Calcif Tissue Int (2008) 83:155–166
DOI 10.1007/s00223-008-9165-y

CLINICAL INVESTIGATIONS

Genetic Variation in Candidate Osteoporosis Genes, Bone Mineral Density, and Fracture Risk: The Study of Osteoporotic Fractures

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Received: 15 May 2008 / Accepted: 19 July 2008 / Published online: 12 September 2008

Abstract Candidate osteoporosis gene variants were examined for associations with fracture risk and bone mineral density (BMD). A total of 9704 white women were recruited at four U.S. clinical centers and enrolled into the Study of Osteoporotic Fractures, a longitudinal cohort study. Genotyping of 31 polymorphisms from 18 candidate osteoporosis genes was performed in 6752 women. Incident radiographic fractures were identified at the third and eighth examinations compared with the baseline examination. BMD was measured at the total hip by dual-energy X-ray absorptiometry. Analyses were adjusted for age, clinic site, and self-reported ethnicity. During a mean follow-up of 14.5 years, a total of 849 hip, 658 vertebral, and 2496 non-hip/nonvertebral fractures occurred in 6752 women. Women carrying the ALOX15_G48924T T/T genotype had a higher rate of hip fracture (hazard ratio [HR] = 1.33;95% confidence interval [95% CI] = 1.00–1.77) compared with the G/G genotype. Compared with those carrying the PRL_T228C T/T genotype, women with either the C/C (HR = 0.80; 95% CI = 0.67–0.95) or C/T (HR = 0.81; 95% CI = 0.68–0.97)

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genotype had a lower rate of nonvertebral/nonhip fractures. Women carrying the BMP2_A125611G G/G genotype had a higher rate of vertebral fracture (odds ratio [OR] = 1.51; 95% CI = 1.03–2.23) compared with the A/A genotype. Women with the ESR1_C1335G G/G genotype had a higher rate of vertebral fracture (OR = 1.64; 95% CI = 1.07–2.50) compared with the C/C genotype. Compared with those with the MMP2_C595T C/C genotype, women with the C/T (OR = 0.79; 95% CI = 0.65–0.96) or T/T (OR = 0.44; 95% CI = 0.27–0.72) genotype had a lower rate of vertebral fracture. In conclusion, polymorphisms in several candidate genes were associated with hip, vertebral, and nonhip/nonvertebral fractures but not with total hip BMD in this large population based cohort study.

Keywords Genetics · Polymorphism · Osteoporosis · BMD · Fracture

Osteoporosis is a disorder characterized by low bone mass and increased risk of fracture. Many factors influence the risk of osteoporosis, including diet, physical activity, medication use, coexisting diseases, and a family history of the disorder. Research into the genetic basis of osteoporosis has been motivated by evidence that bone traits tend to be highly heritable. Whereas genetic factors explain 50% to 80% [1–4] of the biological variation in bone density and other bone phenotypes, these factors are not sufficient to explain fracture risk [5]. Deng et al. [5] report that the genetic correlation between bone density and fracture is not significant and that most genes found to be relevant to bone density may not be important for hip fracture.

Osteoporosis, like other genetically complex diseases, is the product of multiple genetic and environmental factors, and their interactions [6, 7]. To date, several approaches have been attempted to identify osteoporosis-related genes, among them linkage analysis in families and candidate gene association analysis in populations and case–control collections. Genes hypothesized to play a role in osteoporosis include those involved in bone formation and remodeling (e.g., LRP5), those involved in hormone signaling (e.g., VDR and ESR1), and those that code for bone-structure proteins (e.g., COL1A1). However, many of the reported allelic associations with osteoporosis have not been replicated when tested in other cohorts. The inconsistent results may be due in part to the lack of statistical power to detect subtle genetic effects and the different approaches for identifying genes and gene variants as well as differences in study design. In addition, the preference for reporting positive associations and for underreporting of negative results may introduce bias in the genetic epidemiology literature [8].

DNA samples from individuals in the Study of Osteoporotic Fractures (SOF) cohort have been used to evaluate the relationship between allelic variants in several candidate genes and osteoporosis risk. SOF is a multicenter cohort study that was initiated in 1986 to determine risk factors for osteoporotic fractures in elderly women [9]. As a source of DNA samples for an osteoporosis genetic marker screen, SOF has many advantages. Of the approximately 7000 prospectively recruited white women who provided samples with adequate consent for participation in genetic studies, over 800 have had incident hip fractures, over 600 have had incident vertebral fractures, and virtually all had bone mineral density (BMD) measurements at the hip. As such, SOF is the largest and best-characterized U.S. cohort available for studying the genetics of osteoporosis in primarily white women of European ancestry. Associations between allelic variants within the VDR [10, 11], IL6 [12], TNFα [13], NOS3 [14], and OPG [15] genes and fracture risk or BMD have been reported in SOF. Here, we present the results of a genetic association study covering 31 polymorphisms in 18 candidate genes that was performed with the SOF cohort to identify genetic risk factors for osteoporosis.

