Large-Scale Analysis of Association Between *LRP5* and *LRP6* Variants and Osteoporosis

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Context Mutations in the low-density lipoprotein receptor–related protein 5 (*LRP5*) gene cause rare syndromes characterized by altered bone mineral density (BMD). More common *LRP5* variants may affect osteoporosis risk in the general population.

Objective To generate large-scale evidence on whether 2 common variants of *LRP5* (Val667Met, Ala1330Val) and 1 variant of *LRP6* (Ile1062Val) are associated with BMD and fracture risk.

Design and Setting Prospective, multicenter, collaborative study of individual-level data on 37 534 individuals from 18 participating teams in Europe and North America. Data were collected between September 2004 and January 2007; analysis of the collected data was performed between February and May 2007. Bone mineral density was assessed by dual-energy x-ray absorptiometry. Fractures were identified via questionnaire, medical records, or radiographic documentation; incident fracture data were available for some cohorts, ascertained via routine surveillance methods, including radiographic examination for vertebral fractures.

Main Outcome Measures Bone mineral density of the lumbar spine and femoral neck; prevalence of all fractures and vertebral fractures.

Results The Met667 allele of *LRP5* was associated with reduced lumbar spine BMD (n=25 052 [number of participants with available data]; 20-mg/cm^2 lower BMD per Met667 allele copy; $P=3.3\times10^{-8}$), as was the Val1330 allele (n=24 812; 14-mg/cm^2 lower BMD per Val1330 copy; $P=2.6\times10^{-9}$). Similar effects were observed for femoral neck BMD, with a decrease of 11 mg/cm^2 ($P=3.8\times10^{-5}$) and 8 mg/cm^2 ($P=5.0\times10^{-6}$) for the Met667 and Val1330 alleles, respectively (n=25 193). Findings were consistent across studies for both *LRP5* alleles. Both alleles were associated with vertebral fractures (odds ratio [OR], 1.26; 95% confidence interval [CI], 1.08-1.47 for Met667 [2001 fractures among 20 488 individuals] and OR, 1.12; 95% CI, 1.01-1.24 for Val1330 [1988 fractures among 20 096 individuals]). Risk of all fractures was also increased with Met667 (OR, 1.14; 95% CI, 1.05-1.24 per allele [7876 fractures among 31 435 individuals)]) and Val1330 (OR, 1.06; 95% CI, 1.01-1.12 per allele [7802 fractures among 31 199 individuals]). Effects were similar when adjustments were made for age, weight, height, menopausal status, and use of hormone therapy. Fracture risks were partly attenuated by adjustment for BMD. Haplotype analysis indicated that Met667 and Val1330 variants both independently affected BMD. The *LRP6* Ile1062Val polymorphism was not associated with any osteoporosis phenotype. All aforementioned associations except that between Val1330 and all fractures and vertebral fractures remained significant after multiple-comparison adjustments.

Conclusions Common *LRP5* variants are consistently associated with BMD and fracture risk across different white populations. The magnitude of the effect is modest. *LRP5* may be the first gene to reach a genome-wide significance level (a conservative level of significance [herein, unadjusted $P<10^{-7}$] that accounts for the many possible comparisons in the human genome) for a phenotype related to osteoporosis.

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OSTEOPOROSIS IS DISTINguished by low bone mineral density (BMD), worsening bone microarchitecture, and increased risk for fractures. Heritability data show that genetic factors determine up to 80% of the variance in BMD,[1,2] which is a major predictor of osteoporotic fractures. While the genes that contribute to differences in risk for osteoporosis and os-

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teoporotic fractures are for the most part unknown, it is thought that the risk of developing osteoporosis is dependent on several common gene variants, each with modest effects.[3,4]

During recent years, variation in the gene coding for low-density lipoprotein receptor–related protein 5 (LRP5) has been implicated in bone mass accrual and susceptibility to osteoporosis. LRP5, and its closely related homologue, LRP6, function as cell-membrane coreceptors for Wnt proteins in the canonical Wnt signaling pathway.[5,6] Several lines of evidence suggest that LRP5 may be a key determinant of bone mass. Loss-of-function mutations in the *LRP5* gene cause osteoporosis-pseudoglioma syndrome,[6] characterized by severe osteoporosis and blindness. Conversely, activating point mutations in this same gene result in high bone mass.[7,8] Other *LRP5* missense mutations have been described in patients with bone mass disorders, including endosteal hyperostosis, osteopetrosis, and osteosclerosis.[9] Various mouse models have also replicated the bone phenotype of mutated *LRP5*.[6,10]

Common genetic variations in *LRP5* have been proposed as candidates for influencing bone phenotypes at the population level. Some reports have suggested that *LRP5* polymorphisms contribute to variation in BMD in the general population,[11-23] but results are inconclusive. This inconsistency can be explained in part by variations in the examined polymorphisms, the analytical approaches used, and the examined phenotypes. Data on fracture risk are limited, with only 2 reports published so far.[14,22] The most frequently studied polymorphisms in this gene are 2 amino acid substitutions (Val667Met and Ala1330Val),[13,15,17,18,21-25] and there is some additional in vitro evidence that the Ala1330Val variant results in a functional difference of the LRP5 protein.[25]

Mouse studies have shown that point mutations in the *LRP6* gene lead to a low bone mass phenotype.[26] While LRP6-deficient mice have early developmental problems that are not compatible with life, mice that carry (heterozygous) mutations in both *LRP5* and *LRP6* have decreased BMD and limb deformities, which indicates that LRP5 and LRP6 interact in limb development and BMD acquisition.[27] A recent report has identified an inherited mutation in *LRP6* to be linked to coronary heart disease but also to low-trauma fractures and low BMD.[28] In addition, a common protein variant of LRP6 (Ile1062Val) has been found to contribute to fracture risk in elderly men.[22] This same variant was recently shown to have functional consequences in vitro[29].

