Collaborative Meta-analysis: Associations of 150 Candidate Genes With Osteoporosis and Osteoporotic Fracture

Published in final edited form as:
Ann Intern Med. 2009 October 20; 151(8): 528–537.

Collaborative Meta-analysis: Associations of 150 Candidate Genes With Osteoporosis and Osteoporotic Fracture

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Abstract

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Background—Osteoporosis is a highly heritable trait. Many candidate genes have been proposed as being involved in regulating bone mineral density (BMD). Few of these findings have been replicated in independent studies.

Objective—To assess the relationship between BMD and fracture and all common single-nucleotide polymorphisms (SNPs) in previously proposed osteoporosis candidate genes.

Design—Large-scale meta-analysis of genome-wide association data.

Setting—5 international, multicenter, population-based studies.

Participants—Data on BMD were obtained from 19 195 participants (14 277 women) from 5 populations of European origin. Data on fracture were obtained from a prospective cohort (*n* = 5974) from the Netherlands.

Measurements—Systematic literature review using the Human Genome Epidemiology Navigator identified autosomal genes previously evaluated for association with osteoporosis. We explored the common SNPs arising from the haplotype map of the human genome (HapMap) across all these genes. BMD at the femoral neck and lumbar spine was measured by dual-energy x-ray absorptiometry. Fractures were defined as clinically apparent, site-specific, validated nonvertebral and vertebral low-energy fractures.

Results—150 candidate genes were identified and 36 016 SNPs in these loci were assessed. SNPs from 9 gene loci (*ESR1*, *LRP4*, *ITGA1*, *LRP5*, *SOST*, *SPP1*, *TNFRSF11A*, *TNFRSF11B*, and *TNFSF11*) were associated with BMD at either site. For most genes, no SNP was statistically significant. For statistically significant SNPs (*n* = 241), effect sizes ranged from 0.04 to 0.18 SD per allele. SNPs from the *LRP5*, *SOST*, *SPP1*, and *TNFRSF11A* loci were significantly associated with fracture risk; odds ratios ranged from 1.13 to 1.43 per allele. These effects on fracture were partially independent of BMD at *SPP1* and *SOST*.

Limitation—Only common polymorphisms in linkage disequilibrium with SNPs in HapMap could be assessed, and previously reported associations for SNPs in some candidate genes could not be excluded.

Conclusion—In this large-scale collaborative genome-wide meta-analysis, 9 of 150 candidate genes were associated with regulation of BMD, 4 of which also significantly affected risk for fracture. However, most candidate genes had no consistent association with BMD.

Primary Funding Source—European Union, Netherlands Organisation for Scientific Research, Research Institute for Diseases in the Elderly, Netherlands Genomics Initiative, Wellcome Trust, National Institutes of Health, deCODE Genetics, and Canadian Institutes of Health Research.

Osteoporosis and osteoporotic fractures are heritable (1–3), common, and costly. One third to one half of women of European descent experience an osteoporotic fracture in their lifetime (4,5), and the annual direct expenditure for osteoporotic fractures exceeds $17 billion in the United States (6). Morbidity and mortality associated with the disease will increase substantially as populations age (7).

Bone mineral density (BMD) remains the single most clinically useful risk factor for osteoporotic fracture and is the metric on which most therapeutic decisions are based (8). Substantial efforts have been dedicated to understanding whether variants in genes known to influence bone physiology also influence risk for low BMD and possibly fractures—called *candidate genes* (9). The candidate gene approach has relied on the assumption that if a gene is important for biological reasons, it may also affect the trait of clinical interest. Whether these genes do indeed influence propensity to osteoporosis and fracture has remained uncertain because many candidate gene studies lacked sufficient sample sizes and a replication group with which to validate findings (10). Moreover, replication efforts that evaluate 1 or a few

variants at a time are highly susceptible to fragmented, selective reporting of only the most promising results (11,12), and the selection of single or several variants in a gene does not provide information on whether variants elsewhere in the gene may influence the disease of interest. The notable exceptions to this is the set of variants from candidate genes tested in 18 000 to 45 000 participants by the GENOMOS (Genetic Markers for Osteoporosis) Consortium —*ESR1* (13), *COL1A1* (14), *VDR* (15), *TGFB1* (16), and *LRP5* and *LRP6* (17)—which systematically replicated these candidate genes.

Recent advances in microarray technology have facilitated genome-wide association studies, which test genetic variation across the human genome in thousands of individuals without a priori hypotheses. Through the use of dense genotyping, large study samples, and replication studies to confirm results, these studies have led to the discovery of many common genetic variants that have robust statistical evidence for association with various traits and diseases (18).

Genome-wide association studies have left the previous literature on candidate genes in a state of uncertainty (19) because they offer a means to reevaluate how many (if any) of the hundreds of previously proposed candidate gene associations are true. Specifically, genome-wide association data sets cover a large proportion of the common variation across the genome and can be used to systematically replicate previously proposed associations without selective reporting biases. Associations identified in these studies typically suggest a small effect size, an approach that becomes even more statistically efficient if large-scale genome-wide association data are pooled in meta-analyses of studies that use consistent phenotype definitions and analysis methods so that replication power is optimized (20,21). It would be important to know whether even a small proportion of previously identified candidate genes for any disease are valid because they may indicate which biological pathways translate into clinical outcomes, and they might be important for future risk prediction tools. For osteoporosis, it is important to further understand whether candidate genes are associated not only with BMD but also with fracture.

