

PERSPECTIVES

Single Gene Mutations and Variations Affecting Bone Turnover and Strength: a Selective 2006 Update

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Abstract

Osteoporosis is a bone fragility disorder with a strong genetic predisposition. Thus far, most gene variations associated with bone density, turnover and/or fractures have been identified through a candidate gene approach, which presupposes prior knowledge of the gene's function in bone biology and/or of the skeletal phenotype caused by single mutations in the gene. Here we review recent advances in the genetic defects leading to alterations in bone matrix composition; osteoblast function, including mineralization; osteoclast activity; and endocrine regulation of bone turnover and phosphate metabolism, and further summarize the genetic association studies relating common variations (polymorphisms) in these genes to BMD and fracture risk in the general population. *BoneKEy-Osteovision*. 2006 December;3(12):11-29.
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Rare skeletal disorders that follow a dominant (parent-affected) or recessive (parent-carrier) pattern of inheritance represent experiments of Nature that provide crucial insights into the molecular mechanisms governing bone modeling and remodeling (1). By the end of 2006, the Online Mendelian Inheritance in Man database (www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM) contains 11,173 genes with a known sequence, of which only 386 have a known phenotype, as well as 2025 phenotypes with a known molecular basis. The keyword "osteoporosis" retrieved 173 entries including 71 mutations; "bone fragility" 35 entries and 15 mutations; and "osteosclerosis" 51 entries and 23 mutations. Single mutations in genes related to bone matrix composition, osteoblastic and osteoclastic functions, and to the hormonal regulation of bone and mineral homeostasis cause inherited forms of bone fragility, including low bone density/osteoporosis, osteolysis, osteopetrosis and osteomalacia (rickets) (2). Conversely, mutations can result in high bone mass (HBM) syndromes, skeletal dysplasias, bone deformities, and extraskelatal calcifications without prominent bone fragility (for a complete nosology and classification of genetic skeletal disorders,

see Superti-Furga and Unger (3)). Heterozygous carriers of gain-of-function mutations in the low-density lipoprotein (LDL) receptor-related protein 5 (*LRP5*) and those with heterozygous loss-of-function mutations in sclerostin (*SOST*), for instance, have high bone mineral density (BMD) without necessarily a skeletal defect (4;5). As such, they exemplify that individuals at the tails of bell-shaped (normal) distribution for BMD in the general population may be carriers of mutations in skeletal genes. By extension, these individuals could also be carriers of allelic variations, rather than mutations, in these genes.

Accordingly, this review focuses on selective disorders and their single gene mutations affecting bone modeling and remodeling, and the current evidence for the association of allelic variation in these genes with "idiopathic" osteoporosis (see Table 1). Extensive reviews of the new bone biological pathways, and of the genome-wide linkage studies in humans and mice, as well as the association studies of candidate genes for osteoporosis, have recently been published elsewhere (6;7).

| Gene | Protein | Locus | Association studies |
|-----------|--|----------|---------------------|
| COL1A1 | Collagen 1, α 1 chain | 17q21-22 | + |
| COL1A2 | Collagen 1, α 2 chain | 7q22 | + |
| CRTAP | Cartilage-associated protein | 3p22-24 | NA |
| LRP5 | LDL receptor-related protein 5 | 11q12-13 | + |
| SOST | Sclerostin | 17q12-21 | + |
| ALPL | Tissue non-specific alkaline phosphatase | 1p36 | NA |
| RUNX2 | Runt-related transcription factor 2 | 6p21 | + |
| ACVR1 | Activin type 1 receptor | 2q23-24 | NA |
| CA2 | Carbonic anhydrase II | 8q22 | NA |
| CLCN7 | Chloride Channel | 16p13 | + |
| TC1RG1 | Vacuolar proton pump | 11q13 | + |
| CTSK | Cathepsin K | 1q21 | - |
| TNFRSF11B | Osteoprotegerin | 8q24 | + |
| TNFRSF11A | RANK | 18q22 | + |
| MMP2 | Matrix metalloprotease (gelatinase) | 16q13 | NA |
| ESR1 | Estrogen receptor α | 6q25 | + |
| CYP19 | Aromatase | 15q21 | + |
| VDR | Vitamin D receptor | 12q12-14 | + |
| CYP27B1 | 1- α hydroxylase | 12q13 | NA |
| FGF23 | Fibroblast growth factor-23 | 12p13 | NA |
| PHEX | Phosphate-regulating endopeptidase homolog, X-linked | Xp22 | NA |
| DMP1 | Dentin matrix protein 1 | 4q21 | NA |

Table 1. Skeletal genes in this review, locus mapping and availability of related association studies. + indicates significant association; - indicates no association; and NA indicates no association studies available (thus far).

