Genetics of osteoporotic fracture

Chuan Qiu1,2
Christopher J Papasian3
Hong-Wen Deng1,2,3,4
Hui Shen1,2

1Center for Bioinformatics and Genomics, Department of Biostatistics and Bioinformatics, School of Public Health and Tropical Medicine, Tulane University, New Orleans, Louisiana, USA; 2Department of Basic Medical Sciences, School of Medicine, University of Missouri-Kansas City, Kansas City, Missouri, USA; 3Center of System Biomedical Sciences, University of Shanghai for Science and Technology, Shanghai, China; 4Molecular and Statistical Genetics Lab, College of Life Sciences, Hunan Normal University, Changsha, China

Abstract: Osteoporosis is a major public health problem that results in a massive burden to patients and society through associated low-trauma, osteoporotic fractures. Previous studies have shown that osteoporosis-associated traits, such as low bone mineral density, as well as the probability of actually experiencing an osteoporotic fracture, are under strong genetic control. Susceptibility to osteoporosis and osteoporotic fractures is likely to be controlled by multiple genetic and environmental factors, and by interactions between them. Although numerous genetic studies, mainly candidate gene association studies, have attempted to decipher the genetic basis for osteoporosis and osteoporotic fractures, little success has been achieved. Recent advances in high-throughput genotyping technology and knowledge of common human genetic variants have shifted the approach for studying human complex disorders from candidate gene studies to large-scale genome-wide association studies. In the past three years, more than 10 genome-wide association studies have been carried out for osteoporosis. A number of genes that are associated with osteoporosis-related traits, and/or with the probability of actually experiencing an osteoporotic fracture, have been successfully identified and replicated through these studies. In this article, we review the recent progress in the genetics of osteoporosis, with an emphasis on studies that have focused on genes that directly affect osteoporotic fractures.

Keywords: osteoporotic fracture, candidate gene association study, genome-wide association study

Introduction

Osteoporosis, characterized by low bone mass with microarchitectural deterioration and skeletal fragility, is the most prevalent metabolic bone disease in the elderly. Individuals with osteoporosis are at increased risk for low-trauma osteoporotic fractures, particularly at the spine, hip, and wrist.1

It is currently estimated that approximately 12 million people over the age of 50 years in the US have osteoporosis, and an additional 30–40 million people are at risk for osteoporotic fractures.2 The lifetime risk of suffering an osteoporotic fracture is estimated to be approximately 50% in women and about 20% in men.3,4 Osteoporotic fractures are associated with excessive morbidity and mortality.5,6 In fact, over 20% of patients who suffer a hip fracture die within one year of their injury, and half of those who do survive can no longer live independently. The economic burden caused by osteoporotic fractures is substantial, with an estimated annual direct care expenditure of $19 billion in the US,7 and it is estimated that indirect costs (eg, lost productivity for patients and caregivers) add billions of dollars to this figure. Due primarily to the aging population, it is anticipated...
that the number of osteoporotic fractures and associated costs could double or even triple in the coming decades.7

Multiple studies,8–10 including a recent World Health Organisation meta-analysis of 60,000 individuals,11 have suggested that a parental history of fracture is a major risk factor for osteoporotic fractures. Genetic epidemiological studies have provided direct evidence supporting the concept that susceptibility to osteoporotic fractures is under genetic control,12–14 with estimated heritability ranging from 0.2 to 0.68.15,16 Interestingly, although low bone mineral density is a major risk factor for osteoporotic fractures17 and bone mineral density has been used as the predominant surrogate phenotype for osteoporotic fractures in genetic studies,18,19 the genetic correlation between bone density and osteoporotic fractures is actually very low. This suggests that the genetic factors influencing bone density and osteoporotic fractures are largely independent of one another.15,20 It is therefore critical to conduct genetic studies for osteoporotic fractures directly, rather than relying predominantly upon surrogate indicators, such as bone density. Furthermore, all genes that are found to be important for bone density or other risk factors for osteoporotic fractures should be tested for their direct relevance to these fractures.21

In the past three years, a number of genetic factors contributing to risk of osteoporotic fractures have been identified, mainly through genome-wide association studies. In this article, we review recent progress in human genetic studies of osteoporotic fractures, and discuss future directions that can be used to search for genetic factors underlying osteoporotic fractures.