The objective of the current study was to examine the contribution of 2 common amino acid substitutions in the LRP5 protein and of 1 amino acid substitution in the LRP6 protein to BMD and risk of fracture using large-scale evidence.

Some scattered studies[12-23] have tested this association, but results have not been conclusive due to limited sample size. The current collaborative study has the potential to answer this question more definitively because of its large sample size and therefore large power to observe the expected modest associations. In addition, its prospective design, consistent genotyping, and combined analysis of individual-level data diminish bias and the respective noise and heterogeneity that bias might introduce in the outcomes.

We report here on the combined analysis of individual-level data from the full Genetic Markers for Osteoporosis (GENOMOS) consortium, including data from 37 534 individuals. GENOMOS collected standardized data and performed prospective genotyping for these polymorphisms across a large number of teams, only a few of which had previously addressed some of these specific polymorphisms.[22,23,25,30,31]

METHODS

Organizational Issues

The GENOMOS project is a large-scale study of candidate gene polymorphisms for osteoporosis outcomes.[32] This report includes the 12 study populations included in previous collaborative analyses of other gene polymorphisms.[32-34] The decision to study the *LRP5* and *LRP6* polymorphisms in the GENOMOS consortium occurred on June 6, 2004, when the consortium consisted of these 12 European populations.[35-44] At that time, results were available for 1 study (ERGO [Rotterdam, the Netherlands]), so all other populations were genotyped prospectively. During the course of this study, participants from 6 other teams (4 from Europe,[45-48] 1 from the United States,[25] and 1 from Canada[30]) joined the consortium; these teams genotyped all polymorphisms after they joined the consortium, except for FOS, which had already genotyped the 2 *LRP5* polymorphisms.

Participants are still being followed up for all cohorts with incident fracture data, with the exception of some teams in the EPOS multicenter study. The cutoff dates for fracture data in these cohorts are 2002 for APOSS, 2001-2004 for the EPOS centers, 2001 for ERGO, 2002 for LASA, and 2003 for UFO. Data were collected between September 2004 and January 2007, while analysis of the data occurred between February and May 2007. All studied individuals were white, and race/ethnicity was self-reported by study participants.

Details on the design of the 18 studies[25,30,35-40,42-52] are provided in **TABLE 1** and further details in **TABLE 2**, eTable 1, and eTable 2. Participants were unrelated in all studies except FAMOS, for which we selected 1 participant per pedigree using random-number selection. Participating teams contributed information on *LRP5* and *LRP6* genotypes, sex, age, height, weight, menopausal status, use of hormone therapy, activity and ability data (when available), BMD at lumbar spine and femoral neck (in mg/cm^2), and fractures. Bisphosphonate use was very rare and thus was not believed to warrant a separate analysis (although available data are reported herein). Smoking status and exercise were not collected in the same format across cohorts. Nevertheless, exercise and ability-adjusted estimates of effect in single studies were obtained whenever possible. The coding of smoking was heterogeneous; thus, as opposed to exercise and ability data, for which the scales were simply different, the smoking categories in each co-

hort may be overlapping or inconsistent. Therefore, it was believed that adjustment for smoking could not provide meaningful results (although available data are reported herein). For all analyses, participants with missing relevant data were excluded.

This study was approved by the institutional review boards of each local institution, and all individuals provided written informed consent to participate in clinical and genetic studies.

BMD Measurements

Bone mineral density was measured by dual-energy x-ray absorptiometry with different devices (Table 1). Measurements used the same reference device within each population. We interpreted results of the analysis of individual-level data for BMD by comparing within-population absolute differences in the mean values of BMD across genotypes. We do not focus on absolute BMD values, because these values may depend on the measuring device.

Fracture Assessment

Fractures were identified either by questionnaire, medical records, or radiographic documentation. Details of fracture assessment and exclusion of age at fracture, fracture type, and trauma type for each cohort are given in Table 2. Longitudinal studies also had data available on incident fractures that had occurred during the follow-up period. Information on incident vertebral fractures included in the analysis was collected with routine surveillance methods using radiographic examination.

Genotyping

We genotyped *LRP5* Val667Met (dbSNP [http://www.ncbi.nlm.nih.gov/projects/SNP/] ID rs4988321), *LRP5* Ala1330Val (rs3736228), and *LRP6* Ile1062Val (rs2302685) single-nucleotide polymorphisms (SNPs) prospectively. These 3 SNPs were the only ones examined in this study. *LRP5* Val667Met (rs4988321), *LRP5* Ala1330Val (rs3736228), and *LRP6* Ile1062Val (rs2302685) polymorphisms were assessed by Taqman, except for the AOS study, for which fluorescence polarization was used for assessment of the *LRP5* Val667Met and *LRP6* Ile1062Val polymorphisms. We cross-validated genotypes from different laboratories by blinded genotyping of 50 reference samples by all genotyping teams. The coordinating team in Rotterdam evaluated the results and reported any discrepancies in the reference samples in general terms to improve calling of genotypes by failing teams. We repeated genotyping of the reference samples, and teams had to switch genotyping techniques if they were still generating more than 5% errors in the reference samples. In addition, each team checked its own cohort genotyping afterward by reanalyzing at least 5% of their samples selected at random. Genotyp-

