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REVIEW

Osteoporosis genetics: year 2011 in review

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Increased rates of osteoporotic fractures represent a worldwide phenomenon, which result from a progressing aging in the population around the world and creating socioeconomic problems. This review will focus mostly on human genetic studies identifying genomic regions, genes and mutations associated with osteoporosis (bone mineral density (BMD) and bone loss) and related fractures, which were published during 2011. Although multiple genome-wide association studies (GWAS) were performed to date, the genetic cause of osteoporosis and fractures has not yet been found, and only a small fraction of high heritability of bone mass was successfully explained. GWAS is a successful tool to initially define and prioritize specific chromosomal regions showing associations with the desired traits or diseases. Following the initial discovery and replication, targeted sequencing is needed in order to detect those rare variants which GWAS does not reveal by design. Recent GWAS findings for BMD included *WNT16* and *MEF2C.* The role of bone morphogenetic proteins in fracture healing has been explored by several groups, and new single-nucleotide polymorphisms present in genes such as *NOGGIN* and *SMAD6* were found to be associated with a greater risk of fracture non-union. Finding new candidate genes, and mutations associated with BMD and fractures, also provided new biological connections. Thus, candidates for molecular link between bone metabolism and lactation (for example, *RAP1A* gene), as well as possible pleiotropic effects for bone and muscle (*ACTN3* gene) were suggested. The focus of contemporary studies seems to move toward whole-genome sequencing, epigenetic and functional genomics strategies to find causal variants for osteoporosis.

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Osteoporosis and related fractures are major health problems, increasing in magnitude as the population ages. In this review of mostly human genetic studies published in 2011, we use the terms osteoporosis and fractures separately. Osteoporosis (low bone mass) and osteoporotic, or low-trauma, fracture (OF), the ultimate manifestation and dangerous sequel of osteoporosis, are genetically distinct entities. It was shown time and again that genetic contributions to a risk factor may differ from the genetic contribution to the ultimate disease phenotype. This is especially true for a 'proxy phenotype', such as dual-emission X-ray absorptiometry (DXA)-derived bone mineral density (BMD), and a complex event with various etiological factors (not necessarily musculoskeletal), such as OF. Thus it was estimated that $<1\%$ of the additive genetic variance is shared between BMD and fractures at the hip.[1] Similarly, there was only modest co-inheritance between computed tomography-derived vertebral fractures and volumetric BMD at L_3 vertebra (genetic correlation $= -0.37$).[2] As BMD is only one, although major, predictor of OF risk, it cannot individually serve as a perfect surrogate of the skeleton's ability to withstand the forces that produce fractures; neither is it a perfect proxy for the genetic study of OF. Empirically, genes that contribute to variation in BMD do not always contribute to OF (as the overlap in heritability is low, there were only four of nine genes shown to be associated with both phenotypes in a large meta-analysis;[3] in the most recent genome-wide association studies (GWAS),[4] only 14 of 56, the top BMD-associated single-nucleotide polymorphisms (SNPs), were also associated with the fracture). For these reasons, while reviewing the most recent literature, we consider all the following distinct phenotypes that have a role in the osteoporosis of old age: bone mineral density, bone loss (measured as BMD change) and the 'end-point disease', for example, OF *per se*.

Genome-wide Association Studies

In 2011, there was no breakthrough in finding a genetic cause of the osteoporosis or OF despite our best efforts as a community. Accordingly, multiple GWAS and candidate gene studies failed to explain more than a small percentage (single numbers) of the otherwise high heritability of BMD. GWAS use gene chips to scan the human genome, analyzing large amounts of variants in DNA for association with a quantitative trait such as BMD, or with a binary trait like OF (in cases and controls). Contrary to what we expected, GWAS have identified only a small

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number of the causal variants for recently identified genetic loci, which, in turn, explain only a small fraction of the genetic contribution to OF. Several explanations were proposed; for one, common variants account for only a small proportion of genetic components, and the missing heritability comes from the massive class of rare genetic variants that GWAS do not reveal by design.[5] GWAS, however, is a good starting point, as it can define and prioritize regions that can be further explored by targeted sequencing, to detect rare variants. With this in mind, the roles of GWAS and other human association studies are not only in creating a genetic score to predict osteoporosis or OF; they are also an important tool for the discovery of biological pathways relevant to bone health. Thus, a previously unknown gene, *FONG*, was found by GWAS of osteoporosis (defined by BMD) in Japanese.[6] Duncan *et al.*[7] reported a GWAS using an extreme truncate selection design, comparing 900 postmenopausal women (age 55–85 years) with either extremely high or low hip BMD. The study replicated 21 BMD-associated genes and found suggestive association of six new loci, near *CLCN7*, *GALNT3*, *IBSP*, *LTBP3*, *RSPO3* and *SOX4*; these associations were replicated in two consortia.