Candidate gene association studies
As with all other complex disorders, early genetic studies of osteoporotic fractures relied heavily on candidate gene association approaches, and results were largely inconsistent across different studies.18 The lack of reproducibility of early candidate gene association studies is not unique to osteoporosis; this is a common phenomenon in the field of genetics of human complex disorders. As we have discussed previously,22 one of the main reasons for this lack of reproducibility is overinterpretation of nominally significant results from studies with insufficient statistical power. More recently, large collaborative studies with adequate sample size have begun to provide a clearer delineation of the association between osteoporotic fractures and a number of candidate genes.23 A few specific candidate genes are discussed below, and a more comprehensive list can be found in several excellent review articles.18,23,24

Vitamin D receptor gene
The VDR gene is one of the most extensively studied candidate genes for osteoporotic fractures. The largest study of the VDR gene reported to date was performed by the Genetic Markers for Osteoporosis (GENOMOS) consortium, which included 6067 fracture patients (2088 had vertebral fractures) and 20,175 controls.25 In this study, no significant association was detected between osteoporotic fractures and four “classical” VDR polymorphisms, namely, FokI, BsmI, ApaI, and TaqI. However, the Cdx2 polymorphism showed a modest association ($P = 0.039$) with risk for vertebral fractures.25

Grundberg et al26 studied the association between common haplotypes defined by the BsmI, Apal, and TaqI polymorphisms and risk of vertebral fractures in elderly men from Sweden (n = 3014, including 208 vertebral fractures). The baT haplotype was found to be associated with increased risk of vertebral fractures (odds ratio [OR] = 1.65, 95% confidence interval [CI]: 1.146–2.391, $P < 0.01$), independent of body weight, height, age, and bone density. More recently, a literature-based meta-analysis of 17 studies generated a combined sample of 2112 fracture cases and 4521 controls to test for associations between osteoporotic fractures and FokI, BsmI, Apal, and TaqI polymorphisms of the VDR gene.27 When stratifying by fracture type, they found that hip fracture cases had a significantly lower frequency of the bb genotype for BsmI (OR = 0.82, 95% CI: 0.70–0.97) and the Tt genotype for TaqI (OR = 0.65, 95% CI: 0.43–0.97), but a significantly higher frequency of the tt genotype for TaqI (OR = 1.74, 95% CI: 1.05–2.91). Additionally, subjects with the Aa genotype of Apal had a significantly higher risk of vertebral fracture (OR = 1.63, 95% CI: 1.03–2.59).27 Together, these studies suggested that there is a modest, but statistically significant, association between specific VDR polymorphisms and osteoporotic fractures.

Type I collagen α1 gene
The COL1A1 gene encodes the alpha 1 chain of type I collagen, which is the most abundant structural protein in the bone matrix. Mutations in the COL1A1 gene can cause osteogenesis imperfecta, a Mendelian disorder presenting with moderate to severe bone fragility.28–30 The COL1A1 gene has been extensively studied in relation to bone mineral density and osteoporotic fractures.18,19 The majority of these studies have focused on the G/T polymorphism located in intron 1 of the COL1A1 gene, which affects a binding site for transcription factor Sp1.31 The GENOMOS consortium performed a prospective participant-level meta-analysis for the COL1A1 Sp1 polymorphism in relation to fractures in 20,786 unrelated
Caucasian subjects, including 6067 individuals with any fracture, 2088 with vertebral fractures, and 2407 with incident fractures (412 had incident vertebral fractures). Although the COL1A1 Sp1 polymorphism was not associated with overall fractures, there was a nonsignificant trend toward association with vertebral fracture, and a nominally significant association with incident vertebral fractures in females (OR = 1.33, 95% CI: 1.00–1.77, \( P = 0.05 \)). Further adjustment for lumbar spine bone density and postmenopausal status did not considerably alter any of these effects. Similar to what had been conducted for \( VDR \) polymorphisms, a literature-based meta-analysis was also recently published for the COL1A1 Sp1 polymorphism, involving 2294 patients with fractures and 10,285 controls. In contrast with the results from the GENOMOS study, a significant difference in genotype distribution was detected between overall patients with fractures and controls. Further analyses suggested that the SS genotype is associated with reduced risk for both vertebral (OR = 0.58, 95% CI: 0.38–0.90) and nonvertebral (OR = 0.80, 95% CI: 0.68–0.95) fractures. These studies suggest that the COL1A1 Sp1 polymorphism may confer very modest effects on the susceptibility to osteoporotic fractures.