Table 1. General Study Characteristics Among the 18 Participating GENOMOS Teams

Team	Country of Origin	No.	Participation Rate, %[a]	Recruitment		Genotyping Date
				Source	Date	
Cohort Studies						
APOSS[38]	Scotland	3886	67	General population	1991-1992	2006
DOPS[39]	Denmark	2016	58	General population	1990-1993	2006
EPOLOS[49]	Poland	736	16	General population	1999-2001	2005
EPOS[42]	European	3510	NA[b]	General population	1990-1999	2005
ERGO[50]	Netherlands	7983	78	General population	1990-1993	2003
FOS[25]	United States	2188	71[c]	General Population	1996-2001	2003-2006
GEOS[30]	Canada	1909	NA[d]	General population	1996-2001	2005
GOOD[46]	Sweden	1068	49	General population	2003-2007	2005-2006
LASA[43]	Netherlands	1513	82	General population	1992-1993	2005
MrOs-Sweden[47]	Sweden	3014	48	General population	2004	2005-2006
UFO[45]	Sweden	2066	60	General population	1986-2003	2006
Cross-sectional Studies						
AOS[48]	Denmark	783	27	General population	2002-2003	2004-2006
AUSTRIOS-A[36,40]	Austria	755	80	General population	2003	2005
AUSTRIOS-B[51]	Austria	1124	80	Nursing home patients	1998	2005
BARCOS[35]	Spain	876	ND	Patients	1997-2004	2006
FAMOS[44]	European	562	NA	Family study with low BMD	1999-2001	2005
FLOS[52]	Italy	2800	ND	Hospital patients	1994-2005	2005
Case-Control Study						
AROS[37]	Denmark	745	ND	Cases: hospital; Controls: general population	1995-2004	2006

Abbreviations: BMD, bone mineral density; GENOMOS, Genetic Markers for Osteoporosis; NA, not applicable; ND, no data.
[a] Calculated as No. participants/No. eligible for the study.
[b] Study was performed by multiple teams, with varying participation rate.
[c] Participation rate is for the Framingham offspring cohort for the children who had 2 parents in the original Framingham cohort.
[d] Study was advertised; interested women decided to participate.

ing was performed after all prospective radiographic measurements had been performed and had been entered into the databases, so assessment of whether or not a fracture existed would not have been affected by knowledge of genotype.

Outcomes

The main outcomes included BMD of the lumbar spine and femoral neck; all prevalent fractures; and prevalent vertebral fractures by clinical or morphometric criteria.[53] We also conducted sensitivity analyses for incident fractures; incident vertebral fractures; and low- and no-trauma fractures. The latter exclude fractures occurring with high trauma, as assessed by the circumstances in which they had occurred, their location, or both. Information on high- and low-trauma fractures was available for 6 of the 18 studies.

Analyses

Hardy-Weinberg Equilibrium and Haplotype Reconstruction. We performed exact tests for Hardy-Weinberg equilibrium proportions[54] using GENEPOP version 4.0.[55] We reconstructed haplotypes of the 2 *LRP5* polymorphisms using PHASE version 2.0.[56]

Evaluation of Genetic Effects. All analyses were stratified per study and sex (29 study-sex population strata). For single-SNP analyses we obtained summary estimates using inverse-variance random-effects meta-analysis. For haplotype-based analyses we used mixed models, as described below.

Table 2. Assessment Methods and Exclusion Criteria Among the 18 Participating GENOMOS Teams

	Assessment Method				Exclusion Criteria		
		Fracture					
Team	BMD[a]	All	Vertebral	Incident Vertebral	Age at Fracture, y	Fracture Type	Trauma Type
Cohort Studies							
APOSS[38]	Norland	Questionnaire	Questionnaire	ND	<18	NA	NA
DOPS[39]	Hologic	Radiographic documentation	Radiographic documentation	ND	NA	Hands, skull, fingers, feet, clavicle	NA
EPOLOS[49]	Various[b]	Questionnaire; medical records	Questionnaire; radiographic documentation	ND	<18	Fingers, toes, feet, hand, clavicle, skull	High
EPOS[42]	Various[b]	Questionnaire; medical records	Radiographic documentation	Radiographic documentation	<20	NA	High
ERGO[50]	Lunar	Medical records; radiographic documentation	Radiographic documentation	Radiographic documentation	<55	NA	NA
FOS[25]	Lunar	Questionnaire	ND	ND	<30	NA	NA
GEOS[30]	Lunar	Questionnaire	Questionnaire	ND	NA	NA	NA
GOOD[46]	Lunar	Questionnaire	ND	ND	NA	NA	NA
LASA[43]	Hologic	Questionnaire; medical records	Radiographic documentation	Radiographic documentation	NA	NA	NA
MrOs-Sweden[47]	Hologic	Questionnaire	NA	ND	<50	NA	NA
UFO[45]	Lunar	Radiographic documentation	ND	ND	<50	All fractures except wrist and hip	High
Cross-sectional Studies							
AOS[48]	Hologic	ND	ND	ND	NA	NA	NA
AUSTRIOS-A[36,40]	Hologic	Questionnaire; medical records	Radiographic documentation	ND	<20	Fingers, face, skull, clavicle	NA
AUSTRIOS-B[51]	NA	Questionnaire; medical records	Radiographic documentation	ND	<20	Fingers, face, skull, clavicle	NA
BARCOS[35]	Hologic	Medical records; radiographic documentation	Radiographic documentation	ND	<45	Hands, face, skull, fingers, feet	High
FAMOS[44]	Various[b]	Questionnaire	Radiographic documentation	ND	NA	NA	NA
FLOS[52]	Hologic	Medical records; radiographic documentation	Radiographic documentation	ND	NA	NA	High
Case-Control Study							
AROS[37]	Hologic	Radiographic documentation	Radiographic documentation	ND	NA	NA	High

Abbreviations: BMD, bone mineral density; GENOMOS, Genetic Markers for Osteoporosis; NA, not applicable; ND, no data.
[a] Dual-energy x-ray absorptiometry devices used for measurement.
[b] Various methods used with European spine phantom calibration.

