

## EDITORIAL



## Bona Fide Genetic Associations with Bone Mineral Density

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Osteoporosis is a common skeletal disorder characterized by compromised bone strength and increased bone fragility, affecting up to 40% of postmenopausal women and 15% of men.<sup>1</sup> Its clinical significance lies in the occurrence of osteoporotic fractures — most commonly involving the forearm, the vertebral bodies, and the hip — with considerable morbidity, mortality, and cost to the individual and the community.<sup>2</sup>

Many factors, including age, menopausal status, smoking, physical activity, diet, coexisting diseases, and pharmacologic treatments, influence the risk of osteoporosis, but one of the most clinically important risk factors is a family history of the disorder. As in other polygenic disorders, alterations in numerous genes each make small contributions to susceptibility to osteoporosis. With the knowledge that osteoporosis has a strong genetic component, it is natural to ask whether the relevant genes could be identified. In principle, genetic studies can improve the prediction of risk and illuminate the underlying biologic characteristics of disease. In this issue of the *Journal*, Styrkarsdottir et al.<sup>3</sup> identify relevant genes; although their results do not provide substantial predictive power, they may open new windows into the pathophysiological characteristics of osteoporosis.

Researchers seeking to find genes that influence osteoporosis and the risk of fracture have used two main approaches: linkage analysis and association studies.<sup>4</sup> In most of these studies, bone mineral density has been used as a surrogate phenotype, because it has a high heritability and represents an important and measurable clinical predictor of the risk of fracture.<sup>5</sup> However, early studies, like those of other polygenic traits, were fraught with difficulties.<sup>6</sup>

Linkage studies were unsuccessful at identifying a gene influencing bone mineral density. Association studies were even more problematic. Limited by logistic factors, and perhaps assuming that effects of genetic variants on bone mineral density would be large, investigators routinely carried out association studies with, at most, hundreds of patients and used genetic variants that happened to be at hand. In addition, nominally significant results ( $P < 0.05$ ) were usually advanced as evidence of an association. Many early, heralded genetic-association studies of bone mineral density and fracture focused on a few markers in a small number of promising candidate genes such as the estrogen receptor 1 gene *ESR1* or the vitamin D (1,25-dihydroxyvitamin D<sub>3</sub>) receptor gene *VDR*, and the results proved to be difficult to replicate; more recently, larger collaborative studies have started to provide more convincing evidence of association.<sup>7</sup> In retrospect, we now know that the early studies were underpowered to detect the more modest effects that are generally seen with polygenic traits and diseases and that most of the nominally significant  $P$  values were more likely than not false positive results due to statistical fluctuations or technical artifacts.

With improvements in both genotyping technology for single-nucleotide polymorphisms (SNPs) and knowledge of common genetic variation, it has become possible to perform an unbiased search of the entire genome for common genetic risk factors in large cohorts of patients.<sup>8</sup> A preliminary genomewide investigation of 100,000 markers in 1141 subjects in the Framingham Heart Study recently identified 40 SNPs as being potentially associated with several bone phenotypes, but the results did not meet the statistical threshold of genomewide significance and have

not been validated.<sup>9</sup> The study by Stykarsdottir et al. is the first large and comprehensive genome-wide association study of bone mineral density.

Stykarsdottir et al. identified five loci for which there is strong evidence of association with bone mineral density: three previously identified candidate genes (*ESR1*, the osteoprotegerin gene *OPG*, and the receptor activator of nuclear factor- $\kappa$ B ligand gene *RANKL*) as well as two new loci, on 1p36 near the complex of the zinc finger and BTB domain containing 40 gene and the wingless-type MMTV integration site family member 4 gene (*ZBTB40-WNT4*) and on 6p21 in the major-histocompatibility-complex (MHC) region. These associations reach the threshold of genomewide significance set by the investigators ( $P < 1.7 \times 10^{-7}$ ), a finding that suggests that they are probably true associations, although Stykarsdottir et al. note that the evidence for 6p21 is slightly weaker than for the other regions. The location of 6p21 within the HLA region also raises the possibility of population stratification (confounding of the genetic association because of ancestry, which can be seen for genotypes linked to HLA), but the authors took several measures to minimize this potential problem.

The variants with the strongest evidence of association with bone mineral density were tested for association with fracture in an independent cohort. There was modest but significant evidence of association for the two new loci (1p36 and 6p21) and *OPG* as well as for variants near the receptor activator of the nuclear factor- $\kappa$ B gene (*RANK*), the gene encoding low-density lipoprotein receptor-related protein 4 (*LRP4*), and the spectrin, beta, nonerythrocytic 1 gene (*SPTBN*). The effects were modest, with odds ratios for fracture between 1.06 and 1.15. Given these results and the strong evidence of an effect on bone mineral density, it seems likely that these variants have a small but real effect on the risk of fracture.

Three of the reported associations (with *ESR1*, *OPG*, and *RANKL*) identified in this unbiased genetic study involve crucial regulators of bone homeostasis. Decreased estrogen production or impaired estrogen sensitivity represents a major risk factor for osteoporosis and fractures in both women and men. *RANKL*, its cognate receptor *RANK*, and its natural decoy receptor *OPG* have recently been identified as major regulators of osteoclast activity and bone resorption.<sup>10</sup>

As Stykarsdottir et al. state, the value of these particular findings for prediction of risk of frac-

ture is currently limited. It remains to be seen whether future genetic discoveries will add sufficient predictive information to help stratify patients according to risk, in a clinically useful manner. It will also be interesting to test more focused hypotheses, such as whether the *ESR1* variants modulate the effects of estrogen therapy on osteoporosis or on other clinical outcomes. Similarly, the analysis of variants in *OPG*, *RANK*, or *RANKL* could be relevant to the effects of denosumab (a human *RANKL* monoclonal antibody) in preventing fracture as well as other bone-related disorders.<sup>11</sup>

The small amount of variation in the risk of fracture explained by these SNPs is a different concept than the population attributable risk of 17% quoted by Stykarsdottir et al. for one of the variants. The seemingly high value of 17% represents the number of cases that would be avoided if persons with the common, high-risk genotype instead had the same risk as those with the low-risk genotype. This value should not be taken to represent the explanatory power of these variants for the risk of disease.

Probably the most important effect of these and other genomewide association studies is the insight they provide into the underlying biology of disease. Unlike other clinical correlations or data for most animal models of disease, validated genetic associations can provide direct evidence of causality of a particular gene or pathway in a disease process in humans. Many unbiased genomewide screenings have identified genes that are the sites of action for existing therapies — such as *ESR1* and *RANKL*, the sites of action of estrogen and denosumab, respectively, as reported by Stykarsdottir et al. On the basis of this track record, one can only assume that some of the new loci identified by means of such genomewide screening will also be good drug targets and provide leads for the development of improved therapies and preventive measures.

Dr. Hirschhorn reports holding equity in, and receiving consulting fees from, Correlagen and receiving lecture fees from Pfizer. He also reports holding a patent for a genotyping method. No other potential conflict of interest relevant to this article was reported.

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