The value of candidate gene studies, such as those reviewed above, is inherently limited due to their focus on a few specific candidate genes, the biological function of which is known to be related to bone development and metabolism; this approach precludes the identification of novel osteoporotic fracture susceptibility genes. In addition, these studies normally test only a few previously typed polymorphisms, and thus cannot determine whether other variants in the selected candidate genes may contribute to the susceptibility of osteoporotic fractures. Furthermore, most, if not all, of these studies are not properly controlled for population stratification, because they do not have ancestry informative marker data available. For these reasons, results of candidate gene studies, even large collaborative studies, such as GENOMOS, must be interpreted with caution.

**Genome-wide association studies**

Analogous to other polygenic complex diseases, susceptibility to osteoporotic fractures is likely to be influenced by multiple genes, each with small to modest effects. Therefore, it is critical to perform unbiased, hypothesis-free searches across the entire human genome to identify genetic factors underlying osteoporotic fractures. Recent advances in high-throughput single nucleotide polymorphism genotyping technology, and knowledge of common genetic variants in the human genome, have made large-scale genome-wide association studies (GWAS) feasible. Study populations for GWAS often include thousands of individuals, and each individual is typically evaluated for hundreds of thousands to millions of single nucleotide polymorphisms distributed throughout the entire genome. In the past three years, more than 10 GWAS of osteoporosis have been performed, mainly focused on bone mineral density variation rather than osteoporotic fractures per se. In this section, we review GWAS that have identified susceptibility genes of osteoporotic fractures.

**deCODE Genetics**

By genotyping approximately 300,000 single nucleotide polymorphisms in 5861 Icelandic subjects, deCODE Genetics conducted the first large and comprehensive GWAS of bone mineral density. Five genomic regions, encompassing the major histocompatibility complex region and the \( \text{RANKL}, \text{OPG}, \text{ESR1}, \text{and ZBTB40} \) genes, attained genome-wide significant associations with bone mineral density. Twelve of the most significant single nucleotide polymorphisms in these five regions, along with eight other single nucleotide polymorphisms showing suggestive associations with bone mineral density, were tested for associations with low-trauma fracture using 4406 fracture cases collected from Iceland, Australia, and Denmark. Significant associations were detected for the \( \text{ZBTB40} \) gene (\( P = 0.016–2.4 \times 10^{-3} \)) and for the major histocompatibility complex region (\( P = 0.044–0.008 \)) with osteoporotic fractures at specific skeletal sites (hip, forearm, and vertebral) and with broadly defined osteoporotic fractures. The \( \text{OPG} \) gene showed a nominally significant association (\( P = 0.04 \)) with broadly defined osteoporotic fractures, and no association with osteoporotic fractures was detected for the \( \text{ESR1} \) or \( \text{RANKL} \) genes. Interestingly, several genes that did not attain genome-wide significant associations with bone mineral density showed evidence of an association with osteoporotic fractures. These included the \( \text{RANK} \) gene (\( P = 0.005 \)), the \( \text{SPTBN1} \) gene (\( P = 1.8 \times 10^{-4} \)), and the \( \text{LRP4} \) gene (\( P = 0.007 \)). For all of these loci, the effects on osteoporotic fractures were rather modest, with \( \text{OR} \) ranging from 1.06 to 1.15. Following the initial GWAS, the deCODE Genetics group expanded their discovery and replication cohorts, and re-evaluated 100 single nucleotide polymorphisms and 20 nonsynonymous or potentially functional single nucleotide polymorphisms that produced the most significant associations in their initial GWAS. Four new loci attained genome-wide significant associations with bone mineral density, including the \( \text{SOST} \) gene, the \( 