Materials and Methods

Subjects

SOF is a longitudinal epidemiologic study of 9704 women aged 65–99 (mean 71.7, standard deviation 5.3) years recruited from four study centers located in Baltimore, Maryland; Minneapolis, Minnesota; Portland, Oregon; and the Monongahela Valley near Pittsburgh, Pennsylvania. The baseline SOF examinations were conducted from 1986 to 1988 [16]. SOF was originally designed to investigate risk factors for osteoporosis and osteoporotic fractures. Black women were initially excluded from SOF because of their low risk of fractures [17]. Also excluded were women with bilateral hip replacement or those unable to walk without assistance. All participants were community dwellers at baseline. Since then, follow-up examinations have taken place approximately every 2 years. The institutional review boards on human research approved the study at each institution, and all the women provided written informed consent.

Genotyping

Buffy coat or whole blood specimens were collected from a total of 6975 participants at either visit 2 (1989–1990) or visit 6 (1997–1998). In collaboration with Roche Molecular Sciences, the SOF DNA was purified, assayed, stored,

and cataloged. The present analysis was completed by using DNA extracted from either buffy coat or whole blood samples. Among the 6975 participants who provided samples, genotyping was performed in 6752 women who provided adequate consent for participation in genetic studies and had sufficient DNA available. Genotyping of the 31 polymorphisms in 18 genes (Table 1) was performed by Roche Molecular Systems (RMS) in Alameda, California, in 2004–2005. Candidate osteoporosis genes were selected at the time on the basis of the hypotheses that they were involved in bone formation and remodeling; that they were involved in hormone signaling; or that they encoded bone-structure proteins. We selected single nucleotide polymorphisms (SNPs) that were available at the time we initiated the project on the basis of potential function or previous publication in association studies of osteoporosis. Not all candidate SNPs were compatible with the genotyping platform, and we report results for those that were successfully genotyped.

The candidate gene polymorphisms were genotyped in the context of a multiplex polymerase chain reaction (PCR) amplification followed by allele-specific SNP detection with immobilized oligonucleotide probes in linear arrays, as previously described [18]. Primers were modified at the 5′ phosphate by conjugation with biotin. A total of 10 to 50 ng of purified human genomic DNA was amplified in a reaction volume of 50 μL with AmpliTaq Gold DNA polymerase with a GeneAmp PCR System 9600 thermal

Table 1 Candidate genes and polymorphisms

Gene	SNP position	RS no.	Gene name	OMIM accession no.
ALOX15	G48924T	rs7220870	ARACHIDONATE 15-LIPOXYGENASE	152392*
	G49010C	rs2664593		
	C51425T	rs11078528		
	A57901G	rs743646		
BMP2	C117863T	rs1980499	BONE MORPHOGENETIC PROTEIN 2	112261*
	A125611G	rs235764		
	G149529A	rs235739		
	C167584T	rs996544		
CALCR	C1654T	rs1801197	CALCITONIN RECEPTOR	114131*
CASR	C3403G	rs1801726	CALCIUM-SENSING RECEPTOR	601199*
CBFA1	A529 G[a]	NA	CORE BINDING FACTOR 1	600211*
COL1A1	G296T	rs1107946	COLLAGEN, TYPE I, ALPHA-1	120150*
	G2046T	rs1800012		
COMT	G1947A	rs4680	CATECHOL-O-METHYLTRANSFERASE	116790*
CYP1A1	A6570G	rs1048943	CYTOCHROME P450, SUBFAMILY I, POLYPEPTIDE 1	108330*
ESR1	T938C	rs2234693	ESTROGEN RECEPTOR 1	133430*
	A984G	rs9340799		
	C1335G	rs1801132		
FRZB1	A757G	rs9288087	FRIZZLED-RELATED PROTEIN	605083*
	G19524A	rs2242070		
	C26794G	rs7775		
GSTP	A2627G	rs1695	GLUTATHIONE S-TRANSFERASE, PI	134660*
LRP5	G1980A	rs2277268	LOW DENSITY LIPOPROTEIN RECEPTOR-RELATED PROTEIN 5	603506*
MMP1	135792 (−/G)	rs1799750	MATRIX METALLOPROTEINASE 1	120353*
MMP2	C595T	rs243865	MATRIX METALLOPROTEINASE 2	120360*
	A1829G	rs2287074		
MMP13	A326G	rs2252070	MATRIX METALLOPROTEINASE 13	600108*
MTHFR	C677T	rs1801133	5,10-METHYLENETETRAHYDROFOLATE REDUCTASE	607093*
PPAR	C34G	rs1801282	PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR, GAMMA	601487*
PRL	T228C	rs7739889	PROLACTIN	176760*
	G1627T	rs1341239		

