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COMMENTARIES

Genomewide Association Studies in Osteoporosis: Have We Reached the Bottom of the Ocean or the Tip of the Iceberg?

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Commentary on:

- Styrkarsdottir U, Halldorsson BV, Gretarsdottir S, Gudbjartsson DF, Walters GB, Ingvarsson T, Jonsdottir T, Saemundsdottir J, Center JR, Nguyen TV, Bagger Y, Gulcher JR, Eisman JA, Christiansen C, Sigurdsson G, Kong A, Thorsteinsdottir U, Stefansson K. Multiple genetic loci for bone mineral density and fractures. *N Engl J Med*. 2008 May 29;358(22):2355-65.
- Richards JB, Rivadeneira F, Inouye M, Pastinen TM, Soranzo N, Wilson SG, Andrew T, Falchi M, Gwilliam R, Ahmadi KR, Valdes AM, Arp P, Whittaker P, Verlaan DJ, Jhamai M, Kumanduri V, Moorhouse M, van Meurs JB, Hofman A, Pols HA, Hart D, Zhai G, Kato BS, Mullin BH, Zhang F, Deloukas P, Uitterlinden AG, Spector TD. Bone mineral density, osteoporosis, and osteoporotic fractures: a genome-wide association study. *Lancet*. 2008 May 3;371(9623):1505-12.

Tremendous progress has been made in the genetic dissection of many human complex diseases as a result of genomewide association studies (GWAS). However, there are few large-scale GWAS in osteoporosis. Recently, such deficiency was redressed to a certain extent due to the completion of two GWAS projects on bone mineral density (BMD), osteoporosis fracture, and osteoporosis risk. In April and May of 2008, two groups, deCODE Genetics and also a collaborative group involving researchers from TwinsUK, the Rotterdam Study, and the Chingford Study, published their GWAS research findings on osteoporosis in the April 29th edition of the *New England Journal of Medicine* (1) and the May 3rd edition of *The Lancet* (2).

In the *NEJM* paper, 5861 Icelandic subjects (the discovery set) were tested for an association between 301,019 single-nucleotide polymorphisms (SNPs) and BMD at the hip and lumbar spine. Based on the results in the discovery set, researchers further tested an association between 74 SNPs at 32 loci in replication sets of Icelandic, Danish, and Australian subjects (4165, 2269, and 1491 subjects, respectively). Sequence variants in five genomic regions were significantly associated with BMD in the discovery set and were confirmed in the replication sets (combined P values, 1.2×10^{-7} to 2.0×10^{-21}). Three regions are close to or within genes previously shown to be important for the biologic characteristics of bone: the receptor activator of nuclear factor-κB ligand gene (*RANKL*) (chromosomal location, 13q14),

the osteoprotegerin gene (*OPG*) (8q24), and the estrogen receptor 1 gene (*ESR1*) (6q25). The two other regions are close to the zinc finger and BTB domain containing 40 gene (*ZBTB40*) (1p36) and the major histocompatibility complex region (6p21). The 1p36, 8q24, and 6p21 loci were also associated with osteoporotic fractures, as were loci at 18q21, close to the receptor activator of nuclear factor-κB gene (*RANK*), and loci at 2p16 and 11p11.

In the *Lancet* paper, the researchers analyzed 314,075 SNPs in 2,094 women from the United Kingdom (TwinsUK discovery cohort) and identified a set of the most promising SNPs that were then tested for replication in three cohorts involving an additional 6,463 individuals. They also investigated *cis*-acting allelic expression levels in lymphoblast cell lines (LCLs). They reported a non-synonymous SNP in the *LRP5* gene that was associated with decreased BMD (rs3736228: $P = 6.3 \times 10^{-12}$ for lumbar spine (LS) and 1.9×10^{-4} for femoral neck (FN)) and increased risk of osteoporosis (odds ratio (OR) = 1.3, $P = 8.0 \times 10^{-3}$). The risk allele was associated with an increased risk of osteoporotic fractures in two cohorts involving 6,639 individuals (OR = 1.3, $P = 2.0 \times 10^{-3}$). They also found three SNPs near the *TNFRSF11B* (osteoprotegerin) gene that were associated with decreased BMD (top SNP, rs4355801: $P = 7.6 \times 10^{-10}$, $P = 3.3 \times 10^{-8}$ for LS and FN, respectively) and increased risk of osteoporosis (OR = 1.2, P = 0.038). Alleles at this region associated with decreased BMD were associated with decreased osteoprotegerin allelic expression in LCLs ($P = 2.0 \times 10^{-7}$ for rs6469788). More interestingly, they found that 22% of the entire population was at least heterozygous for both risk alleles and these alleles had a cumulative association with BMD (P for trend $= 2.5 \times 10^{-17}$). The presence of both risk alleles increased the risk of osteoporotic fracture (OR = 1.3, $P = 6.0 \times 10^{-3}$) and osteoporosis (OR = 1.5, $P = 3.0 \times 10^{-3}$).