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gene, the \textit{MARK3} gene, and the \textit{RANK} gene.\textsuperscript{43} Notably, single nucleotide polymorphisms in the \textit{SOST} gene and the \textit{MARK3} gene also had modest effects on low-trauma fractures (OR = 1.07–1.10).\textsuperscript{43}

\textbf{TwinsUK and Rotterdam}

In this study of 2094 women from the TwinsUK cohort and 4081 unrelated Dutch subjects from the Rotterdam study, 314,075 single nucleotide polymorphisms were evaluated for association with bone mineral density.\textsuperscript{44} The results were replicated in two independent cohorts. One single nucleotide polymorphism near the \textit{OPG} gene, and one nonsynonymous single nucleotide polymorphism (rs3736228) in the \textit{LRP5} gene, showed genome-wide significant associations with bone density variation.\textsuperscript{44} The risk allele at rs3736228 was also associated with an increased risk of osteoporotic fractures (OR = 1.3, 95% CI: 1.09–1.52, \textit{P} = 0.002). This association persisted after adjustment for bone mineral density (OR = 1.22, 95% CI: 1.02–1.45, \textit{P} = 0.03),\textsuperscript{44} suggesting that this locus may influence the risk of osteoporotic fractures, independent of its effects on bone mineral density.

\textbf{Chinese and US Midwest}

By using a cohort of 700 elderly Chinese Han subjects (350 hip osteoporotic fracture cases and 350 healthy matched controls), our group performed the first GWAS specifically focused on hip osteoporotic fractures.\textsuperscript{45} Major findings in this GWAS were further validated in an independent replication cohort, containing 390 hip osteoporotic fracture patients and 516 matched controls.\textsuperscript{45} Both the GWAS discovery cohort and the replication cohorts were collected from the area of Xi’an, China. Stringent inclusion/exclusion criteria were applied for recruitment of both cases and controls, and all hip fractures were identified or confirmed through diagnosis by orthopedic surgeons or radiologists with radiological evidence.\textsuperscript{46} We found that one single nucleotide polymorphism, rs13182402, within the \textit{ALDH7A1} gene, was strongly associated with hip osteoporotic fractures in both the discovery (\textit{P} = 8.53 \times 10^{-6}) and replication cohorts (OR = 2.25, 95% CI: 1.72–2.94, combined \textit{P} = 2.08 \times 10^{-9}).\textsuperscript{45} This single nucleotide polymorphism also showed a modest association with hip bone density (combined \textit{P} = 6.39 \times 10^{-4}) in additional Chinese and Caucasian samples (n = 9962).\textsuperscript{45} Unfortunately, bone mineral density measurements were not available for the hip osteoporotic fracture cases, so we could not determine whether the effect of this single nucleotide polymorphism on risk of hip osteoporotic fractures was through its influence on bone density or independent of this effect.\textsuperscript{45}

Copy number variation refers to a segment of DNA that is present in varying copy numbers in genomes of different individuals. Copy number variations can influence gene expression, and have been implicated in the etiology of several human complex diseases.\textsuperscript{46–48} Using the same Xi’an hip osteoporotic fracture cohort, we constructed a genome-wide copy number variation map containing 727 copy number variation regions in Chinese individuals, and detected a strong association between a copy number variation on chromosome 4q13.2 and hip osteoporotic fractures (OR = 1.73, 95% CI: 1.22–2.45, \textit{P} = 2.0 \times 10^{-3}).\textsuperscript{49} Subsequent molecular analyses fine-mapped the copy number variation to a 150 kb interval spanning the \textit{UGT2B17} gene.\textsuperscript{49} Furthermore, the association between \textit{UGT2B17} copy number variations with hip osteoporotic fractures was successfully replicated in the independent hip osteoporotic fractures cohort (\textit{P} = 6.34 \times 10^{-3}).\textsuperscript{49} This copy number variation also showed modest associations with hip bone mineral density and femoral neck bone geometry (\textit{P} = 5.0 \times 10^{-4}–0.021) in additional independent Chinese and Caucasian samples.\textsuperscript{49} Because the \textit{UGT2B17} gene encodes an enzyme catabolizing steroid hormones, we also tested the association between this copy number variation and the serum concentrations of testosterone and estradiol. Compared with subjects having one or two copies of \textit{UGT2B17}, subjects with no copies of this gene had significantly higher serum concentrations of testosterone (\textit{P} = 0.005) and estradiol (\textit{P} = 0.01).\textsuperscript{49} Taken together, our results suggested a putative mechanism for osteoporotic fracture by which the \textit{UGT2B17} gene inhibits steroid hormone levels, thereby compromising bone mass and integrity and increasing the risk of osteoporotic fractures.\textsuperscript{49}