Wnt Genes Continue to be Hot

Many members of the Wnt pathway are associated with BMD at a genome-wide significant level; some genes, such as *WNT4*, *SOST*, *LRP5* and *WLS*, were confirmed by many works. At the annual meeting of ASBMR in San Diego, CA (2011), among multiple GWAS presented, the common theme was an association with *WNT16* (wingless-type MMTV integration site family member 16). Thus, a meta-analysis of GWAS, which used a relatively modest discovery sample from four cohorts of the Genetic Factors of OSteoporosis (GEFOS) Consortium ($n=4777$), was able to capture SNP rs2254595 in *WNT16.*[8] This SNP was strongly associated with radial BMD ($P=8.04\times10^{-13}$, $\beta=-0.14$), as well as moderately associated with forearm fracture ($P=9.92\times10^{-3}$, OR = 1.28). Similarly, GWAS of the cortical thickness and cortical volumetric BMD, measured by peripheral quantitative computed tomography in the tibial diaphysis, reported *WNT16.*[9,10] These GWAS used not particularly large sample, of only 5952 Caucasian subjects, aged from 15 to 46 years, pointing out the benefit of studying bone mass and geometry in a relatively younger sample.

Furthermore, in children from different ethnic backgrounds (mean age = 6.2±0.28 years, $n=2660$), another SNP near *WNT16* gene was associated with the total body BMD (replicated in older adults from the Netherlands and in a younger adult sample from the UK, with a joint meta-analysis $P=1.1\times10^{-15}$).[11] This signal was also found among 56 SNPs replicated by Estrada *et al.*[4] who analyzed lumbar spine and femoral neck BMD in a large sample of European-ancestry individuals from the GEFOS consortium (n~32 000). Notably, the consortium also found that *WNT16* contributed to the risk of fracture (any type OF) in 31 016 cases and 102 444 controls.

Functionally, Wnt16 is involved in specification of the sclerotomal somite compartment, which houses vertebral and vascular smooth muscle cell precursors.[12] A non-canonical signaling by Wnt16 seems to be conserved in mammals. GWAS robustly show that *WNT16* possibly exerts an effect on bone mineralization, which is observed in children and still is manifested in adulthood, therefore implying that WNT16 has a role from the early development on. The confluence of GWAS results is an indication of a trade-off between the statistical power advantage of large sample sizes attracted by the GWAS consortia vs a greater homogeneity in (age, geography) and better-defined phenotype (such as peripheral quantitative computed tomography-measured cortical BMD of the tibia); it also attests for a large effect size of *WNT16* signal on the risk for developing osteoporosis. To note, the adequately-powered GWAS of OF is yet to be performed. A modest-size GWAS in a population-based cohort from the Korean Association Resource with 288 cases (with any low-trauma OF) and 1139 healthy controls is one among the first attempts.[13] Their best association with OF ($P=1.27\times10^{-5}$, not GWS) was detected in the vicinity of the genes *FZD8* and *ANKRD30A* on chromosome 10p11.2.

Candidate Genes and Biological Connections

Among multiple gene-based association studies this year, some proposing novel gene candidates for osteoporosis and others replicating previous GWAS,[14] studies of several established genes of interest deserve to be mentioned: for example, peroxisome proliferator-activated receptor gamma (PPARG), a regulator of adipocyte differentiation. Additionally, PPARG has been implicated in the pathology of numerous diseases, including obesity, diabetes, atherosclerosis and cancer. This year, in two Danish cohorts, three SNPs in *PPARG* were shown to be associated with the risk of developing vertebral fractures.[15] Interestingly, an interaction between rs1151999 and diet has been found, similar to Ackert-Bicknell *et al.*,[16] who had previously shown this to be case for the same SNP with dietary fat. In elderly Slovenians, associations of *PPARG* were found with non-traumatic hip OF, but not with BMD.[17] Four studies dealt with *COL1A1*, which encodes the pro-alpha1 chains of type I collagen. In one study, there were associations with BMD and OF (all-type and vertebral) in Danish perimenopausal women;[18] however, in the other, of older Spanish women, no associations were found with hip fracture.[19] Finally, two meta-analyses (mostly Caucasian) found some associations: in one, with 24 511 participants (7864 fractures) from 32 studies, the Sp1 polymorphism (rs1800012) was associated with a modest reduction in BMD and an increased risk of fracture;[20] in the other meta-analysis of cases and controls from 33 studies, the SNP rs1800012 (G2046T) had 1.65 odds ratio for having osteoporosis (clinically diagnosed as present or absent).[21] Less prominent were the results for another known gene candidate, *MTHFR* (methylenetetrahydrofolate reductase). A meta-analysis of 20 studies[22] suggested that *MTHFR* C677T polymorphism was marginally associated with fracture risk. It was also modestly associated with BMD, but only at lumbar spine ($P=0.036$).

