9, TRAP, cathepsin K, MST1R, integrin b3, NFATc1, calcitonin receptor* | | *CD14 antigen, toll-like receptor-6, TNF, STAT-1, Fc receptor 11b, TNF receptor 1B* | 362 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Mouse RAW 264.7 cells | Mouse RAW 264.7 mature osteoclasts | | | | | *SPHK1, Wnt10b, BMP6* | | *Sclerostin* | 363 |
| Mouse RAW 264.7 cell and BMC | Mouse RAW 264.7 cell and BMC | RANKL | | | | *NHA-oc/NHA2* | | | 73 |
| Mouse MC3T3 E1 cells | Mouse osteoblasts MC3T3 E1 cells, clone 4 | Three dishes for 1-h thapsigargin treatment at 10 nM and the other three dishes as control | | | | *ATF4, eIF2α-p, FOS, FGF-9, BMP-2* | | | 364 |
| | Mouse MC3T3-E1 preosteoblasts | Histone deacetylase inhibitors, such as trichostatin A, MS-275, or valproic acid | | | | *Slc9a3r1, sorbitol dehydrogenase 1, glutathione S-transferase α 4* | | *Proteasome subunit, β type 10, adaptor-related protein complex AP-4 σ 1* | 365 |
| | Mouse osteoblastic cell line MC3T3-E1 subclone 4 | Mechanical straining | 674 | *Fos, Ptgs2, Rgs2, Pthlp* | | | | | 366 |
| Mouse bone marrow cells | Mouse osteoclast progenitors | IL-6 and RANKL | | Genes related to MAPK phosphatases and the ubiquitin pathway | | *MKP 1, MKP 7* | | *Senp 2, Cul4A* | 367 |
| | Primary bone marrow cells | IGF2 | | | | *CXCL7, SDF1* | | | 72 |
| Mouse C3H10T1/2 cells | Mouse C3H10T1/2 cells | Runx2 or osterix adenovirus | | | | | | *Wnt4, Bglap1, BMP7* | 69 |
| Mouse C3H/HeJ and C57BL/6J bone | Mouse C3H/HeJ and C57BL/6J femur | Rosiglitazone | | | | Genes associated with PPARG signaling pathway and fatty acid metabolism | | Genes associated with cell cycle | 368 |
| Mouse C57BL/6 tissues | Tibiae from wild-type mice | Mechanical loading for 3 h | 642 | | 324 | *Osteopontin, Postn, Ostn, Dlx5, Bmp4, Bmp10, Sost, Timp1, Timp2, Ctgf, Esr1* | 318 | | 369 |
| | Bone samples from the tibiae | Ankle load | | | 242 | *C-fos, Egr1, Atf3, MMP3* | 199 | *TNF, CRLF1, BARX2* | 370 |
| Mouse breast adenocarcinoma cell lines | Tumor-bone interface and the tumor alone area | Genetic expression at the tumor-bone interface was compared with the tumor alone area | | | | *Cathepsin G, cathepsin K, MMP9, MMP13* | | | 92 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Mouse MSC line, Kusa 4b10 cells | Differentiating osteoblasts | PTH or PTHrP | 4675 | 1444 genes were significantly changed by both treatments, 1384genes changed specifically by PTH, and 403 genes specifically by PTHrP at all time points | | Genes belonging to the family of ephrins and their receptors, such as *ephrinB2* | | *PDZ-RGS3* | 87 |
| Mouse ES cells | Mouse ES cells | Investigate the differentiation of mouse ES cells cultured under three differentiation conditions, embryoid body, gelatin, matrigel | | | | *Spp1, Csf1, Gsn, Bmp8b, Crlf1* | | | 371 |
| Mouse chondrogenic cell line ATDC5 | Mouse chondrogenic cell line ATDC5 | Dexamethasone | 96 | | | *CTGF, integrin*$\alpha$*10, SGCK, DMP1, lipocalin 2* | | *SFRP, IGF-1, Lumican* | 372 |
| | Mouse chondrogenic cell line ATDC5 | Leucine and rapamycin | | 1,571 genes affected by leucine restriction and 535 genes affected by rapamycin | | | | | 373 |
| Mouse VSMCs | Mouse VSMCs | MK 4 | | | | *DT-diaphorase* | | *Osteoprotegrin* | 374 |
| Mouse 129SvEv | 129SvEv Sparc$^{tm1cam}$ null, male *vs.* 129Sv/Ev wild-type, male, femoral midshaft | | | | | | | *Sparc, Zfp162, Bysl, E2F4* | 307 |
| Swiss Webster mouse BVECs and MVECs | VECs from trabecular bone regions and central marrow cavity regions of mouse long bones | Compared the gene expression in BVECs and MVECs | | | 5 | *ALDH3A1, SMOC-2, MMP-13, C/EBP-b, ANX8* | 2 | *Spa, MGP* | 375 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Mouse TM40D and TM40D-MB cells | Mouse TM40D and TM40D-MB cells | Compared the gene expression profile of low (TM40D) *vs.* high metastatic (TM40D-MB) tumor cells | 20 | | | *COX2, Fgf7, Ptx 3* | | *CD1d1 antigen, Cxcl1* | 376 |
| Mouse osteoblasts | Osteoblasts | Wnt3a and FGF alone or in combination | | 70% of the genes induced by Wnt3a were down-regulated by combined FGF treatment | | | | | 377 |
| | Calvarial osteoblasts from 7-d-old CD1 mice | Unit gravity (1 g) *vs.* modeled microgravity (0.0008 g) | | Genes involved in osteoblast differentiation, function, and osteoblast-osteoclast cross-talk | 45 | *IL-6, Lcn2, Nqo1* | 88 | *Penk1, Tnmd, Aspn, Cdh11, Ogn, Wisp2, Sfrp2* | 378 |
| | Primary osteoblast cultures isolated from wild-type and Arrb2 $^{-/-}$ mouse calvaria | Intermittent PTH | | | 215 | *Slc11a1, Unc93b1, Pla2g7, Lgmn, Cebpd* | 200 | *Sept7, Ttc3, Steap4, Bmpr1a, Sh3bgrl, Hltf* | 379 |
| | Osteoblasts and osteocytes from mouse neonatal calvaria | Comparing genes expression between osteocytes and osteoblasts | 385 | | 249 | *Col15a1, Ank, Gnas, ENpp1, Enpp6, Notch1, Dlk1, Ptprz1, Bmp4, Gdf10, Tgfβ3, Fgf1* | 136 | *Col16a1, Mmp9, Mmp23, Adamts18, Fzd1* | 380 |
| Mouse osteocyte Y4 Cells | Osteocyte Y4 cells from the long bone | $10^{-7}$ M risedronate and alendronate for 48 h | | | | Genes encoding zinc ion binding proteins | | | 381 |
| Mouse osteocyte | Mouse osteocyte | Comparing the gene expression profiles of GFP-positive *vs.* GFP-negative cells | | | 269 | *DMP1*, *Sost* | | | 382 |
| Mouse ST2 cells | Mouse ST2 osteoblastic/stromal cells | *P. gingivalis* ATCC33277, gingipain-mutants | | | | | | *Cyclin D, Cyclin E* | 383 |
| Other parts or tissues of mouse | Calvarial cells of Wnt5a$^{-/-}$ and wild-type mice | Gene expression profiles of the Wnt5a$^{-/-}$ calvarial cells as compared to wild-type cells were evaluated | 3528 | | 2002 | *Fb1, Fst, Cccng1, Wisp1* | 1526 | *Runx2, Osterix, ALP, Sfrp2, Igf2, Dhh, Col2a1* | 384 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | Suture-associated dura mater | Compare genomic changes in the dura mater underlying the posterior frontal and sagittal sutures of mice | 6057 | 2529 genes at postnatal d 5, increasing to 3439 genes at postnatal d 10 and 6057 genes at postnatal d 20 | | *Igf2, Tgfb2, Vegf-C, cadherin 1, Collagen 1* | | *Mmp13* | 385 |
| | Cartilage of the distal femur of neonatal mice | Dicer-deficient | | | | *Hmga 2* | | | 386 |
| | p85$\alpha^{-/-}$ and wild-type OCs | M-CSF and RANKL | | | | | 94 | *MITF, JDP2, cathepsin K, TRAP, MMP-9, integrin β3* | 387 |
| | Diabetic mice and control mice | Insulin | | | | | | *FUNX2, RUNX2-related osteogenic genes* | 388 |
| | Mouse femurs and tibiae excluding joints and primary metaphyses | Glucocorticoid treatment | | | | *Csf1, c-fms, Ibsp, Itgb3, disintegrin, Adam8, Trem2, Oscar, PlCγ, c-Fos, Nfatcl, c-Src, Syk, Vav3, ATPase, Ctsk* | | *TGFβ1, BMP-2, LEF1, Akp2, MAPK* | 88 |
| | Region of regeneration with the underlying dura mater in skeletally immature and mature mice with injured calvaria | Parietal bone defects were created by a 4-mm trephine bit | | | 25 | *BMP-2, BMP-4, BMP-7, IGF-2, FGFR-1, Ptn, Acp5, Ctsk, Mmp2, Mnp14, Ttr, Ptgds* | | | 389 |
| | Mouse calvarial bone | Examined the effect of the col9al-null mutation on the expression of osteoblastic genes | 1 | *Col9a1* | | | 1 | *Col9a1* | 267 |
| | Femur of C3H and B6 strains mice | Compared the effect of rosiglitazone in the C3H and B6 strains | | | 7 gene sets | *PPARG signaling pathway and fatty acid metabolism in both C3H and B6 strains with no significant difference between the two strains* | | *Genes associated with cell cycle in the C3H strain* | 368 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| | Mouse wounds | HOXA3 | 837 | | 332 | *Lrp1, MCP-1/Ccl2* | 505 | *Tnf-α, Myd88, Tollip* | 390 |
| Rats of Sprague-Dawley | Kidney of female Sprague-Dawley rats | Ovariectomy and herbal formula | 64 | | 22 | *HSD17B2, rGK-4, PDK4, HMGCS2* | 42 | *PTGER3, NQO1, YC2, UGT1A6, GSTP2, AKR7A3, SREBP-1* | 391 |
| | Both pairs of femora and tibiae of Sprague-Dawley female rats | Three different PTH peptides, PTH (1-34), (1-31), and (3-34) | | *Slpi, tfpi2, socs3, gro1* | | *RANKL, c-fos, phex, Gprc5c* | | *CXCR4* | 86 |
| | Fractured femur of male Sprague-Dawley laboratory rats | TP580 | | | | *PI3KR1,CAMKK1, Fibronectin, NFATC1, NFATC2, VHL, COX2* | | *Dynamin 2, caveolin 1* | 84 |
| | BMSCs of Sprague-Dawley rats | Exposed to simulated microgravity and static controls | 413 | | 206 | *ABI1, PTTG1, PMP22, ETS1* | 207 | *CCND1,CDK5, CDC5L, CATNB, FZD1, GPNMB, WNT5A, WISP1* | 78 |
| | Growth plate chondrocytes from the PC and RZ of Sprague-Dawley rats | Captured the unique features of the PC and RZ | | 8 transcripts showing high expression unique to the PC and RZ, respectively | | *Tpt1, Ctgf, Morf4l1, Gas6, Col5a2, Frzb, Gdi2, Jund; Col9a1, Hapln1, Col1a2, LOC689064, Smoc2, Cast, Rp137, LOC497729* | | | 392 |
| | BMSCs of Sprague-Dawley | α-Minimal essential medium containing β-glyceraldehyde-3-phosphate, L-ascorbic acid, dexamethasone and 1,25-$(OH)_2D_3$ | | | 12 | *Pla2g2a, Expi, Pcp4, Cx3cl1, GluAP, Fcna, Mmp9, Lcn2, Il10, Fmo1* | 1 | | 76 |
| | Cultured bone marrow MSCs of Sprague-Dawley rats | Electromagnetic field | 19 | | 6 | *Bmp1, Bmp7* | 13 | *Egf, Egfr* | 77 |
| Rats of Wistar strain | Femurs | Titanium implants | | | 86 | Collagenous and noncollagenous extracellular matrix-related genes, proteoglycans and bone resorption-related genes | | | 85 |
| | Abdominal aorta tissues | High phosphorus diet | 53 | | | | 53 | *SFRP1, SFRP2, SFRP4, cathepsin K* | 393 |