Inverse-Variance Random-Effects Analyses of Individual-Level Data (Single-SNP–Based Analyses). This is a 2-step approach. Separate regression models were performed in each study-sex population stratum (genetic information was coded using dummy variables, depending on the genetic model assessed). We calculated summary genetic effect as the weighted average of regression coefficients across the different strata using the DerSimonian and Laird random-effects method.[57] This method allows for between-strata heterogeneity (dissimilarity) and incorporates it in the calculations. We tested for heterogeneity using the Cochran *Q* statistic (traditionally considered statistically significant at $P < .10$)[58] and quantified its extent using the I^2 statistic (large heterogeneity for values ≥50%).[59] Results of single-SNP–based analyses with mixed models were identical and thus not shown.

Mixed Models (Haplotype-Based Analyses). Linear mixed models were used for continuous outcomes (ie, BMD measurements), and the corresponding generalized linear mixed models were used for binary outcomes (eg, fractures). Population stratum was treated as a random factor and genetic information (haplotypes) as fixed. All models were fitted using maximum likelihood. We relied on a likelihood ratio test to assess whether a model taking into account the genetic information provided better fit (ie, explained data better) than a similar model without the genetic information (eg, a constant-only model).

Choice of Genetic Model and Adjustments. Since there is no strong evidence in favor of a specific genetic model, main analyses used allele-based contrasts. Additional analyses assumed a dominant model for continuous as well as binary outcomes and a "model-free" approach that considers the 3 genotypes as independent factors. For analysis of incident fractures, the binary variable "fracture: yes/no" was used, and odds ratios (ORs) were calculated and translated into risk ratios (RRs) as described below. There would be no rationale for longitudinal time-to-event analyses (eg, a vertebral fracture identified on a radiograph may have occurred at any point in the period between enrollment and follow-up radiography).

The main analyses were unadjusted for other variables. We also performed secondary adjusted analyses by accounting for age, weight, and height (as continuous variables) in the models. Whenever statistically significant genetic effects were identified, additional adjustments for postmenopausal status and use of hormone therapy among women were undertaken. Fracture-risk analyses were also adjusted for BMD (lumbar spine BMD or femoral neck BMD in separate analyses). The proportion of the fracture risk explained by BMD was calculated from the regression coefficients as $(\beta_{unadjusted} - \beta_{adjusted})/\beta_{unadjusted}$. In additional analyses, we tested for interactions of the 2 *LRP5* SNPs with age (among all individuals) and postmenopausal status (among women).

Effects at the Population Level. For an indicative population-level estimate, the per-allele OR for the significant associations with fractures and vertebral fractures was also converted into an RR[60] considering the median fracture prevalence across the included cohort studies. We calculated the population-attributable fraction using allele frequencies from the median cohort study.

Adjustments for Multiple Comparisons

Adjustment for multiple comparisons is generally not favored for hypothesis-validating studies as opposed to discovery studies. Nevertheless, we have illustratively also adjusted the main estimates for the main analyses for 3 polymorphisms × 4 main outcomes (lumbar spine BMD, femoral neck BMD, all fractures, and vertebral fractures) using the Boole-Bonferroni inequality.[61] We emphasize that because the 4 main outcomes are correlated and 2 of 3 polymorphisms are also in linkage disequilibrium, Bonferroni adjustments are overly conservative. Conventional statistical significance is claimed for $P < .05$ adjusted for multiple comparisons. Genome-wide significance is claimed for unadjusted $P < 10^{-7}$.[62-64] Genome-wide significance accounts for the very large number of polymorphisms and associations thereof that can be tested across the human genome, regardless of whether all or some of them are tested in a study.

Our study is more than 90% powered to detect effect sizes of 0.1 SD in BMD and ORs of 1.20 for fractures and vertebral fractures, if the associations are consistent across different populations. Power would be eroded in the presence of large between-population heterogeneity.[65]

All statistical analyses were performed using Intercooled Stata 8.2 (StataCorp, College Station, Texas) and R 2.4.1 (the R Foundation for Statistical Computing [http://www.R-project.org]). All reported *P* values are 2-tailed.

RESULTS

Database

Data were collected between September 2004 and January 2007. Analysis of the collected data was performed between February and May 2007. Among the 37 534 participants (24 177 women) analyzed, data on lumbar spine BMD, femoral neck BMD, all fractures, and vertebral fractures existed for 28 073, 28 022, 35 762, and 22 580 participants, respectively. There were 8932 participants with any fracture and 2146 with vertebral fractures. Basic characteristics and further details of the cohorts are shown in Tables 1 and 2 and eTables 1 and 2. Genotypic information on *LRP5* Val667Met, *LRP5* Ala1330Val, and *LRP6* Ile1062Val was available for 32 720, 32 423, and 33 038 individuals, respectively. Information on all 3 SNPs was available for 30 989 individuals. The eFigure shows the position of the SNPs in the gene with the haplotypes and its frequencies in the total population studied. The frequency of the Met667 allele ranged

from 2% to 8%, of Val1330 from 10% to 19%, and of *LRP6* Val[1062] from 15% to 23% (for details see eTable 3).

Genotype frequencies were similar across the participating populations (eTable 3). No data set deviated significantly from Hardy-Weinberg equilibrium ($P > .05$), except for *LRP6* Ile1062Val (in AOS and APOSS) and *LRP5* Ala1330Val (in FLOS and LASA). Exclusion of these data did not affect summary estimates or conclusions (not shown). Linkage disequilibrium between the LRP5 polymorphisms was consistently high across all studies ($D' > 0.85$), which allowed inference of haplotypes with high confidence for all cohorts. We consistently identified 3 major haplotypes, and haplotype frequencies were similar across cohorts (eTable 3).