NA, not available

[a] CBFA1_A529G probes target an A/G variant at base 929 in NM_004348.3

cycler (PE Biosystems, Foster City, CA) with the following cycling profile: an initial hold at 94°C for 7 minutes, then 33 two-step amplification cycles of 15 seconds at 95°C for denaturation and 60 seconds at 60°C for annealing/extension, and a final 5-minute product extension step at 68°C. Chromogenic detection of allelic variants following stringent hybridization of the biotinylated PCR products to the immobilized sequence-specific probes was performed on a Profiblot II T24 (Tecan, Research Triangle Park, NC). Roche Molecular Sciences in-house software was used to scan the linear arrays on an Epson Perfection 1670 scanner (Epson, Long Beach, CA) and to assign genotypes.

Fracture Ascertainment

Details of the method for identifying fractures have been previously published [19–21]. Briefly, participants were contacted every 4 months by postcard or telephone to ask whether they had experienced a fracture. More than 95% of these contracts were completed. All fractures are adjudicated by radiographic report, and a detailed description of the implementation and accuracy of SOF fracture data has been previously reported [22]. Fractures that occurred because of major trauma such as motor vehicle accidents were excluded. Vertebral fractures were defined by morphometry by lateral spine radiography collected at the first, third, and eighth clinic examinations. Incident radiographic fractures were identified on follow-up radiographs at the third and eighth examinations compared with the baseline examination. Sample sizes for incident vertebral fractures are lower than for other fractures as a result of deaths, terminations, and loss to follow-up between examinations 3 and 8. All nonvertebral and nonhip fractures were analyzed as a group, and hip and vertebral fractures were analyzed separately.

Bone Density Measurements

Hip BMD were measured by dual-energy X-ray absorptiometry (QDR 1000; Hologic, Bedford, MA) at the second visit. Details of these methods and quality control procedures have been reported elsewhere [23–25]. Details of the densitometry and quality control methods utilized in SOF have been outlined elsewhere [24, 26–28].

Statistical Analysis

Hardy-Weinberg equilibrium of the candidate gene polymorphisms was assessed by the χ^2 goodness-of-fit statistic. We analyzed BMD by genotype with analysis of variance. To determine the relationship between genotype and the incidence of hip and nonspine, nonhip fractures, we used Cox proportional hazard models to estimate hazard ratios (HRs) and 95% confidence intervals (95% CIs). Logistic regression was used to estimate odds ratios (ORs) and 95% CIs to determine the relationship between genotype and risk of vertebral fracture. The wild-type genotype served as the referent group in these analyses. All analyses were adjusted for age, clinic site, and self-reported ethnicity (northern, central, and southern European). The Bonferroni method was used to calculate the corrected P values for multiple testing. Statistical analysis was performed with the statistical software program SAS version 9.1 (SAS Institute, Cary, NC).

Results

HRs and ORs for the polymorphisms within selected candidate genes are listed in Table 2, and all genotypes were in Hardy-Weinberg equilibrium. Five SNPs showed a nominal association with some form of fracture, although no SNPs reached statistical significance after adjusting for multiple testing by the Bonferroni correction with $\alpha = 0.0004$. The CBFA1_A529G polymorphism was monomorphic for the A/A genotype in this population.

Hip Fracture

During a mean follow-up of 14.5 years, a total of 849 hip fractures occurred among the approximately 6600 women with no history of hip fracture. Women with the ALOX15_G48924T T/T genotype had a 33% higher rate of hip fracture, compared with women with the G/G genotype (HR = 1.33; 95% CI = 1.00–1.77).

Nonvertebral and Nonhip Fracture

During the same 14.5-year follow-up, a total of 2496 nonvertebral and nonhip fractures occurred among approximately 6000 women. Compared with women carrying the PRL_T228C T/T genotype, women with either the C/C genotype (HR = 0.80; 95% CI = 0.67–0.95) or the C/T genotype (HR = 0.81; 95% CI = 0.68–0.97) had an approximately 20% lower rate of nonvertebral and nonhip fractures.