(*Continued*)

**TABLE 6.** Continued

| Subject or sample categorization | Cell type or tissues | Treatments | Differentially expressed genes (n) | Important differentially expressed genes | Up-regulated genes (n) | Important up-regulated genes | Down-regulated genes (n) | Important down-regulated genes | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| Rats of Fisher 344 | Irradiated and nonirradiated primary osteoblast cultures | HBO treatments | | | 26 | *Integrin β1, β-tubulin, ADP-ribosylation factor 1, stearoyl-coenzyme A desaturase 2, aldolase A, HSP90 β, peroxiredoxin* | 7 | | 394 |
| Rats of Fisher 344 and LEW strains | Proximal femora | Copenhagen 2331 and Dark Agouti were used as a negative control | 99 | *VEGF, FGF2, IGF2, IGF2BP3, TNF* | | | | | 395 |
| Rats of Fischer 344, LEW, COP, and DA strains | Femoral bone tissues | Comparing the mRNA sequence of QTL regions contributing to the variation in lumbar vBMD with the NCBI database | 285 | *Akap1, Asgr2, Esd, Fam101b, Irf1, Lcp1, Ltc4s, Mdp-1, Pdhb, Plxdc1, Rabep1, Rhot1, Slc2a4, Xpo4* | | | | | 396 |