BMD Analyses

Effects of LRP5 Met667 and Val1330. For the *LRP5* Val667Met and Ala1330Val polymorphisms, highly significant effects on the lumbar spine and femoral neck BMD were observed (**TABLE 3**). The BMD effects tended to be larger for Val667Met than for Ala1330Val. The largest effects were found for lumbar spine BMD, which decreased by 20 mg/cm^2 (n = 25 052 [number of participants with available data]; $P = 3.3 \times 10^{-8}$) per copy of Met667 allele and 14 mg/cm^2 (n = 24 812; $P = 2.6 \times 10^{-9}$) per copy of Val1330 allele. For the femoral neck, the effects were 11 mg/cm^2 (n = 25 193; $P = 3.8 \times 10^{-5}$) and 8 mg/cm^2 (n = 25 026; $P = 5.0 \times 10^{-6}$), respectively. The aforementioned results remained significant after adjusting for multiple comparisons (the adjusted P values were 4.0×10^{-7}, 3.1×10^{-8}, 4.6×10^{-4}, and 6.0×10^{-5}, respectively).

Findings were highly consistent across studies for both *LRP5* variants (**FIGURE 1** and **FIGURE 2**), and no heterogeneity was detected (P for heterogeneity $> .90$ for all analyses). Adjustment of the estimates for age, height, and weight and further adjustment for postmenopausal status and use of hormone therapy in women had no major effect on the associations

Table 3. Unadjusted Difference in Bone Mineral Density (BMD) for *LRP5* Val667Met, *LRP5* Ala1330Val, *LRP6* Ile1062Val, and *LRP5* Haplotypes in Allele-based and Genotype-based Contrasts[a]

	Lumbar Spine			Femoral Neck		
SNP, Contrast, Subgroup	No.	BMD Difference (95% CI), mg/cm^2	*P* Value	No.	BMD Difference (95% CI), mg/cm^2	*P* Value
***LRP5* Val667Met**						
Met vs Val (allele-based)						
Men	9564	−17 (−30 to −4)	.01	9802	−16 (−25 to −6)	.001
Women	15 488	−22 (−30 to −13)	2.9×10^{-7}	15 391	−9 (−15 to −2)	.008
All	25 052	−20 (−27 to −13)	3.3×10^{-8}	25 193	−11 (−16 to −6)	3.8×10^{-5}
MetMet + MetVal vs ValVal						
Men	9564	−18 (−32 to −5)	8.6×10^{-4}	9802	−16 (−25 to −6)	.002
Women	15 488	−22 (−31 to −14)	4.9×10^{-7}	15 391	−10 (−17 to −3)	.004
All	25 052	−21 (−28 to −13)	3.7×10^{-8}	25 193	−12 (−18 to −6)	2.1×10^{-5}
***LRP5* Ala1330Val**						
Val vs Ala (allele-based)						
Men	9619	−10 (−18 to −2)	.01	9871	−9 (−15 to −3)	1.8×10^{-4}
Women	15 193	−16 (−21 to −11)	6.2×10^{-9}	15 155	−7 (−11 to −3)	8.1×10^{-4}
All	24 812	−14 (−18 to −9)	2.6×10^{-9}	25 026	−8 (−11 to −5)	5.0×10^{-6}
ValVal + AlaVal vs AlaAla						
Men	9619	−12 (−21 to −3)	8.9×10^{-4}	9871	−12 (−18 to −5)	6.0×10^{-4}
Women	15 193	−18 (−24 to −11)	2.0×10^{-8}	15 155	−8 (−13 to 4)	4.5×10^{-4}
All	24 812	−16 (−21 to −10)	3.4×10^{-9}	25 026	−10 (−13 to 6)	9.9×10^{-7}
***LRP6* Ile1062Val**						
Val vs Ile (allele-based)						
Men	9662	−1 (−8 to 6)	.80	9890	3 (−2 to 9)	.18
Women	15 673	0 (−4 to 5)	.85	15 673	2 (−2 to 6)	.30
All	25 335	0 (−4 to 4)	.97	25 454	3 (0 to 6)	.09
ValVal + IleVal vs IleIle						
Men	9662	0 (−8 to 8)	.99	9890	4 (−2 to 9)	.25
Women	15 673	2 (−4 to 8)	.50	15 673	3 (−1 to 8)	.13
All	25 335	1 (−3 to 6)	.61	25 454	3 (0 to 7)	.06
***LRP5* Haplotypes**						
(Val667Met-Ala1330Val, allele-based)						
1 (Val667-Ala1330)	23 939	1 [Reference]	NA	24 195	1 [Reference]	NA
2 (Val667-Val1330)		−10 (−16 to −5)	3.6×10^{-4}		−6 (−10 to −2)	.003
3 (Met667-Val1330)		−21 (−29 to −14)	1.7×10^{-8}		−13 (−18 to −7)	5.8×10^{-6}

Abbreviations: CI, confidence interval; NA, not applicable; SNP, single-nucleotide polymorphism.
[a]Results on individual single-nucleotide polymorphisms are based on inverse-variance random-effects analysis of individual-level data. Results on haplotypes are based on linear mixed models.