Incident Radiographic Vertebral Fracture

A total of 658 incident vertebral fractures occurred among the approximately 2500 women who had undergone assessment at visits 3 or 8. Women with the BMP2_A125611G G/G genotype had a 51% higher rate of vertebral fracture, compared with women with the A/A genotype (OR = 1.51; 95% CI = 1.03–2.23). Women with the ESR1_C1335G G/G genotype had a 64% higher rate of vertebral fracture compared with women with the C/C

Table 2 Associations with fractures and total hip bone mineral density[a]

Gene/polymorphism		Hip fracture HR (95% CI)	Nonvertebral, nonhip fracture HR (95% CI)	Vertebral fracture OR (95% CI)	Total hip BMD (g/cm^2) Mean (95% CI)
Total no.		6575	6068	2475	6515
No. of fractures		849	2496	658	
ALOX15_G48924T	GG	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	GT	1.03 (0.9–1.19)	1.02 (0.93–1.11)	1.01 (0.83–1.24)	0.76 (0.76–0.77)
	TT	**1.33 (1.00–1.77)**	1.02 (0.84–1.23)	0.78 (0.49–1.25)	0.75 (0.74–0.77)
ALOX15_G49010C	GG	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	GC	1.06 (0.92–1.23)	1.00 (0.92–1.09)	0.91 (0.75–1.12)	0.76 (0.76–0.77)
	CC	1.25 (0.93–1.68)	0.98 (0.81–1.19)	0.72 (0.44–1.17)	0.75 (0.74–0.77)
ALOX15_C51425T	CC	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	CT	1.06 (0.92–1.22)	0.94 (0.86–1.02)	0.92 (0.75–1.13)	0.76 (0.76–0.76)
	TT	0.92 (0.70–1.19)	1.00 (0.86–1.16)	0.98 (0.69–1.39)	0.76 (0.75–0.77)
ALOX15_A57901G	AA	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	AG	1.10 (0.93–1.29)	1.01 (0.91–1.11)	0.93 (0.73–1.17)	0.76 (0.76–0.77)
	GG	0.62 (0.29–1.31)	0.93 (0.65–1.34)	0.62 (0.23–1.66)	0.76 (0.74–0.79)
BMP2_C117863T	CC	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.77)
	CT	0.84 (0.71–0.98)	0.98 (0.89–1.08)	1.05 (0.83–1.33)	0.76 (0.75–0.76)
	TT	0.78 (0.65–0.94)	0.96 (0.86–1.08)	0.91 (0.69–1.20)	0.76 (0.75–0.77)
BMP2_A125611G	AA	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.75 (0.74–0.76)
	AG	0.78 (0.60–1.00)	0.93 (0.80–1.08)	1.33 (0.90–1.97)	0.76 (0.76–0.77)
	GG	0.98 (0.77–1.26)	0.96 (0.83–1.12)	**1.51 (1.03–2.23)**	0.76 (0.75–0.76)
BMP2_G149529A	GG	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.77)