Monogenic Disorders

Mutations Affecting Bone Matrix Composition

Perhaps the best known case of inherited bone fragility is osteogenesis imperfecta (OI, types 1 to 7), a disorder characterized chiefly by multiple bone fractures, usually resulting from minimal trauma (8). OI features, however, are highly pleiomorphic, ranging from low BMD in adults (9) to lethality at birth. In OI type I, affected individuals have blue sclerae, normal teeth, and normal or near-normal stature. Fractures are rare in the neonatal period, remain constant from childhood to puberty, decrease thereafter, and often increase

following menopause in women, and after the sixth decade in men. Initially, vertebral body morphology in the adult is normal, but then the classic 'cod-fish' appearance often develops. OI should be considered a possible diagnosis in unclear or atypical perimenopausal osteoporosis (9). Mutations in the coding region of the collagen I α 1 chain (*COL1A1*), or α 2 chain (*COL1A2*), that result in 'functional null' alleles altering the triple collagen helix, the major constituent of the bone matrix, are the cause of "classical" forms of OI (types 1 to 4) (10;11). Newer forms of autosomal dominant (type V), recessive (type VII) or unknown inheritance OI (type VI) are not ascribed to primary defects in collagen I genes, but map to

different chromosomal locations (12). Very recently, mutations in cartilage-associated protein (*CRTAP*), a member of a complex of proteins that function in collagen synthesis and in hydroxylation of a single prolyl residue (986) in the type I collagen $\alpha 1$ chain, were found in OI type VII (13). *CRTAP* mutations also seem to be involved in some severe cases of OI type 2 and 3 (14).

In addition, a novel missense mutation in *COL1A1* was incriminated as the cause of infantile cortical hyperostosis (also known as Caffey disease, currently classified in the group of neonatal osteosclerotic dysplasias, next to Blomstrand disease-*PTH1R* mutation (3)). Caffey disease is characterized by profound alterations of the shape and structure of the underlying bones, particularly the long bones, mandible, clavicles, or ribs (15). Since affected patients also suffer from an acute inflammation of soft tissues, a feature that is completely absent in OI, the finding of a *COL1A1* mutation in Caffey disease was surprising and raises new questions about the role of bone collagen in the inflammatory process (16).

Mutations Affecting Osteoblastic Functions and/or Mineralization

A number of loss-of-function mutations in *LRP5* have been reported that affect bone formation and the normal regression of the embryonic capillary network in the eye, causing osteoporosis with blindness (osteoporosis-pseudoglioma, known as OPPG) (17). A related ocular phenotype is familial exudative vitreoretinopathy (FEVR), in which patients also frequently have low bone density and/or a history of multiple fractures (18). Heterozygous mutations in *LRP5* were recently found in 3 out of 20 children with juvenile osteoporosis, providing a new differential diagnosis with OI (see above) (19). Conversely, a number of missense mutations in *LRP5* that increase receptor function were reported to cause high bone mass (HBM) syndromes, *i.e.*, an increase of 4+ SD in total body BMD above the population mean, first in two unrelated families (4;20), including one with torus palatinus in all affected members. Subsequently, additional mutations were

found in unrelated families with increased trabecular bone density (osteosclerosis) and/or cortical bone thickening (hyperostosis), *i.e.*, sclerosing bone dysplasias (21). Based on their radiographic features, the patients thus identified had previously been diagnosed with endosteal hyperostosis/autosomal dominant osteosclerosis, van Buchem disease type 2 (autosomal dominant), and autosomal dominant osteopetrosis type I (ADOI). Although a recent update of skeletal dysplasias still classified these disorders under various nosological groups (3), the discovery of a common molecular determinant should allow for a reuniting of these diverse conditions under one common denominator (the term "cranio-tubular hyperostoses" has been proposed (22)), indicating that they all result primarily from excessive bone formation. Altogether, these studies have led to the recognition of the Wnt-LRP5 pathway as a crucial regulator of osteoblastic activity (23).