In this international collaborative meta-analysis of 19 195 men and women, we used genotyping arrays to systematically assess all common single-nucleotide polymorphisms (SNPs) assessed in the common haplotype map of the human genome (HapMap) CEPH (Centre d'étude du polymorphisme humain) data set in all previously published candidate genes for osteoporosis. We aimed to identify which candidate genes and common genetic variants near those genes influence osteoporosis and to understand whether the candidate genes that influence BMD also alter the risk for fracture.

Context

Although variations in 150 genes have been tested for their influence on bone mineral density (BMD), these studies have generally not been tested for replication in large studies that assess all common genetic variation across these genes.

Contribution

In this analysis of genome-wide association results from 5 large populations, variations in only 9 of 150 genes were associated with BMD, and variations in only 4 of these genes were associated with fracture.

Caution

The findings do not apply to non-European populations, and the effect sizes were very modest.

Implication

Most genes previously tested for their influence on risk for low BMD have no consistent effect on that risk.

—The Editors

Methods

See the **Appendix** (available at www.annals.org) for a glossary of genetic terms and an overview of analytic techniques.

Cohorts

We performed a meta-analysis of SNP-level genome-wide association results from 5 large cohort populations of European descent: the Rotterdam (the Netherlands) (22), Framingham Heart and Offspring (the United States) (23), deCODE (Iceland) (24), Erasmus Rucphen Family (ERF) (the Netherlands) (25), and Twins United Kingdom (TwinsUK) (26) studies. These cohorts were assembled to identify genetic risk factors in the development of complex disorders or to study aging-related diseases and chronic disabling conditions; all participants (23 016 total before exclusions based on genotyping quality control) were unselected for any trait or condition, and all studies collected cross-sectional data on lumbar spine and femoral neck BMD. Table 1 provides details on the cohorts; data on women and men were considered as separate data sets for inclusion in the meta-analysis.

Gene and SNP Selection

To identify studies of candidate genes and SNPs for this analysis, we searched the Human Genome Epidemiology (HuGE) Navigator, which provides a comprehensive, continuously updated archive of studies assessing the relationship between genetic variants and diseases published since 2000 (27). Very few genetic association studies were published before 2000, and it is unlikely that a gene proposed before that time would not have been studied again in at least 1 study since. We used the Phenopedia tool in the Navigator, which lists all studies associated with a particular phenotype, by using the search terms *osteoporosis* and *osteoporosis*, *postmenopausal* on 18 July 2008. During revision of our article, we updated the list of candidate gene studies for osteoporosis (between 18 July 2008 and 30 April 2009) by searching PubMed using the terms *osteoporosis* or *osteoporosis*, *postmenopausal* and *gene* or *genetic* or *candidate gene* in humans and found that 3 additional candidate genes (*CA8*, *CA10*, and *PBX1*) had been assessed for their association with BMD (28,29). None of the SNPs in or near these 3 additional candidate genes achieved a *P* value less than 0.001 in their association with BMD at the lumbar spine or femoral neck (**Appendix Table 1**, available at www.annals.org, lists all genes studied).

Using the second generation of HapMap data (a registry of all common human genetic variants) (30), we then identified all SNPs within 50 kilobase pairs downstream of the stop codons and upstream of the start codons of autosomal genes identified through the HuGE Navigator search. We used autosomal genes only because we could not accurately impute genetic information on sex chromosomes (31). The stop and start codons were identified by using the Ensembl Genome Browser (Ensembl, Cambridge, United Kingdom).

BMD Measurement

All cohorts had measured BMD at the lumbar spine (L1 to L4 or L2 to L4) and femoral neck by using standard manufacturer protocols on a dual-energy x-ray absorptiometry machine (Table 1). Measurements were performed as follows: in the Rotterdam Study, at baseline between 1991 and 1992 (22); in ERF, between 2002 and 2003 (25); in the Icelandic population,

at baseline (32); and in generations 1 and 2 of the Framingham Offspring Study, between 1992 and 1997 (33) and between 1996 and 2001 (34), respectively. In TwinsUK (26), all measurements were obtained from the most recent BMD data to better match the age distribution of the other cohorts.

Genotyping and Quality Control

Appendix Table 2 (available at www.annals.org) describes genotyping, imputation, and association testing in each cohort. Genotyping for the TwinsUK, Rotterdam, and deCODE studies has been described elsewhere (26,32). After we assessed all polymorphic SNPs identified in autosomal chromosomes from the HapMap CEPH phase II panel (release, build 36) and aligned all genotypes to the positive strand, we imputed missing genotypes by using the MACH (Center for Statistical Genetics, University of Michigan, Ann Arbor, Michigan) (35) or IMPUTE (Oxford University, Oxford, United Kingdom) (36) software programs. These programs implement hidden Markov model–based algorithms to impute missing genotypes (37). The imputation allows assaying of most of the common genetic variations (minor allele frequency >1%) in the genome and permits the sharing of data between cohorts that have used different gene array chips to genotype participants. Empirical evidence suggests that these 2 imputation algorithms tend to provide similar results (38,39).