Adapted from Y. Guo *et al*.: *Expert Rev Endocrinol Metab* 3:223–267, 2008 ([3]), with permission from Expert Reviews Ltd. IT, Intertrochanteric; OP, osteoporosis; ZDCs, zirconium dioxide ceramics; ODHPSC, osteoblasts derived from human pulpar stem cell; PDE, phosphodiesterases; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate; MNNG, N-methyl-N′-nitro-N-nitrosoguanidine; MNC, mononuclear cell; KLF, Kruppel-like factor; LPS, lipopolysaccharide; BGP, β-glycerol phosphate; DAPT, N-[N-(3,5-diflurophenylacetate)-L-alanyl]-(S)-phenylglycine t-butyl; RCC, renal cell carcinoma; RBM, RCC bone metastasis; MDNSCs, marrow-derived neural stemlike cell; ES, embryonic stem; VSMC, vascular smooth muscle cell; MK-4, menaquinone-4; BVEC, bone-derived vascular endothelial cell; VECs, vascular endothelial cells; MVEC, marrow-derived vascular endothelial cell; M-CSF, macrophage colony stimulating factor; OC, osteoclast; PC, perichondral zone; RZ, reserve zone; HBO, hyperbaric oxygen: ASCs, adipose tissue-derived stromal cells; GFP, green fluorescent protein; siRNA, small interfering RNA; vBMD, volume BMD.

which is secreted by renal cell carcinomas that metastasize to bone. Collectively, these studies have helped fill important gaps in our knowledge regarding regulation of osteoclastogenesis, thereby enhancing our fundamental understanding of the pathogenesis of osteoporosis.

#### B. Proliferation and differentiation of mesenchymal stem cells (MSCs)

MSCs, which can differentiate into osteoblasts, chondrocytes, adipocytes, and myoblasts *in vivo* and *in vitro*, have been regarded as the most hopeful cell sources for bone tissue engineering, regenerative medicine, and gene therapy. Osteoporosis might be due to defects in MSCs that lead to reduced proliferation and osteoblast differentiation. However, the use of MSCs as seeding cells in bone tissue engineering is hampered by a lack of fundamental knowledge about the molecular mechanisms underlying osteogenic differentiation of MSCs. During the past 2 yr, several studies have explored the molecular mechanisms underlying osteogenic differentiation of MSCs (75). Evidence suggests that the capacity of MSCs for osteogenic differentiation was highly suppressed during late culture stages. The expression of four genes, *EPHA5*, *NOV*, *NDN*, and *RUNX2*, varied depending on culture stage, suggesting that these genes have the potential to act as stage-specific markers during osteogenic differentiation of MSCs (75). Purkinje cell protein 4 (*Pcp4*), a gene involved in the deposition of calcium and the modulation of calmodulin-dependent protein kinase, was found to be increased during osteoblastic differentiation of MSCs *in vitro* (76). *Bmp1* and *Bmp7* were up-regulated, whereas *Egf* and *Egfr* were down-regulated during osteogenesis of MSCs subjected to electromagnetic field treatment (77). In addition, simulated microgravity, inorganic polyphosphate, and phosphodiesterases all play important roles in the regulation of osteoblastic differentiation from MSCs by inhibiting population growth of MSCs or by activating fibroblast growth factor (FGF) signaling pathways to induce both proliferation and mineralization of MSCs (78–80). However, the specific signaling pathways controlling gene expression in the osteogenic differentiation process are still not clear. Further study of the molecular mechanisms underlying osteogenic differentiation of bone marrow cells will have important theoretical and practical significance in bone regeneration and repair.

#### C. Gene expression in healthy *vs.* diseased tissues

Comparative gene expression studies of healthy *vs.* diseased tissues can be quite informative because they provide immediate information about the differential regulation of genes that may contribute to the disease process. Two studies by Hopwood *et al.* (81, 82) used microarray analysis to compare expression levels of thousands of genes from osteoporotic individuals to age-matched osteoarthritic or control individuals; for these studies, they investigated trabecular bone from the intertrochanteric region of the proximal femur. A substantial number of the top-ranking differentially expressed genes identified in osteoarthritic bone are known to play roles in osteoblasts, osteocytes, and osteoclasts. Many of these genes are targets of either the Wnt (*TWIST1*, *IBSP*, *S100A4*, *MMP25*, *RUNX2*, and *CD14*) or TGF-β/BMP signaling pathways (*ADAMTS4*, *ADM*, *MEPE*, *GADD45B*, *COL4A1*, and *FST*) (81).

#### D. Effects of therapeutic agents on the healing of fractures

An enhanced understanding of the molecular mechanisms by which therapeutic agents affect the healing of fractures has provided substantial motivation for performing expression profiling studies of bone repair. TP508, a 23-amino acid peptide that represents the thrombin-binding domain for a specific class of thrombin receptors, has a wide range of therapeutic effects on tissue repair (83). By comparing the translational profiles of fractured femurs treated with TP508 *vs.* saline controls, Li *et al.* (84) demonstrated that TP508 accelerated fracture healing by modulating expression levels of proteins primarily involved in the functional categories of cell cycle, cell growth, proliferation, and cell death. In another study of healing in rats treated by titanium implant *vs.* osteotomy, Kojima *et al.* (85) determined that 86 gene transcripts, including extracellular matrix-related, bone resorption-related, and proteoglycan gene transcripts, were up-regulated in at least one time point. Further studies are needed to identify the biological roles of the transcripts in osteointegration. These data not only provide new insights into fracture healing physiology, but also provide the rationale of potential new treatment strategies for fractures.