In 2011, several interesting biological connections were unearthed by genetic studies:

Molecular link between bone metabolism and lactation. There are established links between the calcium homeostasis and lactation: skeleton is an important source of calcium during lactation, when bone mineral reserves are called on in order to meet the demands of milk production. In humans, lactation is associated with a dramatic 5–8% decline in BMD over 6 months; mice lose ~20–30% of bone mineral over the course of lactation.[23] This is a regulated physiological process, which is believed to be activated by PTHR1 signaling.[24,25]

This year, additional new mechanisms of the lactation–osteoporosis connection were suggested by the genetic studies. Thus, for example, the bovine *RAP1A* gene was found to be associated with milk yield and composition traits in dairy cattle. The gene is mapped to a quantitative trait locus located in bovine chromosome 3 (BTA3).[26] Interestingly, an integration of human GWAS and gene expression profiling performed by Hsu *et al.*[27] revealed a locus on 1p13.2 (orthologous to BTA3) associated with osteoporosis-related traits, which harbors the same gene, *RAP1A.* SNPs within human *RAP1A* are strongly associated with narrow neck width in women.[27] Although RAP1A is a regulator having an important role in the developing mammary gland,[28] not much is known about the mechanism involving this gene with bone metabolism. In their study, Cohen-Zinder *et al.*[26] detected two polymorphisms located in the promoter region of bovine *RAP1A* and in intron 4 of the gene, which were significantly associated with milk yield and composition traits in dairy Holstein population. Note that, similar to this finding, other lactation genes were previously identified as the top signals for BMD. Thus, among the 20 loci associated with BMD in a large-scale meta-analysis of human GWAS, performed by the GEFOS consortium,[29] there was a cluster of bone-tooth mineral-extracellular matrix (ECM) phosphoglycoproteins on 4q21.1.[30] In bovine, this cluster of genes is located on BTA6 in the critical region of a quantitative trait locus affecting milk production traits.[31] Genes belonging to this cluster include *MEPE*, *IBSP*, *DMP1*, *DSPP* and *SPP1*. The latter gene, *SPP1* (a.k.a. osteopontin, OPN or IBSP-1), is well known to be associated with bone and cartilage morphogenesis, and ECM deposition in humans. Notably, ECM expression and deposition are thought to have a key role in regulation of other biological processes, such as branching during tubulogenesis of the ureteric bud in the kidney[32] and of the mammary epithelial ductal system.[33] A versatile function of ECM proteins is exemplified by SPP1, which was characterized as an important factor for kidney function, named uropontin,[34] and was also described as an essential factor for mammary gland differentiation.[35] The above confirmation of the roles of the genes in both human BMD and bovine lactation is an important avenue of interspecies research (especially as the lactation and milk production traits are difficult to be studied in humans).

In this connection, a new genetic link between breast cancer and bone resorption was identified by Mendoza-Villanueva *et al.*,[36] which demonstrated that Runx2, an important determinant of bone metastasis in breast cancer, and its co-activator BCFβ are required for the expression of genes that mediate the ability of metastatic breast cancer cells to directly modulate both osteoclast and osteoblast function. The study also showed that Runx2-dependent inhibition of osteoblast differentiation by breast cancer cells is mediated through the Wnt antagonist, sclerostin.

Muscle-bone pleiotropy. Besides defective bone metabolism, OF has other etiologic factors, including muscle weakness. Muscle loss (sarcopenia) is slowly moving onto the arena of aging-related degeneration, and consensus definitions are being proposed.[37] Joint analysis of bone and muscle phenotypes has been advocated by us,[38] which has potential to elucidate genetic–pleiotropic relationships between the closely-related compartments of the musculoskeletal system. Pleiotropy is one of the most intuitive, but still understudied fundamentals of human biology.[39] To evaluate its extent in the musculoskeletal field, we performed a principal component-based GWAS in participants of the Framingham Osteoporosis Study.[38] Linear combinations of multiple osteoporosis-related phenotypes (17 measures, including BMD (hip and spine), heel ultrasound, leg lean mass and hip geometric indices) were analyzed. There were novel genes not identified in the analysis of primary phenotypes (such as *HTR1E* (that codes for one of the serotonin receptors), *COL4A2* and *AKAP6*). Bioinformatic analyses demonstrated that top associated genes were enriched for the functions in skeletal and muscular system development ($P < 0.05$).