Statistical Analysis of Single Studies (BMD)

Appendix Table 2 and the **Appendix** outline the details of genome-wide association testing and imputation. To lessen population stratification, individuals were excluded from the analyses if they demonstrated evidence of non-European ancestry by use of the following: STRUCTURE (Chicago) program (40) in the TwinsUK ($n = 20$) and deCODE ($n = 0$) studies, identity by state-clustering analysis in the Rotterdam Study ($n = 129$) (41), Gen-ABEL (Aulchenko Y, Struchalin M, Erasmus University Medical Center, Rotterdam, the Netherlands) (42) in the ERF study, and Eigenstrat principle component analysis (43) in the Framingham study. In the Framingham study, the first 4 principal components indicated the presence of population substructure and were statistically significantly associated with BMD. Thus, the first 4 principal components were included as covariates in the association tests between SNPs and BMD. Population stratification had little effect after removal of such individuals (44) (**Appendix Table 2**), and the associations between SNPs and BMD were corrected for the genomic inflation factors in each study.

For each SNP, a linear regression analysis, with the genotype as an additive covariate and standardized BMD (the phenotype) as the response variable, was fitted to test for association with lumbar spine BMD and femoral neck BMD separately in each cohort. The BMD was adjusted for age and weight in all studies (32) ($\text{BMD} = \beta_0 + \beta_1 \times \text{weight} + \beta_2 \times \text{age} + \beta_3 \times \text{age}^2$, where the age^2 term was included if age^2 was significant [$P < 0.05$]). Cohort-specific standardized residuals (where men and women were analyzed separately) with a mean of 0 and an SD of 1 were used to decrease between-cohort heterogeneity by allowing the additive effect size for each genetic variant to be expressed as a function of the number of SDs in BMD. These regression analyses were evaluated separately for lumbar spine and femoral neck BMDs. For all studies, an additive effect of the minor allele was assessed (that is, assuming that persons who have 2 copies of the minor allele have double the effect of those who have 1 copy).

Meta-analysis

We performed a meta-analysis of the additive effect of each allele on BMD (SNP-level effect size) first by using the METAL software package (Center for Statistical Genetics; www.sph.umich.edu/csg/abecasis/Metal/index.html), which performs an inverse-variance method of meta-analysis with fixed effects, by combining effect sizes and weighting them by their variance (standard error of the effect).

To refine this analysis further, all SNPs with a resultant *P* value of 0.001 or less were analyzed by using both fixed- and random-effects methods (45) by using Stata software (Stata, College Station, Texas) (46). These methods combine information on the effect sizes and SEs to arrive at summary effect sizes. In fixed-effects meta-analysis, the assumption is that the true genetic effect is the same for all combined populations and that differences in effect sizes are due to chance alone. Random-effects methods allow the true genetic effect to differ across populations, and the summary effect shows the average of these different effects across different populations (47). In the absence of observable between-study heterogeneity, fixed- and random-effects estimates coincide. Heterogeneity may indicate genuine differences in the genetic effect but may also be due to differences in sample collection or other reasons. Random effects do not indicate the exact source of heterogeneity; thus, they should also be interpreted cautiously. Between-study heterogeneity was evaluated by using the *Q* statistic and the I^2 metric. The *Q* statistic is considered statistically significant with a *P* value less than 0.10, and the I^2 metric shows the extent of heterogeneity that is beyond chance (values range from 0% to 100%). Given the relatively limited number of combined data sets, results for I^2 should also be interpreted cautiously because there is considerable uncertainty in the estimate (48). The results reported in this article are from the fixed-effects analysis unless stated otherwise.

For further information on power calculations and control for multiple testing, see the **Appendix**.

Association With Risk for Fracture

Only the Rotterdam Study had prospectively and systematically collected the data on fractures that were available for study. Fracture definitions have been provided elsewhere (49). Briefly, nonvertebral osteoporotic fractures ($n = 900$) were defined as incident site–specific (excluding fingers, hands, toes, face, ankle, and skull), arose from minimal trauma (such as falling from standing height), and were validated through medical records or radiograph verification (mean follow-up, 7.4 years [SD, 3.3]) from baseline through 31 December 2001. Over the course of our study, the overall dropout rate has been 22%. Vertebral fractures ($n = 329$) were defined by using thoracolumbar radiographs of the spine. The radiographs were scored for the presence of vertebral fractures by using the McCloskey–Kanis method (50).