To identify additional osteoporosis susceptibility genes, we performed GWAS for bone mineral density\textsuperscript{50} and other osteoporosis-related traits (eg, bone size)\textsuperscript{51} in 1000 unrelated Caucasians, recruited from the US Midwest. We observed significant associations between the \textit{ADAMTS18} gene and hip bone mineral density\textsuperscript{50} and between the \textit{PLCL1} gene and bone size.\textsuperscript{51} In addition, both the \textit{ADAMTS18} and the \textit{PLCL1} genes showed modest associations with hip osteoporotic fractures (\textit{P} = 7.66 \times 10^{-3}–0.019).\textsuperscript{50,51} Supporting this finding, a recent study suggested that the \textit{PLCL1} gene may also confer modest effects on the risk of vertebral fracture in postmenopausal Danish women.\textsuperscript{52}

\textbf{Genetic factors for osteoporosis}

Representing a commendable collaborative effort that included 19,195 subjects of Northern European descent, the GEFOS consortium combined five GWAS to perform
a large-scale meta-analysis of bone mineral density.\textsuperscript{53} Twenty genomic loci showed strong associations with bone density, and these loci included 13 genes that had not previously been significantly associated with bone density at the genome-wide level.\textsuperscript{53} After combining the effects of the top 20 bone density loci, the association between the compound allelic scores and the risk of fracture was assessed in 2727 radiographically screened individuals (302 vertebral fracture cases) and in 4865 individuals followed for an average of 8.2 years (672 nonvertebral fractures).\textsuperscript{53} Despite limited power, a significant association was observed between the compound femoral neck bone density allelic score and the risk of incident nonvertebral fracture (hazards ratio [HR] = 1.042, 95% CI: 1.003–1.084, \( p = 0.04 \)).\textsuperscript{53} Similarly, the compound lumbar spine bone density allelic score was significantly associated with the risk of vertebral fracture (OR = 1.061, 95% CI: 1.009–1.116, \( p = 0.02 \)).\textsuperscript{53} Adjustment for bone mineral density showed that approximately 25%–50% of the genetic effect on fracture could not be explained by bone density.\textsuperscript{53}

Using the same GWAS meta-analysis data, the GEFOS consortium also evaluated 36,015 single nucleotide polymorphisms surrounding 150 osteoporosis candidate genes to determine whether they were associated with either bone density or fracture (900 nonvertebral osteoporotic fracture patients and 329 vertebral osteoporotic fracture patients).\textsuperscript{54} Single nucleotide polymorphisms from nine candidate genes were significantly associated with osteoporotic fractures, with an estimated OR ranging from 1.13 to 1.43.\textsuperscript{54} After accounting for their association with bone mineral density, single nucleotide polymorphisms from five candidate genes, specifically LRP5, SOST, SPP1, RANKL, and RANK, were also significantly associated with osteoporotic fractures, with an estimated OR ranging from 1.13 to 1.43.\textsuperscript{54} After accounting for their association with bone mineral density, single nucleotide polymorphisms from the SOST and the LRP5 genes retained statistically significant associations with nonvertebral osteoporotic fractures, and single nucleotide polymorphisms from the SPP1 gene showed persistent associations with vertebral osteoporotic fractures.\textsuperscript{54} These results suggest that some of these genes may have effects on the risk of osteoporotic fractures that are not mediated through bone mineral density, but perhaps through their effects on other aspects of bone strength or nonskeletal factors, such as the propensity to fall.\textsuperscript{55}