#### E. Endocrine regulation of bone remodeling

Systemic effects of hormones and growth factors play an important role in physiological and pathological mechanisms of bone remodeling. PTH, which is currently used in the therapy of osteoporosis, has been studied intensively. One important study of PTH and bone investigated the anabolic and catabolic effects of intermittent and continuous treatments with three different PTH peptides, PTH (1-34), (1-31), and (3-34) (86). This study identified and validated a large number of genes such as *slpi*, *tfpi2*, *socs3*, and *gro1*, which were previously not considered to be expressed in bone or to be regulated by PTH treatment. The precise function of these newly identified genes in the anabolic and catabolic bone state, if any, deserve further investigation. In using mouse whole-genome cDNA microarrays to assess the responses to PTH (1-34) and

PTHrP (1-141) in the Kusa 4b10 mouse marrow stromal cell line, members of the ephrin and Eph family were identified as targets of PTH (1-34) and PTHrP (1-141) (87). Among the regulated genes, ephrinB2 mRNA was up-regulated in response to both PTH and PTHrP. EphrinB2 protein might act in a paracrine or autocrine manner on the osteoblast itself to stimulate osteoblast maturation and/or bone formation under the influence of local PTHrP or administered PTH (87). Glucocorticoid excess can induce alterations in bone metabolism that weaken bone structure and increase fracture risk. Using microarrays in glucocorticoid excess-treated mice, Yao *et al.* (88) revealed that glucocorticoid excess was associated with early activation of genes associated with osteoclastogenesis (*csf1*, *c-fms*, *lbsp*, and *ltgb3*) and adipogenesis (*c/EBPα* and *PPARγ*) and a later suppression of genes associated with osteogenesis and mineralization (*TGFβ1*, *BMP2*, *LEF1*, *Akp2*, and *MAPK*). These gene expression changes may correspond to alterations in bone metabolism with glucocorticoid exposure that result in rapid bone loss. These results enhance our understanding of glucocorticoid-induced bone loss by providing *in vivo* evidence supporting the concept that glucocorticoid excess directly or indirectly regulates the transcription of specific genes associated with bone physiology.

Besides the aforementioned studies, during the past 2 yr, fruitful results were also generated by microarray studies in several additional bone biology research fields, including osteosarcoma biology (89, 90), spine fusion (91), regulation of osteolysis (92), and osteoblast- or chondrocyte-mediated pathological processes in bone diseases such as ossification of the posterior longitudinal ligament (OPLL) of the spine (93) and osteoarthritis (OA) (94–96). These studies will not only illuminate new methods for bone biology research but also are likely to provide significant insights that will contribute to the development of new therapeutic interventions in the near future.

As our previous review pointed out, most high-throughput gene expression studies have used cultured cell lines of humans, mice, and rats *in vitro*. The disadvantage of using cultured cell lines is that gene expression profiles undoubtedly change because cultured cells gradually lose their osteogenic potential due to lack of stimulation by factors that are available *in vivo*, but not *in vitro*. Given this significant limitation, studying fresh cell- or bone-related tissues may be a promising way to obtain data that more closely resemble *in vivo* conditions.

## VII. Proteomics Studies

Because cell recognition and signal transduction pathways occur at the protein level (*e.g.*, receptor/ligand interactions, antigen recognition, and cell adhesion), it is appropriate and important to utilize proteomics approaches to directly investigate protein expression. In addition, because of complicated processes such as alternative mRNA splicing and posttranslational modification of proteins, the correlation between expression levels of mRNA and proteins could be low (97, 98). The Human Genome Project revealed that there are far fewer protein-coding genes in the human genome than there are proteins in the human proteome [~35,000 genes give rise to ~1,000,000 proteins (99)]. This profound discrepancy clearly indicates that the diversity of proteins cannot be fully characterized by gene-expression analysis alone. Consequently, to understand functional genomics, proteomics approaches have become an indispensable complement to mRNA expression microarrays. Combining the data generated by these two approaches provides much richer information than that provided by genomic studies alone.

### A. Studying cultured cells using *in vitro* systems

Proteomics has only recently been applied to the bone field, so only a few relevant studies have been published in the past 2 yr. Most of these studies involve expression proteomics, in which samples from case and control groups are quantitatively analyzed to identify proteins that are differentially expressed between groups. The most common approach was to use *in vitro* cell culture systems, and several proteins that are important for the development of MSCs (100–104), osteoblasts (105–109), osteoclasts (110–113), chondrocytes (114, 115), bone marrow osteoprogenitor cells (116), and osteosarcoma cells (117) were identified in this manner. For example, by applying two-dimensional liquid chromatography/matrix-assisted laser desorption/ionization mass spectrometry (MS) on MSC-derived fast-growing clones (with tripotential differentiation capacity) *vs.* MSC-derived slow-growing clones (with only unipotential differentiation capacity), Mareddy *et al.* (102) identified 11 proteins (*e.g.*, calmodulin, tropomyosin, and caldesmon) with differential expression. Using iTRAQ-coupled two-dimensional-liquid chromatography-tandem MS/MS analysis, Xu *et al.* (105) found several cytoskeletal proteins, metabolic enzymes, signaling and cell growth proteins that were differentially expressed in human osteoblasts cultured on plane HA *vs.* osteoblasts cultured on carbon nanotube-reinforced HA. These findings of *in vitro* proteomics studies, however, may be limited by the fact that factors regulating gene expression in complex physiological/pathological environments *in vivo* can be changed or eliminated *in vitro*. Consequently, *in vitro* proteomics studies are likely to be compromised by this comparative lack of regulatory control.

### B. Studying fresh cells, serum, or tissues

Compared with *in vitro* studies, *ex vivo* studies using fresh cells or tissues are more likely to provide data that represent protein expression under physiological/pathological conditions. Our group recently used two-dimensional electrophoresis (2-DE) coupled with MS to perform a comparative protein expression profiling study of circulating monocytes from premenopausal Chinese females with extremely high BMD *vs.* those with extremely low BMD. In total, 38 differentially expressed proteins were identified, and five of these proteins (GSN, FSU1, SOD2, GPX1, and P4HB) were confirmed by Western blotting (118). Moreover, several additional studies of serum and fresh tissues have been performed in the past 2 yr to investigate the global-scale molecular profiling of bone-related diseases, such as OA (114, 119–121), osteonecrosis of the femoral head (122), and osteosarcoma (123). Using 2-DE, a comparative analysis of the proteins extracted from patients with OA cartilage *vs.* those with normal cartilage was performed (119). After further identification by linear ion trap-Fourier transform ion cyclotron resonance MS, 14 proteins associated with OA were unambiguously determined, including proteins involved in glycolysis and energy production (ADH, ADK, ENOA, KPYM, and FR), signaling (ANNX-I, PEBP, and TUB), Redox (PRDX3 and SODM), and cartilage matrix (COLL-I and COLL-VI). In another study using integrated genetic, bioinformatic, and proteomic approaches, five novel proteins (SOX11, FGF23, KLF6, WWOX, and GDF15) were implicated in the genesis of OA (120). Based on the methods of isoelectric focusing, 2-DE, and silver staining, as well as matrix-assisted laser desorption ionization time-of-flight mass spectrometry, levels of kininogen 1 variant, complement factor C3 precursor, and complement factor H were found to be increased, whereas levels of antithrombin III chain B, apolipoprotein A–IV precursor, and gelsolin isoform $\alpha$ precursor were decreased in osteonecrosis of the femoral head patients (122). By comparing the protein expression profiles of two distinct groups of osteosarcoma biopsy samples, a chemosensitive group *vs.* a chemoresistant group, Kawai *et al.* (123) identified 10 protein spots associated with the chemosensitivity of osteosarcoma to preoperative chemotherapy. The proteins represented by these 10 spots could potentially be new diagnostic or prognostic markers for osteosarcoma, or new therapeutic targets for the disease.