Two closely related disorders of excessive/dysregulated bone formation are endosteal hyperostosis (van Buchem disease) and sclerosteosis, the latter sometimes with syndactyly. In both disorders, the responsible mutations lie within or closely downstream of the *SOST* gene (24;25) that codes for sclerostin, a protein that is highly expressed in osteocytes (26). As mentioned above, heterozygous carriers of *SOST* mutations can present as normal subjects with high bone mass (females > males) (5). Most interestingly, it seems that *LRP5* high bone mass (HBM) mutations can prevent binding of sclerostin to *LRP5* (27), which suggests to the author of this review that gene-by-gene interaction between *LRP5* and *SOST* could influence bone mass even in the "normal" population (see polymorphisms below).

Mutations in the gene encoding tissue-nonspecific alkaline phosphatase (*ALPL*) cause hypophosphatasia (28;29). Three more or less distinct types of hypophosphatasia can be identified: 1. Type 1, with onset *in utero* or in early postnatal life, craniostenosis, severe skeletal abnormalities, hypercalcemia, and death in the first year or so of life; 2. Type 2, the adult

form, with more gradual development of symptoms, moderately severe 'rachitic' skeletal changes, and premature loss of teeth; 3. Type 3, with no symptoms, the condition being identified on routine studies (low serum levels of alkaline phosphatase) (30;31). The dominant disorder may be one of osteoblasts, whereas the recessive form is a defect of alkaline phosphatase, and hence of bone mineralization (32). A correct diagnosis is important, since vitamin D therapy, appropriate for most forms of osteomalacia, is of no benefit in hypophosphatasia and has led to inordinate hypercalcemia with resultant kidney damage (33).

Heterozygous loss-of-function mutations (haploinsufficiency) in the core binding factor $\alpha 1$ /runt-related transcription factor 2 (*CBFA1/RUNX2*) gene, an indispensable transcription factor for osteoblast development (34), cause cleidocranial dysplasia (CCD) (35-38). A few patients with recurrent fractures or osteoporosis have been reported (39). In addition, a patient with severe CCD and a frameshift mutation at codon 402 had osteoporosis leading to recurrent bone fractures and scoliosis, providing the first evidence that *RUNX2* may be involved in the maintenance of adult bone mass, in addition to its function in bone development (40). A recent series identified novel *RUNX2* mutations in 14/20 CCD patients, underscoring the highly variable degree of severity in their phenotypic expression (41).

Although not a disorder of bone fragility or increased bone mass, fibrodysplasia ossificans progressiva (FOP) is a devastating inherited (autosomal dominant) or sporadic disease characterized by extra-skeletal ossifications that can eventually become of extraordinary proportions and completely prevent mobility of the affected subjects. In addition, the development of the toe skeleton is often incomplete, cervical vertebrae can be malformed, and the femur neck can be broad and short. Linkage analysis in 5 affected families led to the identification of a single gene mutation in the activin type 1 receptor gene (*ACVR1*) that codes for a bone morphogenetic protein (BMP) receptor family member (type 1). The

same *de novo* mutation was then also identified in 32 sporadic cases of FOP (42).