### Hong Kong Southern Chinese

Recently, Kung et al carried out a GWAS for bone mineral density using a discovery cohort of 800 unrelated Hong Kong Southern Chinese females with extreme bone density values (bottom 10% or top 16% of the population) and performed a two-phase replication study in six independent populations that included 18,098 subjects.\textsuperscript{56} They identified and replicated a novel association between bone mineral density variation and single nucleotide polymorphism rs2273601 of the JAG1 gene.\textsuperscript{56} In a sample of 244 osteoporotic fracture cases and 1637 controls, they found that this JAG1 single nucleotide polymorphism was also associated with the risk of osteoporotic fractures (\( p = 0.009, \) OR = 0.7, 95% CI: 0.57–0.93, \( n = 1881 \)), and that this association was partly, but not completely, attenuated if bone mineral density was added as a covariate in the analysis (\( p = 0.031, \) OR = 0.8, 95% CI: 0.58–0.97).\textsuperscript{56} Therefore, the JAG1 gene may exert its effects on the risk of osteoporotic fractures through mechanisms that are both dependent and independent of bone density.

### Perspectives

GWAS have dramatically advanced our ability to identify osteoporosis susceptibility genes. To date, associations with bone density variation and/or risk of osteoporotic fractures have been identified and replicated for more than 40 genes or genomic regions by GWAS (Table 1). These implicated genes/regions have included genes in known major bone biology pathways, such as the RANK/RANKL/OPG and Wnt/LRP5 pathways, as well as a number of novel osteoporosis susceptibility genes. Interestingly, these genes are enriched in three major networks (Figure 1), suggesting that they may act interactively to affect the predisposition of osteoporosis. The findings of GWAS have provided new insights into the complex genetic and molecular mechanisms underlying osteoporosis. Importantly, several genes have shown significant associations with osteoporotic fractures, independent of their effects on bone mineral density. Although this result was not unexpected based on the low level of genetic correlation between bone mineral density and osteoporotic fractures, it further highlights the importance of directly targeting the osteoporotic fracture phenotype for genetic studies of osteoporosis.

Despite the tremendous benefits of utilizing GWAS, it is important to recognize that current GWAS are constrained by a number of limitations. For instance, current GWAS only focus on common variants in the human genome, mainly single nucleotide polymorphisms, and the collective effects of the loci identified to date explain less than 5% of the estimated heritability of bone mineral density and an even smaller fraction of the heritability for osteoporotic fractures.\textsuperscript{56} Hence, the vast majority of the heritable component of osteoporosis remains to be discovered. As with other complex traits (eg, height),\textsuperscript{57} it is likely that many additional
Table 1 Osteoporosis susceptibility genes identified by genome-wide association studies

<table>
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<tr>
<th>Gene symbol</th>
<th>Gene description</th>
<th>Associated trait</th>
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<td>TGFBR3</td>
<td>Transforming growth factor, beta receptor III</td>
<td>LS BMD</td>
<td>Xiong et al(^42)</td>
</tr>
<tr>
<td>UGT2B17</td>
<td>UDP glucuronosyltransferase 2 family, polypeptide B17</td>
<td>Hip BMD</td>
<td>Yang et al(^49)</td>
</tr>
<tr>
<td>ZBTB40</td>
<td>Zinc finger and BTB domain containing 40</td>
<td>LS/FN BMD</td>
<td>Strykarsdottir et al(^42); Rivadeneira et al(^42)</td>
</tr>
</tbody>
</table>

Abbreviations: LS, lumbar spine; FN, femoral neck; BMD, bone mineral density.
Figure 1 (Continued)
common variants with smaller effects, and a number of rare variants with larger effects on osteoporosis have, thus far, eluded identification. Moreover, it has also been suggested that gene-gene (epistasis) and gene-environment interactions may contribute significantly to the “missing” heritability of complex human diseases. However, genetic studies of humans rarely investigate these effects, partly because previous statistical methods for detecting these effects on a genome-wide scale are relatively poor. Fortunately, novel analytic approaches for genome-wide gene-gene and gene-environment interaction analyses are quickly emerging, and it is expected that implementation of these methods will lead to novel insights into the pathophysiology of osteoporotic fractures.