Vertebral fractures were evaluated cross-sectionally by radiographic screening at the second follow-up (mean, 6.4 years after baseline). In patients with vertebral fractures, the baseline radiographs were assessed to determine whether the fracture was incident or prevalent. Because of this difference in ascertainment, vertebral and nonvertebral fractures were analyzed separately. Logistic regression analysis was performed with adjustment for sex, age, and weight, both with and without inclusion of BMD to test the relationship between SNPs and vertebral and nonvertebral fractures. Adjustments were made with femoral neck BMD for nonverterbral fractures and lumbar spine BMD for vertebral fractures. Only SNPs that were statistically significantly associated with BMD in both fixed- and random-effects analyses were tested for their relationship with fracture. We performed multiple-testing correction by dividing 0.05 by the number of independent SNPs associated with BMD at the lumbar spine or femoral neck arising from each gene. When a gene possessed SNPs that were associated with both lumbar spine and femoral neck BMDs, we chose the larger number of independent SNPs to control for multiple-testing correction of the association of SNPs with fracture.

Ethical Considerations

All studies were approved by the institutional ethics review committees at the relevant organizations, and all participants provided written informed consent.

Role of the Funding Source

All study investigators from Iceland, except G.S., are employees of deCODE Genetics. All other funding organizations had no role in the design and conduct of the study; data collection, study analysis, and management; interpretation of the data; preparation of the manuscript; or approval of the manuscript. This project was funded in part by the European Union Framework 7 Program (for the Genetic Factors for Osteoporosis project), Netherlands Organisation for Scientific Research, Research Institute for Diseases in the Elderly, Netherlands Genomics Initiative, the Wellcome Trust, the National Institutes of Health, deCODE Genetics, and the Canadian Institutes of Health Research. Funding information for the studies in the meta-analysis is included in the Grant Support section at the end of this article.

Results

Systematic Identification of Candidate Genes

A total of 150 genes had been investigated in at least 1 study for their relationship with osteoporosis in human studies in the HuGE Navigator. This literature included 680 articles. **Appendix Table 1** lists the 150 genes selected, and **Appendix Table 3** lists their putative functions (available at www.annals.org).

Of these genes, only 19 had been evaluated in more than 5 studies: *VDR*, *ESR1*, *COL1A1*, *IL6*, *LRP5*, *TNFRSF11B*, *TGFB1*, *ESR2*, *MTHFR*, *CASR*, *CYP19A1*, *TNF*, *BGLAP*, *APOE*, *CALCR*, *PTH*, *IL1B*, *IL1RN*, and *LEPR*. A total of 36 016 common SNPs were considered for analysis, representing all HapMap SNPs in the 150 genes and their immediate vicinity, as described in the Methods section.

Meta-analysis Database

Table 1 shows the baseline characteristics of the study participants (n = 19 195) from the 5 centers included in the meta-analysis.

Associations With BMD

Of the 36 016 evaluated SNPs, 745 were associated with lumbar spine or femoral neck BMD at a P value of 0.001 or less. We identified 241 SNPs from 9 genes (*SPP1* [osteopontin, or *OPN*]), *ITGA1*, *TNFRSF11B* (osteoprotegerin, or *OPG*), *LRP4*, *LRP5*, *TNFSF11* [*RANKL*], *SOST*, and *TNFRSF11A* [*RANK*] [Table 2]), which were associated with lumbar spine BMD (230 SNPs), femoral neck BMD (100 SNPs), or both (89 SNPs) at a statistical significance adjusted for multiple testing ($P < 2.39 \times 10^{-6}$) (Table 2 and **Appendix Tables 4** and **5,** available at www.annals.org, also list the functional location and amino acid change associated with each SNP). Random-effects calculations pinpointed the same 9 genes. All 9 genes had at least 1 SNP associated with lumbar spine BMD, whereas only 3 had at least 1 SNP also associated with femoral neck BMD (**Appendix Table 6,** available at www.annals.org, lists SNPs associated with both femoral neck and lumbar spine BMDs, with consistent direction of effect alleles). For the 6 genes that reached significance for BMD only at the lumbar spine, only 2 (*LRP5* and *SOST*) had SNPs with a P value of 0.001 or less for association at the femoral neck; the risk allele was the same for both skeletal sites. After men were excluded from the analysis, no additional genes that harbored statistically significant SNPs were identified (results not shown).

Heterogeneity

There was statistically significant heterogeneity between data sets for only 5 of the SNP associations (all at the *ESR1* gene locus). The estimated I^2 exceeded 25% for 71 and 50% for 4 associations at the lumbar spine; in contrast, 7 SNPs at the femoral neck had an estimated

I^2 exceeding 25%, and none exceeded 50%. No SNPs at the femoral neck displayed evidence of statistically significant heterogeneity. Point estimates, CIs, and *P* values were similar between fixed- and random-effects analyses (**Appendix Tables 4** and **5**).

Effect Sizes and Independent Information

The absolute effect size per allele ranged from 0.05 to 0.18 SD (Table 2), and most effect sizes were 0.05 to 0.08 SD (**Appendix Tables 4** and **5**). More than half of the statistically significant SNPs (115 by fixed effects, 100 by random effects) were variants in the *TNFRSF11B* gene (also called *osteoprotegerin*) (**Appendix Figure 1**, available at www.annals.org). These SNPs actually represent the equivalent of 8 independent SNPs because there was a high degree of linkage disequilibrium at this locus (**Appendix Figure 1**). Other statistically significantly associated gene loci represented only 1 to 3 independent SNPs (Table 2).