Proteomics represents a promising field that is poised to boost our understanding of the dynamic nature of protein expression, cellular and subcellular protein distribution, posttranslational modifications, and protein-protein interactions. However, the application of proteomics to the bone field is only in its initial stages. Current proteomics studies in osteoporosis focus mainly on expression proteomics. Because key proteins involved in osteoporosis development generally interact with other proteins, functional proteomics studies will be necessary to provide a global understanding of protein-protein interactions. To better understand and even predict the functions of proteins, additional knowledge regarding three-dimensional structures of the proteome is required. Structural proteomics may prospectively fulfill this goal by mapping out the structures of protein complexes or proteins in a specific cellular organelle.

The impact of advances in the field of proteomics on our knowledge of osteoporosis is relatively small at this point. As new techniques are developed and applied to bone, however, we anticipate that the abundance of new information generated will be crucial toward understanding the pathogenesis of osteoporosis and developing novel therapeutic strategies for treating this disease.

## VIII. Future Directions

### A. Functional studies

After genes for osteoporosis have been identified, it is important to perform functional studies to determine the influence of such genes on the differentiation of osteoblasts or osteoclasts and the effects of such genes on the variation of bone-related traits. The strategies may involve *in vitro* and/or *in vivo* assays to test the effects of the gene of interest in cell cultures or animal models. Strategies for *in vitro* assays often include overexpression or knockdown of gene expression in osteoblast or osteoclast precursor cells, which is followed by evaluation of alkaline phosphatase (ALP) or tartrate-resistant acid phosphatase (TRAP) activity to assess osteoblast or osteoclast differentiation. To determine the potential molecular mechanisms by which the expression of target genes are regulated through specific transcription factors, EMSAs and chromatin immunoprecipitation can also be employed. Should strong functional evidence be obtained *in vitro*, one will consider further *in vivo* investigations using knockout and/or transgenic approaches to examine how the target gene changes the bone-related phenotypes, *e.g.*, BMD or BS. *In vivo* functional analyses are admittedly challenging for complex traits partially due to biological redundancy and the significant potential for knockdown of critical genes to prevent *in utero* development.

Furthermore, the specific impact of potential functional variants from the gene of interest will be ascertained through, *e.g.*, promoter assay, RNA splicing assay, and gene activity assay. First, if SNPs change recognition sequences of potential transcriptional factors, binding affinity to the transcriptional factors may be modified, lead-

ing to changes in transcriptional efficiency. Investigators can perform EMSAs and luciferase reporter assays to examine this potential effect. Second, for SNPs located in the splice-site, one can detect alternative mRNA splicing using mRNA phenotyping protocols (124). Third, if SNPs lie in the coding region, constructs that differ in the target SNPs can be prepared and expressed *in vitro*. The activity of different protein products can then be compared using appropriate assays for determining the function of that specific protein.

### B. Epigenetic variation

Epigenetics refers to reversible, heritable changes in gene regulation that occur without a change in DNA sequence. Epigenetic regulation has been implicated as a key regulatory mechanism in the etiology of human complex diseases (125). miRNA regulation, DNA methylation, and histone methylation are three common types of epigenetic modifications. Previous pioneering studies have shown that DNA methylation may be involved in the osteoclastogenesis (126), and acetylation of histone H3 and H4 may be involved in osteogenesis (127). Genome-wide measurement of epigenetic variation has recently been made possible using techniques such as Affymetrix Human Tiling 2.0R Array Set, which will allow us to progress toward a thorough understanding of the roles of epigenetics in osteoporosis.

### C. New phenotypes

Active shape modeling has been used to create a template describing the outline of the hip joint from dual energy x-ray absorptiometry images. Using this method, Goodyear *et al.* (128) identified risk factors for hip fractures independent of BMD. Composite indices of FN strength that are also constructed from hip dual energy x-ray absorptiometry images, such as compression strength index (CSI), bending strength index, and impact strength index, have the potential to improve hip fracture risk assessment (129). Although two studies have made efforts in finding genetic variants for CSI (130, 131), more genetic studies for these new phenotypes are necessary.

## IX. Future Prospects for the Application of Genetic Risk Assessment in Osteoporosis Prediction and Treatment

Genes that have been implicated in osteoporosis by genetic studies only make minor contributions individually to bone density and fracture risk. For example, the five regions identified in the study of Styrkarsdottir *et al.* (39) accounted for only approximately 3% of the total variation in hip and spine BMD. Similarly, the contribution of the CNV of *UGT2B17* gene to variations in BMD, cortical thickness (CT), and buckling ratio (BR) in Caucasians was only 0.67, 0.71, and 0.77%, respectively (47). It is thought that many genes, each with small effects, may be responsible for osteoporosis, rather than a small number of genes with large effects (132). Thus, osteoporosis predictive tests are likely to involve many genes, and tests for single genes are unlikely to be of clinical significance. Therefore, in theory, if most of the major genes that cause osteoporosis can be identified and their interaction with each other and with environmental factors can be understood, this information can be used to identify those who are at risk. However, the depth of our knowledge currently falls far short of this goal. Because molecular genetic studies in the field of osteoporosis are coming at a rapid rate, we are hopeful that, in the next one or two decades, sufficient genetic information will become available to develop genetic algorithms to assess the risk for osteoporosis.

Genetic studies have established a link between osteoporosis in humans and the Wnt, BMP-Smad, and 12/15-lipoxygenase pathways. Recently, the EphrinA-EphR pathway was found to be associated with FN bone geometry section modulus by pathway-based GWA analysis (133). It is likely that new pathways related to osteoporosis will continue to be identified by genetic studies, such as pathway-based GWA analysis. Furthermore, proteomics approaches could characterize the disease process directly by finding sets of proteins that work together to produce disease. Although our fundamental knowledge is fairly limited, there is a general feeling that bone-related pathways provide a substantial potential for developing novel ways to treat osteoporosis (134). Current emphasis in the development of new anabolic therapies for osteoporosis is directed at modifying the effects of Wnt pathway on osteoblast differentiation and bone formation (135). However, due to the pleiotropic function of these various pathways, there is substantial concern that it will be difficult to manipulate these pathways for osteoporosis treatment without producing unwanted side effects on other cells and tissues. In the coming years, basic and clinical efforts will be required to explore new pathways and modes of translating knowledge about the contribution of these pathways to the pathogenesis of osteoporosis into therapeutic applications.