It is similarly important to exercise caution when carrying out GWAS meta-analyses for osteoporotic fractures. The current trend in genetics of osteoporosis is to perform larger and larger GWAS by pooling more and more individual studies into meta-analyses. Although such collaborative efforts are laudable and potentially important for the study of osteoporotic fractures, simply pooling samples together does not always improve statistical power. For instance, it is common to identify individuals with osteoporotic fractures through self-reporting and subsequent

**Figure 1** Three networks enriched for known osteoporosis susceptibility genes. The 40 known osteoporosis genes were evaluated for network analysis using Ingenuity Pathway Analysis software (Version 8.8, Ingenuity Systems Inc, Redwood City, CA). Three networks showed significant enrichment for osteoporosis susceptibility genes (highlighted in gray), including A) a network related to connective tissue development and function, skeletal and muscular system development and function, and tissue morphology, B) a network related to drug metabolism, lipid metabolism, and small molecule biochemistry, and C) a network related to lipid metabolism, small molecule biochemistry, and cellular response to therapeutics.
verification through review of medical records. However, the accuracy of this approach has been questioned.\textsuperscript{65,66} This is particularly true of vertebral osteoporotic fractures for which multiple identification methods have been proposed, and for which the agreement between these different methods is rather poor.\textsuperscript{67,68} Jiang et al compared three methods, namely, qualitative visual assessment, semiquantitative assessment, and an algorithm-based qualitative method, for identifying vertebral osteoporotic fractures.\textsuperscript{67} Of women with vertebral fractures identified by semiquantitative assessment, 53% and 70% were categorized as negative for fracture by the algorithm-based qualitative method and qualitative visual assessment, respectively.\textsuperscript{67} The discrepancy is mainly due to difficulties in differentiating true fractures from nonfracture deformities or normal variants. In addition, osteoporotic fractures at different skeletal sites may have different underlying genetic mechanisms. Therefore, pooling vertebral osteoporotic fracture samples from studies which have used different ascertainment methods, or combining patients who had osteoporotic fractures at different skeletal sites (often referred to as broadly defined osteoporotic fractures), may dramatically increase phenotypic and/or genetic heterogeneity. This increased heterogeneity can in turn diminish rather than increase statistical power and generate false-negative and/or false-positive results.\textsuperscript{69,70} Furthermore, genotype imputation is commonly used for GWAS meta-analysis to accommodate differences in genotyping platforms used by different studies. However, inaccuracies in the imputed genotypes may also increase genetic heterogeneity, thereby increasing false-positive/negative results (unpublished data). Due to these substantial limitations, current GWAS meta-analyses should not be considered a “gold standard” for determining whether a gene does or does not contribute to osteoporosis.

It is also essential to recognize that association does not imply causation. Indeed, none of the currently identified single nucleotide polymorphisms can be definitively designated as the actual causal or functional variant driving genetic associations. It is more likely that they are just “markers” that are in linkage disequilibrium with true functional variants that have not yet been identified. Refined deep-sequenceing for the loci identified by GWAS, followed by cellular and molecular functional studies of selected variants, is required to identify and confirm the actual causal variants. Recent advances in next-generation sequencing technology\textsuperscript{71} combined with “systems genetics” or “systems biology” approaches,\textsuperscript{72,73} which apply sophisticated causality modeling and gene network analyses to integrate genomic, transcriptomic, proteomic, and metabolomic data, would represent powerful strategies for identifying and characterizing causal variants for osteoporotic fractures.

We expect continuous improvements in technology, study design, and statistical methods to greatly facilitate our ability to define the genetic architecture and mechanisms underlying osteoporosis. Furthermore, we expect the insights gained from these studies to be ultimately translated into improved methods for identifying individuals at risk for osteoporotic fractures, and new therapeutic interventions for preventing and treating these injuries.

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