Previous Reports

As of the end of 2008, the 9 genes associated with BMD had been evaluated in a median of 3 previous studies (interquartile range, 2 to 21 studies) indexed in HuGENet Navigator. The median was only 1 for the other 138 genes (interquartile range, 1 to 2 studies) ($P = 0.002$, Mann–Whitney test). However, the most intensely studied gene locus, the *VDR* gene (51) (107 relevant studies indexed in HuGE Navigator by the end of 2008), had no SNP in this study that showed association after adjustment for multiple testing. The lowest uncorrected *P* value was 0.009, which is more than 1000-fold higher than the required significance threshold (**Appendix Figure 2**, available at www.annals.org). For example, the previously studied SNPs in *VDR*, Bsm1(rs1544410) (52), Cdx2(rs11568820) (53), and Taq1(rs731236) (52), were all assessed for their relationship with BMD but did not achieve a *P* value of 0.001 or less in the meta-analysis. Similarly, the extensively studied 677C → T polymorphism (rs1801133) in the *MTHFR* gene (53) did not achieve statistical significance. Because the Sp1 binding-site polymorphism in the *COL1A1* gene (rs1800012) is not recognized by HapMap and has no validated proxy in HapMap, it was not analyzed in this study. This means that the associations previously reported (14) can be neither confirmed nor excluded by the approach used here.

Association With Fracture Risk

Among the SNPs that were statistically significant with BMD by both fixed and random effects, 60 were also significantly associated with risk for fracture (**Appendix Table 7,** available at www.annals.org) at the nominal *P* value of 0.05 or less. These SNPs arose from 5 genes, *SOST, SPP1 (OPN), LRP5, TNFRSF11A (RANK),* and *TNFSF11* (*RANKL*), of which only the *SPP1* gene was associated with both vertebral and nonvertebral fractures. The effect of these SNPs on fracture ranged between an absolute odds ratio of 1.13 (95% CI, 1.01 to 1.27) and an odds ratio of 1.43 (CI, 1.16 to 1.77) for the allele that was associated with decreased BMD (Table 3). Although several SNPs from the *SPP1* and *SOST* loci influenced risk for fracture, these SNPs were in tight linkage disequilibrium and represented only 1 genetic signal. After accounting for the number of independent SNPs associated with BMD at each gene, *TNFSF11* did not remain statistically significant in its association with fracture; the other associations did.

Discussion

In this large collaborative study assessing the effect of common genetic variants (polymorphisms) in and near previously described candidate genes for BMD, we found that most SNPs at genes previously identified as associated with osteoporosis were not associated with the disease. We confirmed that variants at 9 genes (*ESR1, LRP4, ITGA1, LRP5, SOST, SPP1, TNFRSF11A, TNFRSF11B, and TNFSF11*) influence BMD and that variants at 4 of the genes (*SPP1, SOST, LRP5,* and *TNFRSF11A*) also influence the risk for fracture. Three of

these genes (*TNFRSF11A*, *TNFRSF11B*, and *TNFSF11)* reside in the same biological pathway, the *RANK/RANKL/osteoprotegerin* pathway, which influences bone resorption.

The *RANK/RANKL/osteoprotegerin* ligand pathway consists of *TNFRSF11B* (also called *osteoprotegerin*), *TN-FRSF11* (also called *RANKL*), and *TNFRSF11A* (*RANK*). Briefly, this pathway is central to bone physiology because RANKL is a ligand that interacts with the RANK receptor on osteoclast precursors, leading to the activation, differentiation, and fusion of cells of the osteoclast lineage, which promotes bone resorption (54). Osteoprotegerin acts as a dummy decoy in this pathway and binds to RANKL, thereby preventing its association with its natural receptor, RANK. Consequently, osteoprotegerin acts to prevent bone resorption (55). Previous association studies of these genes were mostly underpowered and inconsistent, whereas recent genome-wide association studies have identified this pathway as being among the most important determinants of BMD in the genome (24,26,32). Finally, the exploitation of this pathway has led to the design of a medication (denosumab) that mimics the action of *TNFRSF11B* and reduces the risk for osteoporotic fractures (56).

Our results highlight several loci that have recently been reported in genome-wide association studies as being associated with BMD (including *ESR1*, *TNFSF11*, *TNFRSF11A*, *TNFRSF11B*, *LRP4*, *LRP5*, and *SOST*) and fracture (*TNFSF11*, *TNFRSF11A*, and *LRP5*) (24,26,32). Of note, *ESR1* was identified as being associated with BMD in this study and previous genome-wide association studies (24,32), but a recent large-scale candidate gene study of 3 SNPs at this locus demonstrated associations with fracture but not BMD (13). Thus, *ESR1* remains an intriguing locus. When comparing the magnitude of effect sizes and *P* values between this study and previous genome-wide association studies, we note that although these estimates were of similar magnitude, this comparison is not entirely independent because data from these genome-wide association studies have been included in the current analysis. In addition, replicated candidate loci should be considered along with the additional novel loci from these genome-wide association studies (24,26,32) and their meta-analysis, which is the subject of a different report from our consortium (57).