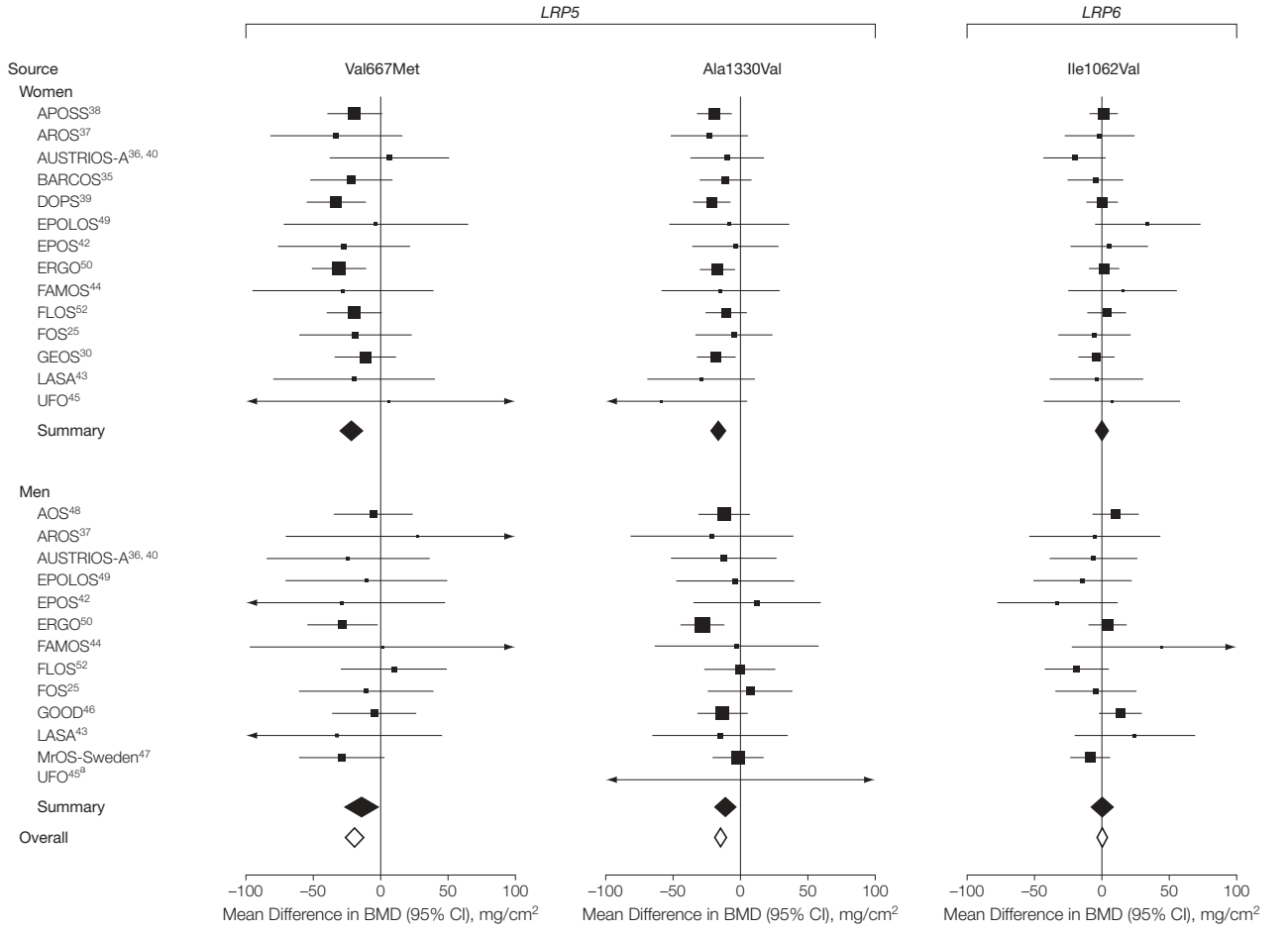
(eTable 4). Teams used very different scales to measure activity or ability as shown in eTable 1, but stratum-specific adjustments using mean-centered scores did not appreciably alter the within-strata estimates of the genetic effects ($P > .10$ by likelihood ratio test compared with corresponding models without the exercise and ability information). We could not detect a sex difference in the association between *LRP5* variants and BMD, but modest sex-specific associations cannot be excluded.

LRP5 haplotypes were highly significantly associated with lumbar spine BMD and femoral neck BMD overall ($P = 9.3 \times 10^{-10}$ and $P = 8.4 \times 10^{-6}$, likelihood ratio tests vs similar models without the *LRP5* haplotypes). Using haplotype 1 (Val667-Ala1330) as a reference, each copy of haplotype 2 (Val667-Val1330) and haplotype 3 (Met667-Val1330) was associated with a lower lumbar spine BMD of 10 mg/cm^2 ($P = 3.6 \times 10^{-4}$) and 21 mg/cm^2 ($P = 1.7 \times 10^{-8}$), respectively (Table 3). The corresponding decreases in femoral neck BMD were 6 mg/cm^2 ($P = .003$) and 13 mg/cm^2 ($P = 5.8 \times 10^{-6}$).

Effects of *LRP6* Val1062. The Ile1062Val polymorphism of *LRP6* did not show a significant association with BMD (Table 3 and Figures 1 and 2). No significant between-study heterogeneity was detected ($P > .57$ for all analyses). Adjusted analyses showed similar results (eTable 4). There was no significant interaction between the *LRP5* haplotypes and the *LRP6* Ile1062Val polymorphism on BMD based on likelihood ratio tests vs similar mixed models without the interaction terms.

Fracture Analyses. *Effects of* LRP5 *Met667 and Val1330.* Both *LRP5* variants were significantly associated with fracture risk (FIGURE 3, FIGURE 4, and TABLE 4). For each Met667 allele, the odds for any prevalent fracture in-

Figure 1. Differences in Bone Mineral Density at the Lumbar Spine Between Individuals, per Copy of the Risk Allele



Results based on inverse-variance random-effects analysis of individual-level data. The size of the data markers is proportional to the weight (inverse of the variance) of each study. AUSTRIOS-B did not have available data on bone mineral density measurements and therefore is not included in this analysis. BMD indicates bone mineral density; CI, confidence interval.

[a]Estimates for UFO (men) could not be obtained for *LRP5* Val667Met and *LRP6* Ile1062Val because all analyzed individuals had the same genotype. For *LRP5* Ala1330Val, mean difference in BMD was 163 mg/cm^2 (95% CI, −537 to 862).