	GA	1.05 (0.84–1.31)	1.01 (0.88–1.15)	0.87 (0.63–1.19)	0.76 (0.75–0.76)
	AA	0.93 (0.74–1.17)	0.99 (0.86–1.13)	0.89 (0.65–1.23)	0.76 (0.75–0.76)
BMP2_C167584T	CC	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.76–0.76)
	CT	0.94 (0.77–1.15)	1.06 (0.94–1.19)	1.03 (0.77–1.37)	0.75 (0.75–0.76)
	TT	0.63 (0.20–1.96)	1.15 (0.68–1.95)	2.13 (0.75–6.02)	0.77 (0.73–0.82)
CALCR_C1654T	CC	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.77)
	CT	1.05 (0.78–1.42)	1.01 (0.85–1.21)	1.11 (0.73–1.68)	0.76 (0.75–0.76)
	TT	1.00 (0.75–1.33)	1.00 (0.84–1.19)	0.93 (0.62–1.40)	0.76 (0.76–0.76)
CASR_C3403G	CC	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.76–0.76)
	CG	0.85 (0.65–1.10)	0.97 (0.83–1.12)	1.12 (0.79–1.60)	0.76 (0.75–0.77)
	GG	0.31 (0.04–2.19)	1.53 (0.87–2.70)	1.38 (0.32–5.91)	0.75 (0.7–0.8)
COL1A1_G296T	GG	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.76–0.76)
	GT	0.91 (0.77–1.07)	0.98 (0.89–1.07)	1.07 (0.85–1.33)	0.76 (0.75–0.76)
	TT	0.98 (0.63–1.53)	0.90 (0.67–1.21)	1.56 (0.82–2.97)	0.76 (0.74–0.78)
COL1A1_G2046T	GG	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.76–0.76)
	GT	1.07 (0.93–1.24)	1.05 (0.96–1.14)	1.09 (0.89–1.34)	0.76 (0.75–0.76)
	TT	1.03 (0.71–1.51)	1.15 (0.93–1.42)	0.94 (0.56–1.58)	0.76 (0.74–0.77)
COMT_G1947A	GG	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.77)
	GA	1.26 (1.05–1.51)	1.00 (0.9–1.11)	1.18 (0.92–1.52)	0.76 (0.75–0.76)
	AA	1.17 (0.96–1.44)	0.98 (0.87–1.10)	1.09 (0.83–1.44)	0.76 (0.75–0.77)
CYP1A1_A6570G	AA	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.76–0.76)
	AG	0.91 (0.68–1.22)	1.02 (0.87–1.21)	0.97 (0.65–1.44)	0.75 (0.74–0.77)
	GG	1.40 (0.58–3.38)	0.76 (0.36–1.59)	1.42 (0.26–7.92)	0.79 (0.75–0.84)
ESR1_T938C	TT	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	TC	1.00 (0.85–1.17)	1.01 (0.92–1.11)	1.18 (0.94–1.48)	0.76 (0.76–0.76)
	CC	0.96 (0.79–1.17)	0.93 (0.83–1.05)	1.11 (0.84–1.46)	0.76 (0.75–0.77)
ESR1_A984G	AA	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	AG	1.14 (0.99–1.32)	0.93 (0.86–1.02)	1.21 (0.99–1.48)	0.76 (0.75–0.76)
	GG	1.01 (0.80–1.26)	0.91 (0.79–1.04)	1.10 (0.80–1.51)	0.76 (0.75–0.77)