## X. Summary

In this article, we have updated the progress of genetic studies of osteoporosis published from October 2007 to November 2009 at three levels, corresponding to DNA, mRNA, and protein. Since our last update toward the end of September 2007, remarkable progress has been made in revealing the genetic basis of osteoporosis. A number of

promising candidate genes, genomic regions, or proteins were identified, and several of them have been replicated by multiple studies. However, the majority of these findings are still inconclusive, pending further investigation. Efforts must be made in various aspects, including controlling population stratification and genetic heterogeneity, performing larger-scale studies in possibly hundreds of thousands of subjects, exploring new phenotypes, considering gene-gene and gene-environment interactions, deep resequencing of the genome, investigating the contribution of CNVs, DNA methylation, and histone modifications, and identifying the causal variants and their biological roles in osteoporosis. We expect that the results of these studies will be used to develop rational approaches toward predicting whether or not individuals are predisposed to developing osteoporosis and novel therapeutic approaches toward the treatment of this important disease.

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# *What's in a name? What constitutes the clinical diagnosis of osteoporosis?*

**E. S. Siris, S. Boonen, P. J. Mitchell, J. Bilezikian & S. Silverman**

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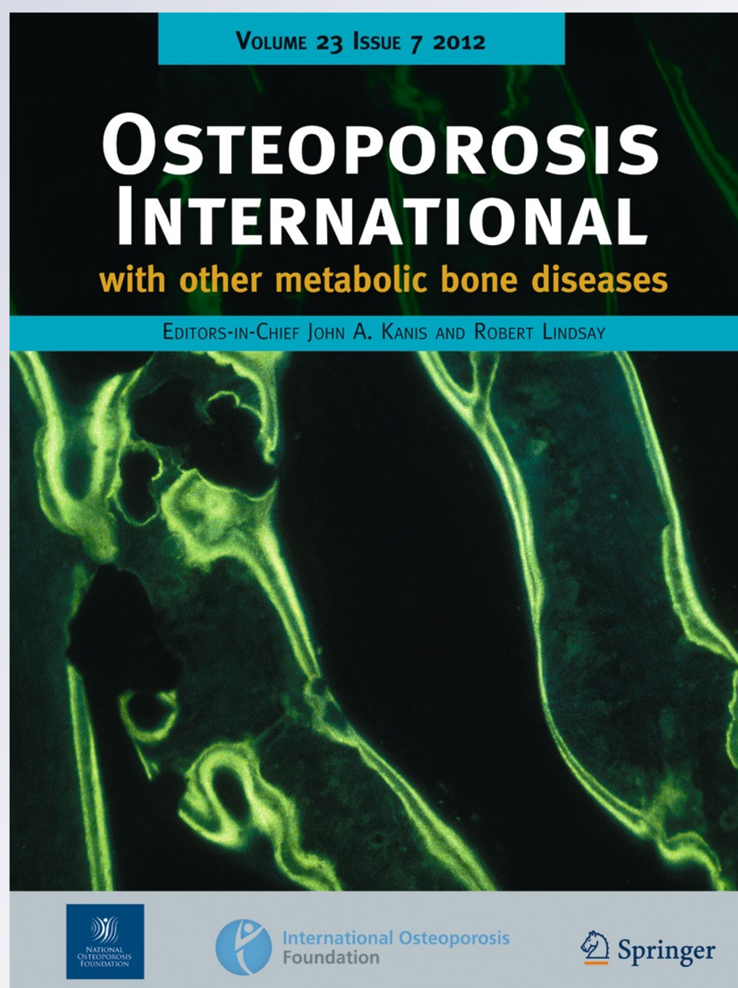


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OPINION PAPER

# What's in a name? What constitutes the clinical diagnosis of osteoporosis?

E. S. Siris · S. Boonen · P. J. Mitchell · J. Bilezikian · S. Silverman

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**Abstract** Osteoporosis is a skeletal disorder in which reductions in bone strength predispose to an increased risk for fractures. Currently, the diagnosis is officially made based exclusively on bone mineral density T-scores that are ≤−2.5 at the spine or hip. Limiting the clinical diagnosis of osteoporosis solely to a T-score-based criterion, which is the official convention in the USA, creates uncertainty about the use of the term osteoporosis to diagnose older women and men who have T-scores >−2.5, but either have already sustained low-trauma fractures or are recognized as having high fracture risk based on absolute fracture risk calculations from FRAX or other algorithms. A failure to diagnose such patients as having osteoporosis may be one component of the well-documented underdiagnosis and undertreatment of this disease which limits our ability to reduce the burden of fractures worldwide. There is a need to expand the criteria for making a clinical diagnosis and to codify these changes in order to help patients, physicians, policy makers, and payers better understand who has this disease and the elevated risk for fracture that it represents.

**Keywords** Bone mineral density · Fracture risk · Fractures · FRAX · Osteoporosis diagnosis

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## Introduction

A National Institutes of Health Consensus Conference convened in 2000 defined osteoporosis as a skeletal disorder characterized by compromised bone strength predisposing to an increased risk for fracture [1]. We have come to recognize that bone strength is a function of both the "quantity" of bone—estimated by measuring bone mineral density—and the "quality" of bone, a complex and multidimensional set of properties including bone microarchitecture, turnover, mineralization, and damage accumulation. The criterion for making a diagnosis of osteoporosis today officially rests upon the quantity component, the bone mineral density T-score measured at the lumbar spine, total hip, or femoral neck. A T-score of −2.5 or lower at one or more of these sites is the arbitrary but not unreasonable cut point for categorizing an older individual as having osteoporosis, and it is an indication for the use of medical treatment to lower the risk of future fractures. Making this BMD-based diagnosis is also critical in terms of using, at least in the USA, an ICD-9 code that definitively labels the patient as having osteoporosis and is necessary for third party payers who are billed for services associated with the disease and for reimbursement for medication given for the disease. Estimates

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of the prevalence of osteoporosis in both postmenopausal women and older men are based upon estimates of the number of individuals with T-scores at or below −2.5 at the hip, and these estimates influence health care policy and public perceptions about this disorder.

The T-score criterion for osteoporosis remains its key characteristic diagnostically, even though it has been clear for some time that most fractures that are considered linked to osteoporosis occur in individuals with bone densities that are better than −2.5 [2–4]. For older persons at high risk for fracture based on non-BMD clinical risk factors (including advanced age, frailty, prior fractures, certain medications, or comorbidities) who happen to have a T-score in the osteopenia range, the diagnostic term that best describes such patients is unclear—can we call it osteoporosis in the high-risk patient if the T-score is not less than −2.5? Should the sole diagnosis be osteopenia when that term may be understating the high risk for fracture that exists in these patients and be misunderstood by both patients and payers?