Mutations Affecting Osteoclastic Function

Pathogenic mutations affecting osteoclast activity may lead to bone fragility due to altered bone turnover, material properties, and microarchitecture (43). These alterations occur either in the context of apparently increased bone mass or, conversely, in the context of prominent osteolysis. The osteopetroses are a heterogeneous group of genetic disorders characterized by increased bone density due to impaired bone resorption by osteoclasts (44;45). As mentioned above, ADO1 is not a true type of osteopetrosis, as it results primarily from an *LRP5* mutation and therefore from an osteoblastic defect (21). Hence, it appears to be the only type of osteopetrosis not associated with an increased fracture rate. The first genetic defect identified in osteopetrosis (renal tubular acidosis variant) involved carbonic anhydrase II deficiency (*CA2*) (46). In this disorder, cerebral calcification appears by early childhood and the osteosclerosis and skeletal modeling defects may gradually resolve by adulthood. Type II ADO (ADO2 or OPTA2), which is due to chloride channel gene (*CLCN7*) mutations (47;48), is characterized by sclerosis predominantly involving the spine, the pelvis, and the skull base. OPTA2 may be severe, possibly reflecting a dominant negative effect of some heterozygous *CLCN7* mutations (49), or relatively asymptomatic (50), particularly with loss-of-function mutations in *CLCN7*. Dental abscesses and fragility of bones are leading complications, with up to 78% of patients having fractures that may heal slowly (51). In contrast, homozygous mutations in the *CLCN7* gene cause autosomal recessive infantile (or malignant) osteopetrosis (ARO or OPTBI), a severe form of the disease which is often lethal in infancy (49). However, about 50% of malignant ARO is due to mutations in the T-cell immune regulator 1 gene (*TCIRG1*), coding for the vacuolar proton pump (ATP6i) (49;52;53). The vast majority of *TCIRG1* mutations identified so far are predicted to cause severe abnormalities in the protein product and likely represent null alleles.

Pycnodysostosis is an autosomal recessive osteochondrodysplasia characterized by deformity of the skull (including wide sutures), maxilla and phalanges (acroosteolysis), osteosclerosis, fragility of bone, and short stature. In the past, a number of these cases have probably been diagnosed as osteopetrosis (54). In this disorder, osteoclasts, which are involved in bone resorption, are normal in number and in ruffled borders and clear zones, but the region of demineralized bone surrounding individual osteoclasts is increased. Ultrastructural studies demonstrate that, in pycnodysostosis, osteoclasts function normally in demineralizing bone but do not adequately degrade the organic matrix (55). Mutations in the cathepsin K gene (*CTSK*), coding for a cysteine protease gene that is highly expressed in osteoclasts, have been identified (56;57).

A number of syndromes characterized by increased, rather than decreased, osteoclastic activity has been reported (58). Juvenile Paget disease (JPD), also known as hyperostosis corticalis deformans juvenilis, is an autosomal recessive osteopathy characterized by rapidly remodeling woven bone, osteopenia, fractures, and progressive skeletal deformity. A homozygous deletion was found in the tumor necrosis factor receptor superfamily, member 11b gene (*TNFRSF11B*) coding for osteoprotegerin (OPG) (59), a molecule that normally suppresses bone turnover by functioning as a decoy receptor for the osteoclast differentiation factor receptor activator of NF- κ B ligand (RANKL) (60-62). Progressive osteoclastic resorption, accompanied by medullary expansion leading to severe, painful, disabling deformity and a tendency to pathologic fracture, is also a feature of familial expansile osteolysis (FEO), caused by activating mutations of the RANK gene (*TNFRSF11A*), which is the receptor for RANKL (63). Other types of inherited osteolysis ('vanishing bone' syndromes) characterized by destruction and resorption of affected bones, such as the Saudi type of idiopathic osteolysis and Winchester syndrome, are due to mutations in the matrix metalloproteases, in this case the gelatinase gene (*MMP2*) (64;65).

Mutations in the Estrogen Pathway and Their Role on the Male Skeleton

Mutations in the estrogen pathway have demonstrated the physiological relevance of this hormone for the acquisition and maintenance of bone mass in males. Smith *et al.* described a 28-year-old man with estrogen resistance due to a mutation in the estrogen receptor α (*ESR1*) (66). He was 204 cm tall and had incomplete epiphyseal closure, with a history of continued linear growth into adulthood despite otherwise normal pubertal development and normal masculinization. Serum estradiol and estrone concentrations were elevated, and serum testosterone concentrations were normal. BMD at the lumbar spine was 3.1 standard deviations (SD) below the mean for age-matched normal women; there was no biochemical evidence of increased bone turnover. Administration of estrogen had no detectable effect. Another man with continued longitudinal growth (170 cm at 18 years of age, 190 cm at the age of 38) and low bone mass was identified with a mutation in the aromatase gene (*CYP19*), that decreased conversion of androgens to estrogens (67). Androgen therapy was ineffective; estrogen therapy resulted in increased spinal BMD, and complete epiphyseal closure after 9 months. The increases in BMD, serum levels of alkaline phosphatase and osteocalcin, and urinary excretion of pyridinoline were similar to those that occur during normal skeletal maturation during puberty. For a comprehensive review of the phenotypes associated with aromatase deficiency in humans and mice, see Jones *et al.* (68).