We observed that many more SNPs were associated with lumbar spine BMD than with femoral neck BMD. These results are consistent with recent genome-wide association studies (24,26, 32) and may reflect biological differences between the sites, a lower heritability for the femoral neck site (58), or higher measurement error at this site. The SNPs associated with an increased risk for fracture were associated with BMD at the lumbar spine, but this finding is influenced by the smaller number of SNPs that were associated with BMD at the femoral neck.

The described SNP associations with BMD and fracture in this study are limited in their ability to improve predictive testing. This is primarily because the documented effect sizes for fracture associations reflect modest effects. Such effects are unlikely to be clinically informative when considered one at a time, but they may acquire greater importance for predictive purposes in combination, particularly if additional genetic variants that predict fracture can be identified (59). Some of these genes may have effects on fracture risk that are not mediated through BMD alone, but may entail other effects (pleiotropy), perhaps on diverse aspects of bone strength (for example, bone geometry, bone matrix, and other features of bone physiology). In our analyses, these additional effects were suggested by the observation that associations with fracture persisted even after adjustment for BMD. Therefore, associations with fracture risk for these variants could reflect effects that are mediated through an effect on BMD or various other pathways. The set of genetic variants influencing BMD and fracture risk are likely to have only partial overlap. In addition, the power to detect effects on vertebral fracture risk was limited by the relatively smaller number of vertebral fractures. Previous reports of candidate genes (13–17) have been better able to address this issue by using more fracture cases, such as

a recent examination of the effect of *LRP5* variants and bone traits involving more than 45 000 participants (17).

Our approach has several other limitations. This study was a thorough analysis of common SNPs assessed in the most recent version of HapMap in and near candidate genes, and we did not consider the effect of rare variants. Thus, it remains possible that rare variants have large effects in these genes or that common SNPs not assayed by HapMap also influence BMD. Interested readers can download the full list of SNPs evaluated in our study (www.gefos.org). As the number of validated SNPs grows with further sequencing efforts, future studies will be required to investigate whether these recently described common SNPs are associated with BMD. We have assessed genes on the autosomes because imputation techniques to assess X-chromosomal polymorphisms are still in development. We also could not rule out the possibility that low-frequency SNPs, even if present, had extremely weak effects; however, these SNPs would be less important clinically. Moreover, consistent with previous genome-wide association studies (60), the effect sizes associated with BMD in our study were generally small. The median effect size in Table 3 was 0.08 SD per risk allele. Generally, a 1-SD decrease in BMD has been associated with a doubling in the risk for osteoporotic fracture (61). Nevertheless, even small effects may indicate that a gene product is biologically relevant, even if its clinical significance is limited. We have assessed candidate genes that had been studied for BMD and thus did not examine candidate genes hypothesized to influence risk for fracture independent of BMD. Our analyses, which included elderly individuals, may be influenced by artifactual changes related to other abnormalities, such as osteophytes, particularly at the lumbar spine site. Moreover, we included body weight as a covariate in our analysis; it is possible that our study did not detect variants influencing BMD through body weight. As were other genome-wide association studies, our study was underpowered to assess for gene–gene interactions (20,62–65). Finally, we have assessed only individuals of European descent and cannot comment on the effect of these genes in populations of different ancestry.

In summary, our study provides direct evidence that most of the common SNPs in previously proposed candidate genes do not actually influence BMD. This finding may be common to other common complex diseases. Conversely, the 9 loci identified, which influence BMD and possibly fracture risk, may have potential clinical utility if medicines can be safely used to influence their function.

Acknowledgments

The authors thank Pascal Arp, Mila Jhamai, Dr. Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for their help in creating the Genome-Wide Association Studies (GWAS) database. They also thank the study participants, the staff from the Rotterdam Study, and the participating general practitioners and pharmacists. For genotyping of TwinsUK samples, the authors thank the staff from the Genotyping Facilities at the Wellcome Trust Sanger Institute for sample preparation, quality control, and genotyping; Centre National de Génotypage; Duke University; and Institute for Molecular Medicine Finland, Finnish Genome Center, University of Helsinki.

Grant Support: By the European Union (grant FP7-Health-F2-2008-201865-GEFOS), and in part by the European Union FP5 (grant HEALTH-LRP4-2008-201865), the Wellcome Trust, the National Institutes of Health, the Canadian Institutes of Health Research, deCODE Genetics, Netherlands Organisation for Scientific Research (NWO), Research Institute for Diseases in the Elderly, and Netherlands Genomics Initiative (NGI). For more details of the GEFOS Consortium, see www.gefos.org. The TwinsUK study was funded by the Wellcome Trust, European Commission Framework (FP7/2007-2013), ENGAGE project HEALTH-F4-2007-201413, and the FP5 GenomEUtwin Project (QLG2-CT-2002-01254). It also receives support from the Arthritis Research Campaign, Chronic Disease Research Foundation, the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's and St Thomas' NHS Foundation Trust in partnership with King's College London, and a Biotechnology and Biological Sciences Research Council project grant (G20234). Dr. Spector is an NIHR senior investigator. The Framingham Osteoporosis Study was funded by grants from the National Institute of Arthritis and Musculo-skeletal and Skin Diseases and the National Institute on Aging (R01 AR/AG 41398 [Dr. Kiel] and R01 AR 050066 [Dr. Karasik]). The Framingham Heart Study was supported by the National Heart, Lung, and Blood Institute (contract N01-HC-25195) and by Affymetrix (contracted for genotyping services; contract N02-HL-6-4278). Analyses reflect

intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource project. A portion of this research was conducted by using the Linux Cluster for Genetic Analysis, funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center. The generation and management of GWAS genotype data for the Rotterdam Study are supported by the NWO Investments (175.010.2005.011, 911-03-012). The Rotterdam Study is funded by the Research Institute for Diseases in the Elderly (014-93-015); NGI/NWO (project 050-060-810); NGI/Netherlands Consortium on Healthy Ageing; Erasmus Medical Center and Erasmus University; the Ministry of Education, Culture and Science; the Ministry for Health, Welfare and Sports; the European Commission (DG XII); and the Municipality of Rotterdam.

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Appendix

Potential Conflicts of Interest: *Employment:* U. Styrkársdóttir (deCODE Genetics), B.V. Halldórsson (deCODE Genetics), A. Kong (deCODE Genetics), U. Thorsteinsdottir (deCODE Genetics), G. Thorleifsson (deCODE Genetics), K. Stefansson (deCODE Genetics). *Consultancies:* S.H. Ralston (Novartis, Procter & Gamble, Merck). *Stock ownership or options (other than mutual funds):* U. Styrkársdóttir (deCODE Genetics), B.V. Halldórsson (deCODE Genetics), A. Kong (deCODE Genetics), U. Thorsteinsdottir (deCODE Genetics), G. Thorleifsson (deCODE Genetics), K. Stefansson (deCODE Genetics). *Grants received:* U. Styrkársdóttir (GEFOS Consortium), B.V. Halldórsson (GEFOS Consortium), U. Thorsteinsdottir (GEFOS Consortium), S.H. Ralston (Wyeth, Novartis), K. Stefansson (GEFOS Consortium). *Patents received:* S.H. Ralston (*COL1A1* as a diagnostic marker).

Reproducible Research Statement: *Study protocol:* Not available. *Statistical code:* Available from Dr. Ioannidis (jioannid@cc.uoi.gr). *Data set:* Certain portions are available to approved individuals through written agreements with the GEFOS Consortium through Dr. Uitterlinden (a.g.uitterlinden@erasmusmc.nl).

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Table 1

Characteristics of Each Cohort Included in the Meta-analysis*

Study, Year (Reference)	Sex	Participants, *n*	Mean Age (SD), *y*	Mean Height (SD), *cm*	Mean Weight (SD), *kg*	Mean Lumbar Spine BMD (SD), *g/cm²*	Mean Femoral Neck BMD (SD), *g/cm²*	Data Collection Time Frame	Participant Description	Densitometer Used†
Rotterdam Study, 2007 (22)	Female Male	2861 2126	68.3 (8.2) 67.3 (7.5)	161.9 (6.4) 175.1 (6.7)	70.0 (10.9) 79.0 (10.6)	1.04 (0.18) 1.17 (0.20)	0.83 (0.13) 0.92 (0.14)	1989–present	Individuals age≥55 y from the Ommoord district of Rotterdam	GE-Lunar DPX-L
Erasmus Rucphen Family study, 2005 (25)	Female Male	740 488	49.9 (15.8) 51.1 (15.7)	161.5 (7.0) 174.0 (7.7)	68.3 (13.3) 83.5 (14.9)	1.12 (0.17) 1.17 (0.18)	0.90 (0.13) 0.96 (0.15)	2002–2005	22 families with ≥5 children baptized in community church between 1850 and 1900	GE-Lunar Prodigy
TwinsUK study, 2008 (26)	Female	2734	49.5 (13.2)	162.4 (6.1)	67.2 (12.8)	0.99 (0.14)	0.80 (0.13)	1993–present	Population- based study of British twins	Hologic QDR-4500W
deCODE study, 2009 (24)	Female Male	5934 809	59.5 (14.0) 65.2 (14.7)	164.2 (6.7) 176.7 (6.8)	70.5 (13.2) 83.7 (14.4)	0.95 (0.17) 1.03 (0.18)	0.70 (0.14) 0.77 (0.16)	1998–present	Inhabitants of Iceland	Hologic QDR-4500W
Framingham Study, 2007 (23)	Female Male	2008 1495	64.8 (11.5) 64.5 (10.9)	159.8 (6.8) 173.9 (7.0)	69.8 (14.9) 86.0 (14.9)	1.13 (0.21) 1.33 (0.21)	0.83 (0.16) 0.96 (0.14)	1971–present	Adult children of the Framingham Study participants and spouses	GE-Lunar DPX-L

BMD = bone mineral density.