Table 2 continued

Gene/polymorphism		Hip fracture HR (95% CI)	Nonvertebral, nonhip fracture HR (95% CI)	Vertebral fracture OR (95% CI)	Total hip BMD (g/cm^2) Mean (95% CI)
ESR1_C1335G	CC	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.76–0.76)
	CG	0.93 (0.81–1.08)	1.00 (0.91–1.09)	1.16 (0.95–1.42)	0.76 (0.75–0.76)
	GG	1.23 (0.91–1.64)	1.06 (0.88–1.28)	**1.64 (1.07–2.50)**	0.77 (0.76–0.79)
FRZB1_A–757G	AA	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.77)
	AG	0.89 (0.66–1.20)	0.92 (0.77–1.11)	0.74 (0.5–1.09)	0.76 (0.76–0.77)
	GG	0.94 (0.71–1.26)	1.01 (0.84–1.20)	0.70 (0.48–1.03)	0.76 (0.75–0.76)
FRZB1_G19524A	GG	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	GA	0.96 (0.83–1.11)	0.95 (0.87–1.03)	1.13 (0.92–1.38)	0.76 (0.76–0.77)
	AA	1.01 (0.76–1.35)	0.97 (0.82–1.16)	1.44 (0.99–2.09)	0.76 (0.75–0.77)
FRZB1_C26794G	CC	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.76–0.76)
	CG	0.95 (0.78–1.15)	0.96 (0.86–1.07)	0.79 (0.60–1.04)	0.76 (0.75–0.77)
	GG	1.05 (0.44–2.54)	0.98 (0.56–1.73)	1.78 (0.48–6.52)	0.74 (0.7–0.78)
GSTP_A2627G	AA	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	AG	0.92 (0.80–1.07)	0.96 (0.88–1.04)	0.96 (0.78–1.17)	0.76 (0.76–0.76)
	GG	0.89 (0.71–1.12)	1.04 (0.91–1.19)	1.05 (0.77–1.43)	0.76 (0.75–0.77)
LRP5_G1980A	GG	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.76–0.76)
	GA	1.05 (0.86–1.28)	1.07 (0.95–1.21)	1.17 (0.87–1.56)	0.76 (0.75–0.76)
	AA	0.69 (0.22–2.14)	0.98 (0.54–1.78)	2.04 (0.53–7.96)	0.76 (0.71–0.8)
MMP1_135792 (−/G)	1G/1G	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	1G/2G	0.94 (0.80–1.10)	0.99 (0.9–1.09)	1.02 (0.82–1.28)	0.76 (0.76–0.77)
	2G/2G	1.03 (0.85–1.25)	1.03 (0.92–1.15)	1.25 (0.96–1.64)	0.76 (0.75–0.76)
MMP2_C595T	CC	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	CT	0.98 (0.85–1.14)	1.00 (0.92–1.09)	**0.79 (0.65–0.96)**	0.76 (0.76–0.77)
	TT	1.20 (0.92–1.56)	0.89 (0.74–1.07)	**0.44 (0.27–0.72)**	0.76 (0.75–0.78)
MMP2_A1829G	AA	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.77)
	AG	0.92 (0.77–1.10)	1.02 (0.92–1.14)	1.21 (0.94–1.57)	0.76 (0.75–0.76)
	GG	0.93 (0.77–1.13)	1.04 (0.93–1.17)	1.24 (0.94–1.64)	0.76 (0.75–0.76)
MMP13_A326G	AA	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.76–0.77)
	AG	1.01 (0.87–1.17)	1.05 (0.96–1.14)	1.16 (0.95–1.41)	0.76 (0.75–0.76)
	GG	1.21 (0.97–1.50)	1.07 (0.93–1.22)	0.87 (0.62–1.22)	0.75 (0.74–0.76)
MTHFR_C677T	CC	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.76–0.77)
	CT	0.96 (0.83–1.11)	1.00 (0.92–1.09)	0.97 (0.80–1.19)	0.76 (0.75–0.76)
	TT	1.09 (0.88–1.36)	1.07 (0.94–1.22)	0.94 (0.68–1.29)	0.75 (0.75–0.76)
PPARγ_C34G	CC	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	CG	0.80 (0.67–0.95)	1.06 (0.97–1.17)	0.82 (0.66–1.04)	0.76 (0.76–0.77)
	GG	1.21 (0.75–1.97)	1.29 (0.97–1.73)	0.92 (0.44–1.92)	0.76 (0.73–0.78)
PRL_T228C	TT	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.75 (0.74–0.77)
	TC	0.85 (0.63–1.15)	**0.81 (0.68–0.97)**	0.74 (0.48–1.15)	0.76 (0.76–0.77)
	CC	0.87 (0.65–1.16)	**0.80 (0.67–0.95)**	0.78 (0.51–1.19)	0.76 (0.75–0.76)
PRL_G1627T	GG	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	0.76 (0.75–0.76)
	GT	0.89 (0.77–1.03)	1.03 (0.94–1.13)	0.84 (0.68–1.03)	0.76 (0.76–0.76)
	TT	0.99 (0.81–1.21)	1.10 (0.97–1.24)	1.10 (0.83–1.46)	0.76 (0.75–0.76)