Mutations Affecting Phosphate Metabolism: Hereditary Rickets

Inherited rickets may be caused by a variety of molecular defects. In vitamin D-dependent rickets type II (VDDRII), also called vitamin D-resistant rickets, osteomalacia, hypocalcemia, and secondary hyperparathyroidism in association with normal serum 25-hydroxyvitamin D and markedly increased serum 1,25-dihydroxyvitamin D are present with (type IIB) or without (type IIA) alopecia (69). Elevated serum alkaline phosphatase, x-ray

prominent trabeculation of long bones, 'rugger-jersey' changes in the vertebrae, and increased density of the skull may be demonstrated. Biopsy of the iliac crests shows wide osteoid seams resulting from the mineralization defect. Secondary hyperparathyroidism may be reflected by bone erosions. Mutations in the vitamin D receptor gene (*VDR*) itself that prevent its normal function are involved (70-73). In VDDR1, also known as pseudo-vitamin D deficiency rickets, osteomalacia, hypocalcemia, and secondary hyperparathyroidism occur without alopecia. This disorder is caused by mutations in the renal 1- α hydroxylase enzyme (*CYP27B1*) and is accompanied, therefore, by a low serum level of 1,25(OH)₂D (74;75). The findings in this disorder differ from those in X-linked vitamin D-resistant rickets (XLH) by the severity and the accompanying myopathy, earlier onset, and depression of calcium, as well as phosphorus, in the blood. The response to vitamin D is better in this disorder than in the X-linked condition. The molecular defects of XLH and autosomal dominant hypophosphatemic rickets (ADHR) have been recently elucidated (76;77), establishing a possible mechanistic link between the two (78). XLH and ADHR are both characterized by a primary defect in the renal conservation of phosphate (79). Thus, ADHR is characterized by isolated renal phosphate wasting and inappropriately normal calcitriol concentrations, rickets, osteomalacia, lower limb deformities, short stature, bone pain, and dental abscesses. In contrast to XLH, this disorder has incomplete penetrance and, occasionally, delayed onset. In some instances, affected children have lost the phosphate wasting defect after puberty. The ADHR Consortium (77) identified missense mutations in a gene encoding a member of the fibroblast growth factor family, FGF23, that alter cleavage and inactivation of this molecule (80). Subsequently, FGF23 has been shown to play a physiological role by inhibiting tubular reabsorption of phosphate in response to diet (81). The phenotypic expression of XLH is similar to ADHR, including suboptimal growth and bone healing despite oral phosphate and 1,25-dihydroxyvitamin D₃ treatment, which may result in hypercalcemia, hypercalciuria,

nephrocalcinosis, and hyperparathyroidism (82). The HYP (X-linked hypophosphatemic rickets) Consortium (76) isolated a gene that exhibited homology to a family of endopeptidase genes, members of which are involved in the degradation or activation of a variety of peptide hormones, which was called *PEX* (or *PHEX*, for 'phosphate regulating gene with homologies to endopeptidases, on the X chromosome'). In 2000, 131 mutations in the *PHEX* gene had been reported (83). Some mutations in secreted PHEX abrogate catalytic activity, whereas others alter the trafficking and conformation of the protein (84), thus providing a mechanism whereby missense mutations result in loss-of-function of the PHEX protein. This might possibly cause accumulation of intact FGF23 (85;86), although it remains controversial whether FGF23 is the substrate of PHEX (87). The implication of FGF23 and other "phosphatonin" genes in disorders of phosphate metabolism has recently been reviewed (88). In addition, two late publications identified mutations in the dentin matrix protein 1 gene (*DMP1*), which codes for a molecule secreted by odontoblasts and osteocytes, to be responsible for a recessive form of hereditary rickets (ARHR) with urinary phosphate wasting (89;90).