*__Appendix Table 2__ (available at www.annals.org) contains additional information on quality control and inclusion and exclusion criteria for each study.

†GE-Lunar: General Electric, Madison, Wisconsin; Hologic QDR-4500W: Hologic, Waltham, Massachusetts.

Ann Intern Med. Author manuscript; available in PMC 2010 March 22.

Table 2

Summary Information for Statistically Significant Genes for Bone Mineral Density*

Variable	*SPP1* (*OPN*)	*ITGA1*	*ESR1*	*TNFRSF11B* (*OPG*)	*LRP4*	*LRP5*	*TNFSF11* (*RANKL*)	*SOST*	*TNFRSF11A* (*RANK*)
Total SNPs tested, *n*	244	609	619	349	82	161	258	138	346
Lumbar spine									
Significant SNPs, *n*†	14 (14)	1 (1)	36 (2)	115 (100)	1 (1)	39 (14)	11 (8)	11 (10)	2 (1)
Estimated independent SNPs, *n*	2	1	2	8	1	3	2	2	1
Median effect size‡	0.06	0.07	0.08	0.09	0.07	0.07	0.07	0.06	0.08
Maximum effect size‡	0.08	0.07	0.08	0.09	0.07	0.09	0.18	0.06	0.08
Lowest *P* value§	6.0×10^{-8}	9.6×10^{-7}	6.1×10^{-11}	3.5×10^{-16}	1.8×10^{-6}	4.7×10^{-8}	1.9×10^{-11}	1.0×10^{-7}	9.4×10^{-9}
Femoral neck									
Significant SNPs, *n*†	0 (0)	0 (0)	18 (19)	70 (70)	11 (11)	0 (0)	0 (0)	0 (0)	0 (0)
Estimated independent SNPs, *n*	–	–	3	4	2	–	–	–	–
Median effect size‡	–	–	0.06	0.06	0.07	–	–	–	–
Maximum effect size‡	–	–	0.07	0.07	0.07	–	–	–	–
Lowest *P* value§	–	–	3.0×10^{-8}	7.1×10^{-9}	4.0×10^{-9}	–	–	–	–

OPG = osteoprotegerin; *OPN* = osteopontin; *RANK* = receptor activator for nuclear factor-κB; *RANKL* = receptor activator for nuclear factor-κB ligand; SNP = single-nucleotide polymorphism.

*Common gene names are in parentheses.

†Data are from the fixed-effects calculations; random-effects calculations are in parentheses. The other data in the table are similar between fixed- and random-effects models (**Appendix Tables 4 and 5**, available at www.annals.org).

‡The effect size is the change in SDs of bone mineral density associated with the protective allele. A change in bone mineral density by 1 SD is often associated with a 2-fold increase in risk for fracture (61).

§The *P* value closest to 0 among SNPs across each gene, for their association with bone mineral density, in the fixed-effects meta-analysis. For the *LRP4* (11p11.2) locus, the most significant association (rs2070852[G]; $P = 4.0 = 10^{-9}$; effect size, 0.07 SD [95% CI, 0.05 to 0.10 SD]) with the femoral neck site reflects an SNP that lies within the *F2* gene. This locus contains many genes in the same linkage disequilibrium block, including the *LRP4, F2, ZNF408, ARHGAP1,* and *CKAP5* genes.

Table 3

Summary Information for Gene Loci Associated With Risk for Fracture

Variable	*SPP1*	*SOST*	*LRP5*	*TNFRSF11A*	*TNFSF11*
Nonvertebral fracture (*n* = 900)					
Statistically significant SNPs at $P \leq 0.05$, *n*	11	10	22	0	0
Estimated independent SNPs, *n*	1	1	1	–	–
Absolute median OR	1.13	1.16	1.15	–	–
Absolute maximum OR (95% CI)	1.13 (1.01–1.27)	1.18 (1.05–1.32)	1.16 (1.01–1.33)	–	–
Lowest *P* value*	0.04	0.005	0.01	–	–
SNPs remaining significant after adjustment for BMD, *n*	0	10	19	–	–
P value threshold, accounting for independent SNPs within gene†	0.025	0.025	0.017	–	–
Vertebral fracture (*n* = 329)					
Statistically significant SNPs at $P \leq 0.05$, *n*	14	0	0	1	2
Estimated independent SNPs, *n*	1	–	–	1	1
Absolute median OR	1.33	–	–	1.23	1.19
Absolute maximum OR (95% CI)	1.43 (1.16–1.77)	–	–	1.23 (1.04–1.47)	1.19 (1.01–1.41)
Lowest *P* value*	0.007	–	–	0.02	0.04
SNPs remaining significant after adjustment for BMD, *n*	14	–	–	0	0
P value threshold, accounting for independent SNPs within gene†	0.025	–	–	0.05	0.025

BMD = bone mineral density; OR = odds ratio; SNP = single-nucleotide polymorphism.

**P* value for the association with risk for fracture closest to 0 among SNPs across each gene.

†0.05 divided by the number of independent SNPs found in the gene to be associated with BMD at the lumbar spine or femoral neck.