BMD, bone mineral density; HR, hazard ratio; 95% CI, 98% confidence interval

[a] All analyses adjusted for age, clinic site, and self-reported ethnicity (northern, central, and southern European)

Bold values are statistically significant results

genotype (OR = 1.64; 95% CI = 1.07–2.50). Women with the MMP2_C595T C/T genotype had a 21% lower rate of vertebral fracture (OR = 0.79; 95% CI = 0.65–0.96) compared with women with the C/C genotype, while women with the T/T genotype had a 56% lower rate of vertebral fracture (OR = 0.44; 95% CI = 0.27–0.72).

From the approximately 6500 participants who had a total hip BMD measurement, there was no significant association between the previously unreported candidate genes and total hip BMD (Table 2). For example, the strongest association with BMD was found for the CYP1A1_A6570G polymorphism (Table 2). The CYP1A1_A6570G A/A and G/G genotypes were associated with mean BMD measures of 0.76 g/cm^2 (95% CI = 0.76–0.76 g/cm^2) and 0.79 g/cm^2 (95% CI = 0.75–0.84 g/cm^2), respectively.

Discussion

We analyzed 31 polymorphisms in 18 candidate genes within the SOF cohort to identify genetic risk factors for osteoporosis. It was of particular interest that women with the ALOX15_G48924T T/T genotype had a 33% higher rate of hip fracture in this study. This polymorphism was the only one with an allelic association with hip fracture in this study. ALOX15 and ALOX12 are contiguous genes located within the 17p13 region of the human genome, which contains a quantitative trait locus that affects BMD in the hip, spine [29], and wrist [30]. However, previous studies have found inconsistent associations between SNPs in ALOX15 and BMD or fracture data [31–34]. The ALOX15_G48924T SNP is located within the 5′ flanking region (−272 bp) of ALOX15. This polymorphism is of interest because a C-to-T substitution at ALOX15 position −292 was shown to create a novel transcription factor binding site for SPI1 [35]. SPI1 selectively binds to the −292 T allele, and transcription assays in primary human macrophages showed that −292 C/T heterozygous individuals expressed three times more ALOX15 mRNA than −292 C/C individuals [35]. Higher ALOX15 mRNA levels were also observed in monocytes from heterozygous −292 C/T carriers [36]. The ALOX15_G48924T SNP (G-272T) examined herein may be in linkage disequilibrium with the functional C-292T polymorphism, leading to differential ALOX15 expression and increased risk of fracture for the variant allele. Alternately, G-272T may itself be functional. Consistent with our findings in the female SOF cohort, there were significant associations between SNPs within the 5′ flanking region of ALOX15 and BMD in Japanese women in two different studies [32–34]. By contrast, two studies did not observe associations between 5′-flanking ALOX15 SNPs and BMD in Chinese women [33] or BMD and fracture in postmenopausal white women [31]. Taken together, the results of genetic association studies performed in this and two other female populations indicate that genetic variation within the 5′ promoter region of ALOX15 may contribute to osteoporosis-related traits. Measuring associations between 5′-flanking ALOX15 SNPs and BMD and fracture in emerging genome-wide association study data sets may help confirm these associations.

Prolactin is a peptide hormone that when present at high levels is associated with decreased levels of estrogen and testosterone. Prolactin may also have direct effects on osteoblast function and bone formation [37–39]. In this study, women carrying one or two copies of the PRL_T228C C allele had a ∼20% lower rate of nonvertebral and nonhip fractures. The function of the intronic T228C polymorphisms is currently unknown. High prolactin levels have been associated with osteopenia, decreased bone density, and increased osteoporosis risk, possibly as a result of a reduction in estrogen levels [40, 41]. In addition, long-term administration of raloxifene, which has been shown to decrease fracture risk in postmenopausal women with osteoporosis, decreases serum prolactin levels [42].

Bone morphogenetic protein 2 is a growth factor belonging to the transforming growth factor beta superfamily that plays a role in osteoblast differentiation. The gene for bone morphogenetic protein 2 (BMP2) was identified as an osteoporosis candidate locus by genome-wide linkage mapping in human populations [43]. To date, two BMP2 SNPs have been associated with fracture [43] and BMD [44]; however, the associations are not consistent [43, 45]. In the present study, women with the intronic BMP2_A125611G G/G genotype had a 51% higher risk of vertebral fracture. The function of the BMP2_A125611G polymorphism is unknown, and it is possible that this SNP is functional or in linkage disequilibrium with a functional variant.

MMP-2 is a determinant of bone remodeling and mineralization and plays a crucial role in forming and maintaining the osteocytic canalicular network [46]. Serum concentrations of MMP-2 have been related to markers of bone turnover including bone alkaline phosphatase, osteocalcin, and cross-linked N-telopeptides of type I collagen [47]. A previous study also found that serum MMP-2 levels may also increase with increasing bone turnover [48]. In the present study, women with one copy of the MMP2_C595T T allele (located in the 5′ promoter at position −1586) had a 21% lower adjusted rate of vertebral fracture, and women with two copies had a 56% lower adjusted rate.

Several studies have investigated the association between estrogen receptor alpha (ESR1) gene variants and osteoporosis [49–51]. In the present study, neither the *Pvu*II (rs2234693) nor the *Xba*I (rs9340799) polymorphisms were associated with hip or nonvertebral/nonhip fracture risk.

Women with the ESR1_C1335G G/G genotype had a 64% higher rate of vertebral fracture, compared with women with the C/C genotype. The ESR1_C1335G variant codes for a synonymous P325P substitution in exon 4. A previous study of late postmenopausal women found that mean femoral neck BMD, but not lumbar spine BMD, was significantly lower in the homozygous G/G women compared with the homozygous C/C women [52]. Another study found that after 6 months of treatment with raloxifene, subjects with C/C or C/G genotype of P325P mutation had significantly lower total cholesterol and low-density lipoprotein cholesterol concentrations, and higher decreases of total cholesterol when compared with those with the G/G genotype [53]. Our results and those of Jurada et al. [52] suggest that the codon 325 G/G genotype is associated with increased risk of vertebral fracture and lower femoral neck BMD.