These two projects are timely and are excellent GWAS studies in the osteoporosis research field. There are many strengths of these studies. Using the Illumina Infinium platform for GWAS, each study produced GWA data over 300,000 SNPs chosen on the basis of the HapMap SNP dataset. The genomic coverage achieved by their marker sets is therefore quite high (3). Each study has a large pool of DNA samples in the GWAS cohort and thus has adequate power to identify the genes of certain effect size operating in osteoporosis. There is formal assessment of the presence of population stratification, which has been adequately controlled by well-developed methods in each study. The statistical associations with BMD, osteoporosis risk and osteoporosis fractures are strong. Positive findings from the GWAS scan were successfully replicated in each study. And such replications were obtained by different centers using different genotyping technologies. Furthermore, one of the studies also confirmed the results of a prospective meta-analysis that recently reported the association of LRP5 alleles with both BMD and fractures in men and women (4). In addition, the study published in *Lancet* showed that the expression of OPG genes was significantly affected by the risk alleles of the identified SNPs, giving more convincing molecular evidence regarding the function of the candidate genetic variants.

However, like most GWAS research, there are some limitations of these two studies on osteoporosis. First, all of the tested subjects were whites of European descent, especially in the *Lancet* study, and most of the participants were white women. Thus the effects of the identified genes like *RANKL*, *ESR1*, *LRP5* and *TNFRSF11B* still need to be investigated in individuals of non-European ancestry. With the exception of LRP5 (see above), it also remains unknown whether or not genetic factors conveying osteoporosis risk are the same in men and women (5). Indeed, methods for formally testing sex differences in genetic association studies have been proposed (6), but have rarely been implemented, including in the studies discussed here. More broadly, expression of allelic variants may vary according to the environment, which is notoriously different among geographic regions and ethnic groups. For instance, the level of physical activity has been reported to influence association of LRP5 polymorphisms with BMD (7). Second, most

of the identified osteoporosis genes are not novel and have been studied extensively before (8-11). Although the presented GWAS results strengthen our confidence in those previous, famous osteoporosis genes, little new information is added to the overall understanding of the genetic architecture of osteoporosis. In contrast, the GWAS analyses did not confirm some known loci and genes associated with osteoporosis and fractures, such as 20p12 and BMP2, which had previously been found in the same Icelandic population and by the same investigators as the current *NEJM* paper (12). As the authors admit, "there are many more sequence variants relevant to osteoporosis to be identified" (1); "many other loci impart smaller effects on bone mineral density" (2). Third, the variation in hip and spine BMD that is accounted for by all the implicated SNPs in these two studies is small (namely <1-3%). Although the contribution to fracture risk might be greater than explained by allele-driven differences in BMD, the clinical values of these studies are not obvious at present. In particular, the *NEJM* study used self-reported fractures that were not necessarily validated, and neither study performed systematic spine X-rays. Considering that vertebral fractures probably represent the most specific osteoporosis phenotype, future GWAS using morphometric vertebral fractures as the phenotype may offer more insight into the value of genetic markers for clinical prediction.

Hence, in many regards, and despite the huge investment and progress these GWAS studies represent, their main results suggest that we have just scraped the tip of the iceberg. To reach the bottom of the ocean where most of the genetic variation underlying osteoporosis risk still lies, not only more GWAS, but also (re)sequencing selected genome regions from GWAS (*e.g.*, 1p36), as well as other approaches, such as gene expression analyses in human and mice, are needed. The risk is that in the end, as we will discover more gene variants associated with the disease, it should appear that most of the population might carry one or more of these risk alleles. However, to know which specific gene(s) allele(s) increase risk in a given individual, *i.e.*, which mechanisms (increased bone turnover, as might be the case with some OPG variants, or decreased bone formation, as might be the case with some LRP5 variants) most likely cause his or her fracture susceptibility, may be most relevant to targeting specific drugs, hence maximizing the benefits and minimizing the risks of osteoporosis therapy.

Conflict of Interest: Dr. Ferrari reports that he receives research support from Amgen and consultancy/speaker's fees from Merck Sharp & Dohme, Eli Lilly, and Amgen**.** Dr. Xiong and Dr. Deng report no conflicts of interest.

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