The soundness of this heuristic integrative approach was also supported by several studies published this year. Thus, Saint-Pierre *et al.*[40] applied a sophisticated statistical approach to GWAS of the lumbar spine and at the femoral neck BMD in a sample of men with extreme truncate design (with either low (LS Z-scores $\leqslant -2$) or high BMD (LS and FN Z-scores >0.5)). This bivariate GWAS found suggestive SNPs in three genomic regions (6q22.1, 15q14 and 22q11); these SNPs have not been reported by previous—univariate—GWAS of BMD. In spite of combined analyses being potentially more powered than univariate ones, there is a chance of spurious findings (a replication is necessary). Interestingly, two new genes, *SLC2A11* on 22q11 and *RYR3* on 15q14-15, are expressed in skeletal muscle.

Muscle-to-bone cross-talk is getting more wings recently. For example, α-Actinin-3 (*ACTN3* gene) is expressed in muscle and its deficiency is detrimental to sprint and power performance in humans. Yang *et al.*[41] had shown that Actinin-3 is also expressed in osteoblasts; its deletion in mice leads to a low bone mass phenotype with decreased bone formation, and a stop polymorphism (R577X) in humans is associated with lower BMD. Furthermore, one among the genes found by the GWAS[7,29] to be associated with BMD was *MEF2C* (myocyte enhancer factor 2C). Besides being involved in myogenesis[42] like the other myogenic basic helix–loop–helix proteins, the MEF2 family of regulatory proteins are involved in bone-relevant pathways including endochondral ossification.[43] Lately, Kramer *et al.*[43] identified a novel function for *MEF2C* in adult bone mass by regulation of osteoclastic bone resorption. Notably, by virtue of being very pleiotropic, *MEF2C* was associated with different traits, such as platelet count, retinal vascular caliber, tonometry and adult height. Future research should be performed to decipher a 'real' musculoskeletal pleiotropy of this molecule, as opposed to a mediation effect by some primary basic mechanism. For example, it was shown[44] that there is co-heritance between vascular and skeletal tissue development during postnatal regeneration; thus, bone and vascular regeneration are coordinated through expression of common sets of transcription and morphogenetic factors.

Another evidence for the importance of muscle-to-bone cross-talk in bone health and disease comes from the rare diseases of muscles. Thus, two studies investigated bones affected by dystrophin deficiency, which is a basis for Duchenne Muscular Dystrophy. Both used *mdx* mice, a model of human Duchenne Muscular Dystrophy. Thus, the study of Novotny *et al.*[45] showed that tibiae of *mdx* mice had up to 50% lower strength and stiffness compared with wild-type mice; they had reductions of 6–57% in cortical cross-sectional moment of inertia and cross-sectional area, and up to 78% reduction in trabecular bone. Importantly, this compromised bone strength was more obvious in very young mice,[45] which corresponds to poor bone health in

boys with Duchenne Muscular Dystrophy. The second group[46] investigated the changes that occur in the femur of *mdx* mice at 21 days of age. They also demonstrated a lower strength, stiffness and energy absorption capacity in *mdx* femora, which were shorter, had a smaller cortical area and thickness, as well as manifested changes in the ECM and collagen organization. Interestingly, at 3 weeks of age the muscle damage in mice is still not significant; thus, the bone seems to be affected even in the absence of significant muscle fiber degeneration.[46]

The above studies make an interesting connection between the muscle and bone biology, offering segue into the musculoskeleton working as a whole.