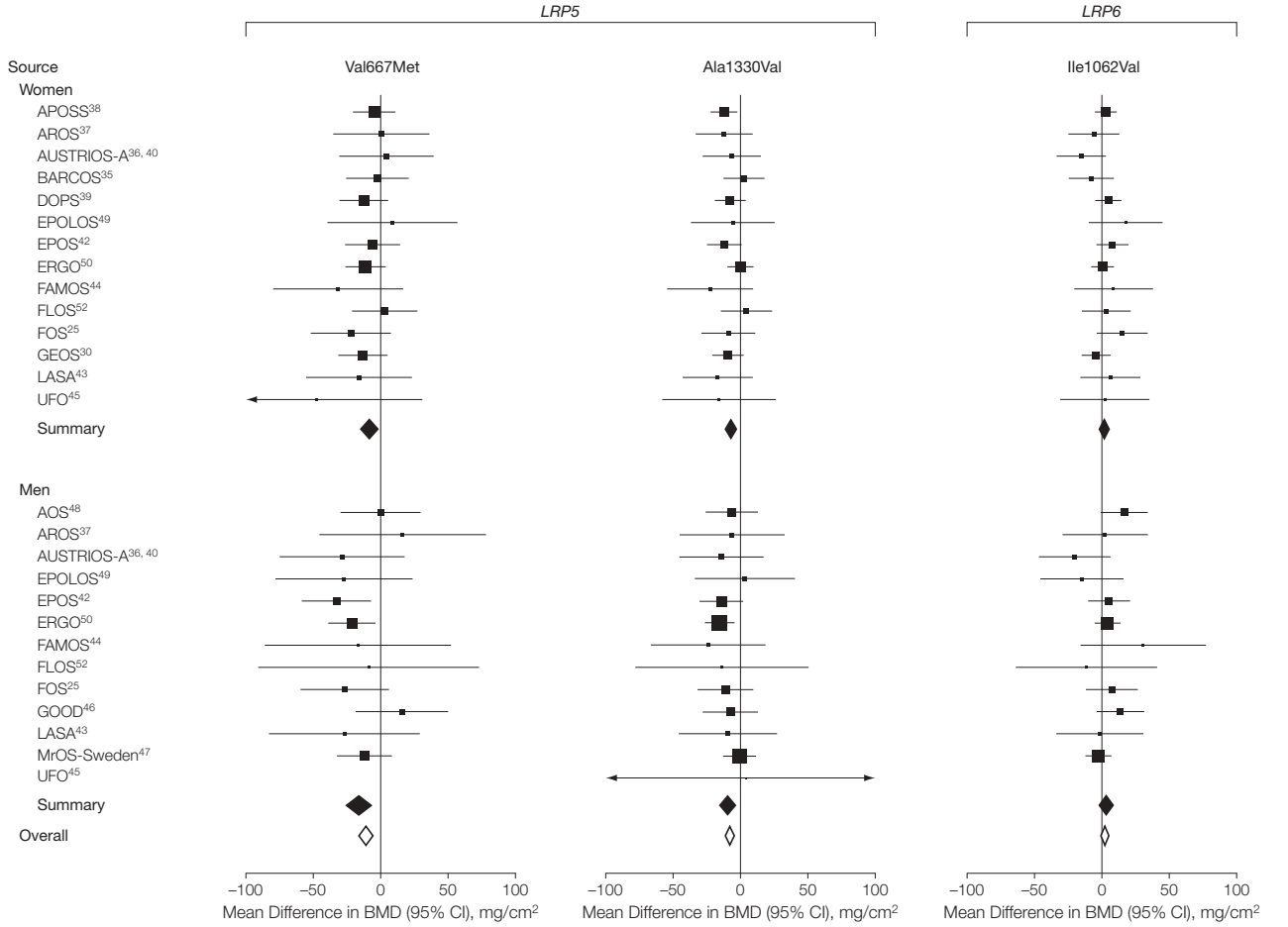
creased by 14% (7876 fractures among 31 435 individuals: OR, 1.14; 95% CI, 1.05-1.24; *P* = .002), and for prevalent vertebral fractures by 26% (2001 fractures among 20 488 individuals: OR, 1.26; 95% CI, 1.08-1.47; *P* = .004). The increased risk for prevalent vertebral fractures was found mainly in women (OR, 1.29; 95% CI, 1.08-1.54; *P* = .004). After adjusting for multiple comparisons, the *P* values for the association of Met667 with all fractures and vertebral fractures became .02 and .048, respectively.

A borderline significant association was found between the Val1330 variant and overall fracture risk. Participants carrying the Val1330 allele had 6% higher odds for any prevalent fracture (95% CI, 1.01-1.12; *P* = .02) (analysis of 7802 fractures among 31 199 individuals). Again, a larger effect was seen for vertebral fracture risk (1988 fractures among 20 096 individuals): carriers of the Val1330 allele had an OR of 1.12 (95% CI, 1.01-1.24; *P* = .03). The effects were no longer significant after adjustments for multiple comparisons (adjusted *P* values became .30 and .31, respectively).

The median prevalence of all fractures and vertebral fractures among cohort studies was 27% and 2.7%, respectively. The calculated RRs for each copy of the Met667 allele on the population level were 1.10 and 1.25 for all fractures and vertebral fractures, respectively. The corresponding RRs for the Val1330 allele were 1.12 and 1.04. The population-attributable risk for both Val1330 and Met667 was approximately 1% for fractures and 3% for vertebral fractures.