To help the clinician, the patient and the payer, as well as those designing clinical trials and the regulatory authorities that approve new therapeutic agents, it is time to readdress what we mean by the *clinical* diagnosis of osteoporosis. Limiting that diagnosis and the use of the 733.0 ICD-9 code to only those with a T-score of −2.5 or below is no longer sufficient based upon our understanding of what osteoporosis represents. To begin this we have to return to the definition of osteoporosis and re-emphasize the words "increased risk of fracture." For the purposes of the position we wish to consider in this paper, let us begin by stating that we are describing the increased risk of fracture that begins to be clinically relevant in postmenopausal women and older men in whom age-related changes in hormonal function, calcium and vitamin D sufficiency, physical activity, and potentially other secondary factors, varying widely in older individuals, lead to a progressive rise in the possibility of fracture as these women and men grow older.

## Who should be diagnosed as having osteoporosis?

Which of these patients have osteoporosis? An otherwise healthy 74-year-old woman has a current BMD test with T-scores of −2.4 at both the total hip and femoral neck. A 62-year-old woman has "osteopenia" by BMD at the spine and hip but recently sustained a vertebral fracture when she slipped and struck her back on a concrete floor. A 57-year old with no prior fracture history, who is 60 in. tall and weighs 99 lb., has a spine T-score of −3 and a femoral neck T-score of −2.3. An active 83-year-old man has never had a BMD test, but he recently fractured his hip after a sideways fall when he lost his balance. A 71-year old with osteopenia at the spine and hip with a T-score at both sites of −2.2 has had multiple falls from a standing height due to a neurodegenerative disease and has already fractured her proximal humerus and her pelvis on separate occasions.

The answer is that all of these patients have a diagnosis of osteoporosis because all of them have an increase in the risk for future fractures presumptively associated with compromised bone strength. For the 74-year old, who is a Caucasian American, the increased risk is apparent when FRAX is applied. FRAX [5] is a country-specific risk assessment tool from the World Health Organization. It combines BMD at the femoral neck with a series of well-validated clinical risk factors that are largely independent of BMD to predict the absolute 10-year risk of both hip and major osteoporotic fractures in previously untreated patients between ages 40 and 90. Even in the absence of any additional FRAX risk factors, whether she is 5′2″ and 120 lb. or 5′8″ and 150 lb., she has a 10-year probability of hip fracture between 4.4 and 4.5 %, which in the USA, based on the cost-effectiveness analysis that underlies the National Osteoporosis Foundation (NOF) Guide [6, 7], calls for treatment to lower her increased risk of having a hip fracture. Notably, neither FRAX nor the NOF Guide clearly refers to such a person as having "osteoporosis." The former allows a calculation of risk, and the latter defines the level of risk that is high enough to treat.

The osteopenic 62-year old has experienced a low-trauma vertebral fracture. With or without other benign risk factors such as the use of corticosteroids or other medications that are detrimental for bone or a medical condition with adverse consequences for bone, and in the absence of osteomalacia or cancer-mediated destruction of bone, she has, based on a large body of evidence, a fracture type that predicts an increased risk of more fractures, especially vertebral fractures as soon as in the next year [8], and virtually all treatment guidelines would recommend she be treated to lower that increased risk for fracture.

Interestingly, the 57-year old—who actually carries a BMD-based diagnosis of osteoporosis—is a bit more intriguing to think about in terms of the implications of her diagnosis. Assuming she, too, is a Caucasian American and that she is early postmenopausal, by FRAX her 10-year risk for hip fracture is 1.4 % and for major fractures 8.3 %, both below the treatment threshold in the NOF Guide. However, by T-score she has a diagnosis of osteoporosis (and any physician who fails to document this would be considered to be missing an obvious diagnosis), and her long-term risk of fracture—assuming she lives for another 20–30 years—is likely fairly high; whether or not she receives treatment now, she has the "disease."

The 83-year-old man fractured his hip. The absence of a BMD test is not an impediment for assessing his future fracture risk. Certainly he needs an evaluation to rule out secondary factors, but the presence of a hip fracture in an older individual is a powerful predictor of future fractures, and his diagnosis is osteoporosis, with or without a BMD

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test. He may think his problem was due to bad luck and an awkward fall, and his orthopedic surgeon may or may not use the word osteoporosis, but he has the disease, and virtually all guidelines recommend that patients like him be treated to lower the high risk of additional fractures.

Finally, the 71-year-old woman who tends to fall has already sustained two osteoporosis-associated fractures after falls from a standing height. If she is an average-sized Caucasian American, with or without BMD, even without our ability to enter falling as a risk factor into FRAX, she has an elevated risk for both hip and major osteoporotic fractures by FRAX and warrants treatment. True, she has osteopenia by BMD—but her clinical diagnosis is osteoporosis. She, like the other patients who are described, has compromised bone strength predisposing to an increased risk for fracture, compounded in her case by frequent falling on the weaker bone.

## There is a need to resolve the diagnostic dilemma for patients, clinicians, policy makers, and payers

Clinicians see patients like these individuals every day, and we are currently unable to use the term osteoporosis with the clear meaning of increased risk for fracture unless the T-score is −2.5 or lower. This dilemma impacts the perceptions of patients and many physicians, may lead to undertreatment when treatment is needed, and certainly affects reimbursement for services and medications. We believe that our field needs to rethink the requirements for making a clinical diagnosis of osteoporosis. A T-score at or below −2.5 in an older person is certainly one way to make the diagnosis. However, if the clinical diagnosis is limited to a T-score diagnosis, a great many patients at risk for fractures will have their risk go unrecognized. FRAX and other risk assessment algorithms have helped to identify those at high fracture risk with or without a T-score measurement. Shouldn't an older individual determined to be at high risk based upon FRAX be diagnosed as having osteoporosis even if the T-score is not −2.5 or lower or if it is not even known?

Additionally and critically, there is general agreement in the literature that a hip or vertebral fracture is associated with very high risk of additional fractures, and either fracture type should, after secondary factors are excluded, unambiguously confer a diagnosis of osteoporosis. A recent report [9] from the multinational Global Longitudinal Study of Osteoporosis in Women has shown an elevation of future fracture risk after any of several prevalent fracture types in postmenopausal women, further supporting the well-recognized adage that fractures beget fractures and that not just hip and vertebral fractures predispose to more fractures. Indeed, half of hip fracture patients have a history of some other type of prior fracture [10]. Before the presence of one or more low-trauma non-hip, non-spine fractures in older patients was to be invoked as diagnostic of osteoporosis, a consensus would be needed regarding which fracture types should be included. This would depend on the published evidence regarding the level of future fracture risk associated with each prevalent fracture type, and judgments about how much risk is sufficient to be viewed as high risk with each type—and therefore be called osteoporosis—would be needed.