Gene Variations and Osteoporosis

Most of the genes described above, whose mutations cause distinct syndromes characterized by altered bone turnover and/or strength, and many other genes known to be implicated in the physiological regulation of bone modeling and remodeling, have been explored as candidate genes for "idiopathic" (common, post-menopausal, senile) osteoporosis (7;91). In this case, polymorphisms along the gene coding, non-coding (introns and 3'-UTR), and/or regulatory (5'-promoter) regions have been associated with BMD, markers of bone turnover and/or fractures, and often with non-bone related features as well (pleiotropic effects) (92). A meta-analysis of association studies of *VDR* 3'-UTR polymorphisms (*Bsm*, *Apa*, and *Taq*) show an estimated 2.4% ($p=0.028$) lower spine BMD in BB compared to Bb/bb (*i.e.*, a

recessive effect of the B allele) (93). Moreover, in 9 eligible cohort studies, women with the BB and Bb genotypes had a significantly greater bone loss at lumbar spine compared to bb (-0.43%/yr, $p=0.011$, *i.e.*, a dominant effect of the B allele). In contrast, this analysis did not confirm association in pre-menopausal women or at the femoral neck (93). Observational and prospective intervention studies indicate an interaction between dietary calcium intake and *VDR* polymorphisms on their association with bone mass in both children (94) and post-menopausal women (95-98). Two large cohort studies, the Nurses Health Study and the Study of Osteoporotic Fractures (SOF), reported discordant results concerning association of *VDR* alleles and fracture risk (99;100). A French study recently confirmed this association, BB having an up to 2-fold increase in fracture risk, independent of BMD (101). Others, however, have reported that fracture risk is decreased with the BAT allele but increased with the baT allele (102). Eventually, two retrospective and prospective meta-analyses, the latter on more than 20,000 individuals from across Europe (GENOMOS), found no association between *Bsm1* alleles and fracture risk (103;104). The latter studies, however, may be hampered by some heterogeneity in the definition and collection of fractures among the various participating centers.

A meta-analysis of the association between *ESR1* genotypes and BMD including more than five thousand women from 22 eligible studies ($n=11$ in Caucasians, and $n=11$ in Asians) concluded that homozygotes for the *XbaI* XX genotype (intron 2) have a modestly but significantly higher BMD (+1-2%) at the lumbar spine or hip compared to xx (105). Moreover, differences in fracture risk were disproportionately high compared to the small differences in bone mass observed between genotypes (odds ratio, 0.66 in XX vs xx) (105). These results have recently been confirmed in a large meta-analysis of individual-level data involving standardized genotyping of 19,000 individuals in 8 European centers (106). An aromatase (*CYP19*) gene tetranucleotide simple tandem repeat polymorphism has also been associated with estradiol levels

and osteoporosis in post-menopausal women (107-109) and, most interestingly, in aging men (110-112). A recent study found a more specific association of *CYP19* polymorphisms with cortical bone size (cortical thickness and cross-sectional area) in men (113).

The *COL1A1* Sp1 variant "s" was shown to produce too many collagen I $\alpha 1$ molecules compared to the S allele *in vitro*, causing bone to be less resistant to mechanical stress (114). A meta-analysis of published data concluded that there was a modest effect of the s allele on BMD (equivalent to 0.1 - 0.2 SD decrease compared to S), but a more prominent association with fracture risk (OR 1.5 and 1.9 in Ss and ss compared to SS genotypes, respectively) (114). Of note, another large meta-analysis looking at hundreds of genetic association studies across various complex disorders and polymorphic genes confirmed a significant association of *COL1A1* Sp1 alleles with fracture risk (OR, 1.6) (115). Most recently, a large prospective meta-analysis of more than 20,000 individuals from several European countries confirmed a slightly but significantly lower BMD at the spine and femoral neck in ss homozygotes, whereas the increased risk of fractures was limited to incidental vertebral fractures in females (116). Some studies in Chinese and in white children have shown association of *COL1A2* genetic variation with BMD (117;118).