There were no significant genetic associations with total hip BMD. The strongest association with BMD was found for the CYP1A1_A6570G polymorphism with a 4% increase in BMD for the G/G genotype compared with the A/A genotype. The inconsistency of genetic associations with fracture and BMD and across fracture types is consistent with studies in mice, which indicate that there are skeletal-site-specific genetic loci for bone mass and strength [54–57]. Previous findings have demonstrated that a wide array of skeletal phenotypes were polygenic with complex segregation patterns [57]. Beamer et al. [55] showed that several quantitative trait loci were responsible for both femoral and vertebral measures of BMD, whereas other quantitative trait loci were unique to femurs or vertebrae. Unique genetic factors contributing to trabecular and cortical bone mass have also been identified [54]. Another possibility is that the fracture findings are possibly spurious as a result of the multiple comparisons that were made.

Although this study found several positive genetic associations with osteoporotic outcomes, most of the investigated polymorphisms were not associated with fracture risk or BMD, and none of the previously unreported polymorphisms were consistently significantly associated with multiple fracture types or BMD sites. Previously reported associations between polymorphisms in COL1A1 [58–64], LRP5 [65–71], CASR [72], CALCR [73], and MTHFR [74–81] and BMD or fracture were not replicated in this study. Inconsistencies between this and previous studies may be due in part to differences in study size, specific SNPs assayed, sex-specific effects, ethnic background, and menopausal status, all of which influence genetic associations with BMD and fracture risk. Several previous studies [58–61, 66, 67, 72, 73, 77, 79] had small sample sizes (<300 participants), which may have led to spurious associations. For several genes, we examined different SNPs than those previously reported [63, 65–67, 73]. Two of the previous studies only found associations in men [65, 67] or premenopausal women [72]. Several studies were conducted on nonwhite participants [67, 68, 76, 79].

This study is limited in making conclusions regarding whether the examined genes play an important role in fracture risk or BMD because of the limited number of polymorphisms per gene studied in this population. Even though the selected polymorphisms based on prior investigation seemed to be promising, no single SNP can explain the variation of an entire gene. Although the SOF cohort is a well-characterized and appropriate cohort to use for osteoporosis-related studies, particularly within the population of elderly white women, results from a single population likely cannot be generalized to all possible populations. Finally, interactions between environmental factors and other genes may have obscured important subgroup associations with the candidate gene polymorphisms.

In the past decades, several approaches have been attempted to identify osteoporosis genes; however, the genes contributing to osteoporosis risk remain poorly defined. As with most complex diseases, it is generally assumed that many gene variants are responsible, with each contributing a subtle effect. Inconsistent results may be due to a lack of statistical power to detect the subtle effects of the responsible gene variants, a lack of standardized methods and approaches to identify the variants, or the selection of the wrong candidate genes. Recently the consortium approach to genetic studies, as exemplified for osteoporosis by the "genetic markers for osteoporosis" (GENOMOS) consortium [51, 64, 69, 82, 83], has remedied some of the most important pitfalls of candidate gene studies by standardizing phenotypes and genotypes, increasing sample sizes, improving power, and reducing false discovery rates. In addition, replication has become well established as the gold standard in genetic association studies to overcome problems with multiple testing and false-positive discoveries. The increasing use of genome-wide screening approaches, which exacerbate the discovery of false-positive findings, requires well-conducted replication studies in a variety of populations to confirm true novel genetic associations and increase generalizability of findings to more than one population. Making genotype data available from phenotypically well-characterized individual studies (such as those reported here) not only provides an opportunity for future confirmation of genome-wide association study results for specific genes, but also contributes to future meta-analyses. In addition, disclosure of negative as well as positive associations is essential to minimize the risk of publication bias. Ioannidis [8, 84] argues that the large majority of molecular epidemiology results should be null and that scientific journals should publish all studies with null results, provided study limitations are acknowledged. Rebbeck et al. [85] provide a framework for prioritizing the publication of reports that

are likely to provide more meaningful information about disease etiology.

Acknowledgments Supported by NIH grants AG05407, AR35582, AG05394, AR35584, AR35583, AR46238, AG005407, AG027576-22, AG005394-22A1, and AG027574-22A1. J. A. Cauley receives funding from Merck & Company, Eli Lily & Company, Pfizer Pharmaceuticals, and Novartis Pharmaceuticals. K. E. Ensrud is a federal employee of the Veterans Affairs Medical Center in Minneapolis, Minnesota, and has received research support from California Pacific Medical Center, who receives funding from Roche Molecular Systems. M. C. Hochberg acts as a consultant for Amgen. J. Li, B. Rhees, and H. Erlich are all employees of Roche Molecular Systems, which provided genotyping reagents and services for this study at no cost under a research collaboration. G. Peltz is a former employee of Roche Palo Alto. S. Cummings, L. Lui, and G. Tranah are employees of the California Pacific Medical Center and receive research support from Roche Molecular Systems.

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