Thus, whether it is based upon a T-score less than −2.5 or by a FRAX level of risk above a declared threshold or by the presence of one or more of several types of prevalent fractures, including but not limited to prevalent hip or vertebral fracture, such patients are at high short- or long-term risk for fracture and should be diagnosed as having osteoporosis. Their doctors need to understand this, the patients need to understand this, and both the policy makers and the payers need to understand this.

## What are some of the implications from expanding the criteria for a diagnosis of osteoporosis?

There are several practical implications that would emerge if we reconsider the basis for the clinical diagnosis of osteoporosis in the way we have proposed. We know that currently, osteoporosis is underdiagnosed and undertreated [11]. Part of the reason may be that many older women never undergo a BMD test [11], and if the diagnosis is limited to a T-score-based diagnosis, many such women will be completely missed. If the three indications for treatment of postmenopausal women and older men in the US NOF Guide, namely a T-score of −2.5 or lower, or a prevalent vertebral or hip fracture, or a FRAX score of 3 % or more 10-year risk for hip fracture or 20 % or more for major osteoporotic fracture [6], were all clinically defined as diagnostic for osteoporosis, 19 % of older men and 30 % of older women in the USA would carry this diagnosis [12].

How many women with a prior fracture (not just hip or spine) are not being recognized as being at risk because we fail to diagnose them as having osteoporosis? In one study of nearly 70,000 women over age 60 living in Australia, 29.1 % reported having previously experienced at least one fracture; 66 % of them had a single prior fracture, 22 % had two, and 12 % reported three or more [13]. The well-documented failure to treat the older postfracture patient to prevent the next fracture is multifactorial, including a lack of awareness that such patients are at high risk for more fractures and the overall absence of a consistent health care systems approach worldwide to move the patient from orthopedic care that treats the fracture to medical intervention to prevent the next fracture [14]. If the postfracture patient is appropriately diagnosed as having osteoporosis, it may be that those who determine health policy or the payers (or perhaps the malpractice lawyers) but

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certainly the clinicians and these patients should recognize that such individuals have the disease and need to be treated.

## Do anti-osteoporosis medications work in patients diagnosed as having osteoporosis based on prior fractures or FRAX?

Will current treatments be effective in patients whose diagnosis is not based exclusively on a T-score of −2.5 or below? Although most clinical trials that established the effectiveness of the therapies available today had entry criteria requiring a T-score −2.5 or lower at spine or hip, others included prevalent fracture as an alternative basis for enrollment. For example, the HORIZON-PFT trial with zoledronic acid allowed enrollment for women with a T-score of −1.5 or less with radiologic evidence of at least one prevalent vertebral fracture [15]. Similarly, the HORIZON-RFT trial of patients following hip fracture that established the efficacy of zoledronic acid to lower the risk of subsequent fractures did not require a BMD as part of the entry criteria [16]. In that trial only about 40 % of hip fracture patients had a femoral neck T-score below −2.5. The Fracture Intervention Trial with teriparatide enrolled women based on number and severity of previous vertebral fractures, with or without a T-score of −1.0 or lower [17]. Not all trials have shown antifracture benefit when the entry level T-score was higher than −2.5 in the absence of a prevalent vertebral fracture, including studies with alendronate [18], but others have shown benefit [19, 20].

Is FRAX risk a basis for determining who will respond to treatment? Clinical trials with clodronate [21], bazedoxifene [22], and denosumab [23] found that the effect of these drugs on reducing fracture risk was greater at higher FRAX probabilities, though this was not the case with raloxifene [24] and alendronate [25]. Both sponsors and regulatory bodies in Europe and the USA are beginning to collect FRAX risk factor information for clinical trials in osteoporosis. Ultimately treatment decisions made by clinicians whose patients are diagnosed with osteoporosis based on FRAX or prior fractures rather than BMD will need to take into account the probability of a given therapy being effective in lowering fracture risk in such patients, based upon data from clinical trials that enrolled these types of patients.

## We understand what we mean by osteoporosis clinically, and we need to codify the expanded basis for the diagnosis

In conclusion, we believe that it is time for our field to revisit the criteria for making a clinical diagnosis of osteoporosis. We must be able to identify all individuals who are at increased fracture risk, not just those with T-scores at or below a specific threshold. We will need to consider whether having increased risk from differing contributions of skeletal and nonskeletal risk factors necessarily implies that current treatments will lower fracture risk in all circumstances. Some patients with osteoporosis may not respond to certain treatment strategies based upon the criteria used to make the diagnosis and may require other treatment approaches—but at least, the risk of fracture will be recognized as diagnostic for osteoporosis, and management to prevent fractures will be more thoughtfully applied. Perhaps we will determine that osteoporosis is not a disease but a syndrome, with the need for differing approaches to achieve the desired outcome of fewer fractures. In fact, some clinicians are likely doing this already in their practices, but we need to *codify* these concepts so that the various stakeholders—including the payers—all speak and understand the same clinical and coding language and agree on the definitions being utilized. If we are ever going to reduce the current burden of fractures, we need to be able to make a clinical diagnosis of osteoporosis when it is present, one that accurately identifies the patient at increased risk for fracture by using this term. In our opinion, we need to start this discussion now.

**Conflicts of interest** Ethel S. Siris is a consultant of Amgen, Benvenue, Eli Lilly, Merck, Pfizer, and Novartis; lecture fees are from Amgen and Eli Lilly. Steven Boonen received research grants from Amgen, Novartis, and Servier; consulting fees are from Amgen, Novartis, and Servier; lecture fees are from Amgen, Novartis, and Servier. Paul J. Mitchell is a consultant of Amgen, GSK, and Daiichi Sanryo. John Bilezikian received grants from NPS and Amgen, and is a consultant of GSK, Lilly, Merck, Amgen, NPS and Warner Chilcott; lecture fees are from Lilly, Amgen, and Novartis. Stuart Silverman received research support from Lilly, Merck, Alliance for Better Bone Health, Roche Genentech Pharmaceuticals, Roche Diagnostics, Novartis, Pfizer, and Medtronics, and is a consultant of Merck, Amgen, Roche Diagnostics, Novartis, Pfizer, and Lilly; he is part of the speaker's bureau of Lilly, Amgen, Warner Chilcott, Roche Genentech, and Pfizer. Dr. Boonen is senior clinical investigator of the Fund for Scientific Research, Flanders, Belgium (F.W.O.-Vlaanderen) and holder of the Leuven University Chair in Gerontology and Geriatrics. No funding was provided by that source for work on this manuscript.

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