Fracture healing. Alongside the etiologic factors leading to OF, sequels of fracture (which include delayed fracture healing and a high incidence of non-union or pseudarthrosis) became of interest to several groups. Two papers dealt with the role of bone morphogenetic proteins in fracture healing and non-union. In the Grimes *et al.*[44] study, 15 SNPs within four genes of the BMP pathway (*BMP-2*, *BMP-7*, *NOGGIN* and *SMAD6*) were examined in the patients with long bone fractures (64 patients with atrophic non-union (cases) and 47 with fracture union (controls)). Despite the relatively small sample size, two SNPs, one in *NOGGIN* and the other in *SMAD6*, were found to be associated with a greater risk of fracture non-union. The authors thus suggest that a simple genetic testing may contribute to the early identification of non-healing. Their biological hypotheses[44] were confirmed in the mouse model. It was noted that, during fracture healing, C57/B6 (B6) mice initiate chondrogenesis earlier and develop more cartilage than C3HeJ (C3H), whereas C3H in turn develop more bone than cartilage. By comparison of the transcriptomes of fracture healing between these strains, Dimitriou *et al.*[47] identified genes that showed differences in timing and quantitative expression, pinpointing the genes associated with BMP/TGFβ signal transduction pathway.

Effects of antiosteoporotic treatment. The frequently asked question this year remains whether genetic studies in humans and animal models have any practical value other than biological discovery. As implied above, one of the premises underlying GWAS is an expectation that this screening technique will discover genes causing the disease, and later lead to patient stratification and individualized therapies. Indeed, a variation in response to treatment, most of which cannot be explained by the patient's clinical and demographic characteristics, is a motivation for continuous genetic exploration. In 2011, there were studies looking at the genetic determinants of anti-osteoporotic treatment and prevention, although modest in their scope and power.

One study focused on a bisphosphonate-induced osteonecrosis of the jaw (BONJ), a complication in patients taking bisphosphonates. In the study by Katz *et al.*,[48] patients with multiple myeloma receiving intravenous bisphosphonate therapy were enrolled; of the 78 patients enrolled, 12 had BONJ. The authors revealed a significant association between BONJ and smoking ($P = 0.048$) and type of bisphosphonate treatment ($P = 0.03$). They also tested 10 SNPs from seven candidate genes. A trend for higher odds for BONJ was found for SNPs in five genes: *COL1A1*, *RANK*, *MMP2*, *OPG* and *OPN*. A genetic score of all five SNPs together was a very strong risk factor for BONJ, with adjusted odds ratio 11.2 (95% confidence interval, 1.8–69.9; *P*-value 0.0097).[48] Another paper studied menatetrenone, a vitamin K2 homolog that has been used as a therapeutic agent for osteoporosis. The study[49] evaluated the influence of γ-glutamyl carboxylase (*GGCX*) gene on the response of serum undercarboxylated osteocalcin and bone turnover markers 3 months after the menatetrenone treatment, in postmenopausal Thai women. The *GGCX* gene was chosen as its rare mutations cause defects in vitamin K-dependent proteins. Nevertheless, the response to vitamin K2 was independent of the *GGCX* genotypes. Such studies are probably underpowered and there is a real chance for the results to be spurious. Therefore, it is important to perform further studies in larger samples for a more definitive result.

Conclusions: More Success to Expect in 2012?

The 'tip-of-the iceberg' studies reviewed above taught us that both planned efforts and serendipitous discoveries are an important outcome of the human genetic studies. One among the surprising biological discoveries last year was that the skeleton—via the bone hormone osteocalcin—modulates fertility.[50] Molecular underpinning of the osteoporosis and metabolic syndrome, and, more globally, of bone, energy and fat metabolism, is discernable by means of genetic study. Similarly, debates about the pleiotropic role of vitamin D, which is shown to be actively involved in the etiology of cancer, inflammation and in skeletal muscle maintenance, are not settled yet.[51] Areas of future interest should include, among other ongoing efforts, a possibility of genetic difference between the cervical and trochanteric hip fractures.[52] We also should expect studies of genetic predisposition to atypical (for example, subtrochanteric) femoral fractures due to exposure to bisphosphonates. Another outstanding question concerns a need for improved understanding of the biological aspects of fracture healing. As risk factors for common diseases are identified, larger clinical trials with quantitative measures of response should be stratified by participant's genetic background.

Five years of GWAS produced many leads, and the tool is well established in the field; however, it will gradually relinquish its position to the sequencing. Whole-genome sequencing supports the idea that rare variants are more likely to be functional or causal than common ones.[53] Development of the sequencing tools is paralleled with a push to create analytical pipelines that are able to evaluate the biological functions of the discovered variants. Even though whole-genome sequencing is believed to have better resolution than GWAS, it will face the same set of problems, including a need for replication in independent samples. Therefore, finding possible remedies for the above issues will leverage epigenomic and functional genomics strategies to find causal variants in etiology of osteoporosis.

Note: A list of the gene acronyms can be found in the supplementary information.

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the BoneKEy website (http://www.nature.com/bonekey)