A number of recent studies has shown association and/or linkage of *LRP5* polymorphisms, particularly missense substitutions, in exon 18 (A1330V) and/or 9 (V667M) with BMD in various populations (see Ferrari *et al.* (23) for review). One study found associations with BMC and projected bone area in children and adult males, but not females, suggesting that *LRP5* genetic variation could contribute to gender-related differences in bone size and, thereby, fracture risk (119). Consequently, the A allele at exon 9, and T allele at exon 18, and their related haplotypes, have been associated with an increased risk of idiopathic osteoporosis in men (IOM), *i.e.*, osteoporosis in men occurring before 65-70 years of age in the absence of known risk factors (alcohol) and hormonal deficiencies

(testosterone) (120). This result provides the first potential molecular mechanism for the previous observation of defective bone formation indices in biopsies from IOM. However, an *LRP5* GG genotype in exon 15 has also been associated with a 60% increase in fracture rate among Australian women (121). Two recent studies analyzed *LRP5* genetic variation simultaneously in women and men from the same area/genetic background. In the Rotterdam cohort, the *1330Val* allele in *LRP5* and *1062Val* allele in *LRP6*, a highly homologous gene of *LRP5*, were associated with lumbar spine bone area and fragility fractures in men, whereas no such association was found in women (122). In the Framingham cohort, *LRP5 1330Val* was also associated with lower BMD in men, in whom an interaction with the level of physical activity was also found; in women, there was also some association with BMD, but this involved different polymorphisms (intron 2) (123). The latter study also provided some evidence for differences in Wnt signaling among *LRP5* haplotypes. Although it appears that *LRP5* non-synonymous SNPs in exon 9 and 18 influence bone mass and/or fracture risk more prominently in men than women, it is possible that different polymorphisms in *LRP5* are more important in women. In this case, the molecular mechanisms remain unknown (gene-by-gene interactions? Imprinting? Gene-environment interactions?).

In addition to the largely investigated *VDR*, *ESR1*, *COL1A1*, and *LRP5* genes mentioned above, a 3-bp insertion ($f=0.38$) in the presumed *SOST* promoter region (SRP3) was associated with a 0.2 SD decreased BMD in women at the femoral neck and lumbar spine, whereas a G variant ($f=0.40$) in the van Buchem deletion region (SRP9) was associated with increased BMD in men at the femoral neck and lumbar spine (124). In this study, no genotype effects on fracture risk were observed. Allelic variations in many other genes implicated in bone modeling and remodeling are beginning to be associated with BMD and/or osteoporotic fractures, including *CBFA1/RUNX2* alleles (125), the osteopetrosis genes *CLCN7* (126; 127), and *TC1RG1* (promoter alleles) (128).

This is consistent with the latter mapping at 11q13 (the same locus as *LRP5*), a strong quantitative trait locus (QTL) for normal BMD in humans (129;130). Several studies have found association with *OPG* genetic variation (131-134), but a large study in Swedish elderly women failed to find association of *OPG* with BMD or fractures (135). Some studies in Asians very recently reported association of *RANK* polymorphisms with BMD in men (136) and postmenopausal women (137). In contrast, no association was found between cathepsin K (*CSTK*) alleles and BMD in a large cohort of Scottish women (138), and, to our knowledge, no other association study with this gene has been reported so far. The *ALPL* gene maps at 1p36, a strong QTL for idiopathic osteoporosis (91;139), and analyses of BMD association with *ALPL* polymorphisms are ongoing, but none has yet been published (to our knowledge).

In conclusion, the rapidly increasing number of single gene mutations identified as responsible for rare Mendelian skeletal disorders has a major impact on our understanding of the pathophysiology of bone modeling and remodeling. In turn, association studies using allelic variations in these genes have started to delineate some genetic markers underlying the population-based susceptibility to osteoporosis. At this stage, it would be necessary to design specific studies to evaluate which and how many genetic markers are needed to improve the prediction of fracture risk beyond BMD and other known risk factors.

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