Excluding patients with vertebral fractures, the per-allele ORs for nonvertebral fractures were found to be 1.12 (95% CI, 1.02-1.23) and 1.05 (95% CI, 0.99-1.12) for the *LRP5* Met667 and *LRP5* Val1330 alleles, respectively. Effects on fractures were unaltered when adjustments were made for age, weight, height, and postmenopausal status; no

Figure 2. Differences in Bone Mineral Density at the Femoral Neck Between Individuals, per Copy of the Risk Allele



Results based on inverse-variance random-effects analysis of individual-level data. The size of the data markers is proportional to the weight (inverse of the variance) of each study. AUSTRIOS-B did not have available data on bone mineral density measurements and therefore is not included in this analysis. BMD indicates bone mineral density; CI, confidence interval. Estimates for UFO (men) could not be obtained for *LRP5* Val667Met and *LRP6* Ile1062Val because all analyzed individuals had the same genotype.

between-study heterogeneity was detected ($P > .33$ for all analyses). When adjustments for each individual's BMD were performed at either the lumbar spine or femoral neck, the formal significance of the overall effects on fracture was lost for most of the associations. The effect of the Val1330 allele on all fractures was unaltered by adjustment with lumbar spine BMD, while approximately 30% of the increased risk for vertebral fractures conferred by the *LRP5* Met667and *LRP5* Val1330 alleles was explained by the lumbar spine BMD. Similarly, lumbar spine BMD explained approximately one-third of the effect of the Met667 allele on risk of all fractures and vertebral fractures (see also eTable 5).

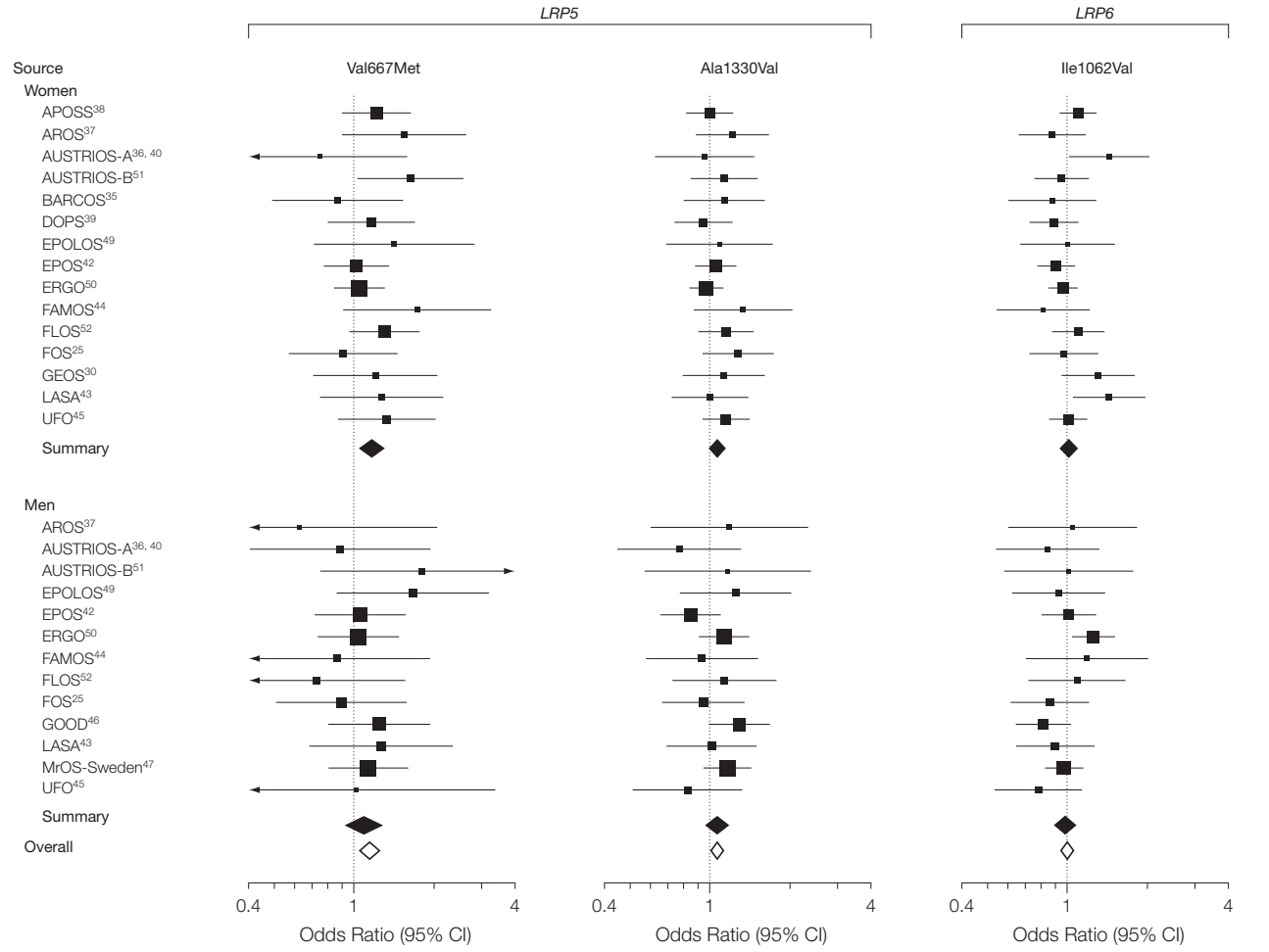
Overall, *LRP5* haplotypes were marginally associated with the risk for all fractures ($P = .05$; likelihood ratio test) and with the risk for vertebral fractures ($P = .02$; likelihood ratio test) (Table 4). Using the most common haplotype (haplotype 1, Val667-Ala1330) as reference, carriage of each copy of haplotype 3 (Met667-Val1330) was associated with an increase in the odds for vertebral fractures of 28% (95% CI, 1.08-1.55; $P = .006$). Associations with any prevalent fracture were not beyond what would be expected by chance.

Effects of LRP6 *Val*^{1062}. The *LRP6* Ile1062Val polymorphism was not associated with fractures overall (Table 4). The CIs excluded 6% differences in the OR for any prevalent fracture between alleles. Adjustment for age (as well as sex, weight, and height) did not appreciably change any of the summary estimates for fracture risk. There was no significant heterogeneity between studies in any analysis.

eTable 6 depicts analyses for incident and low-energy fractures. Results were not conclusive, given the availability of much more limited data.

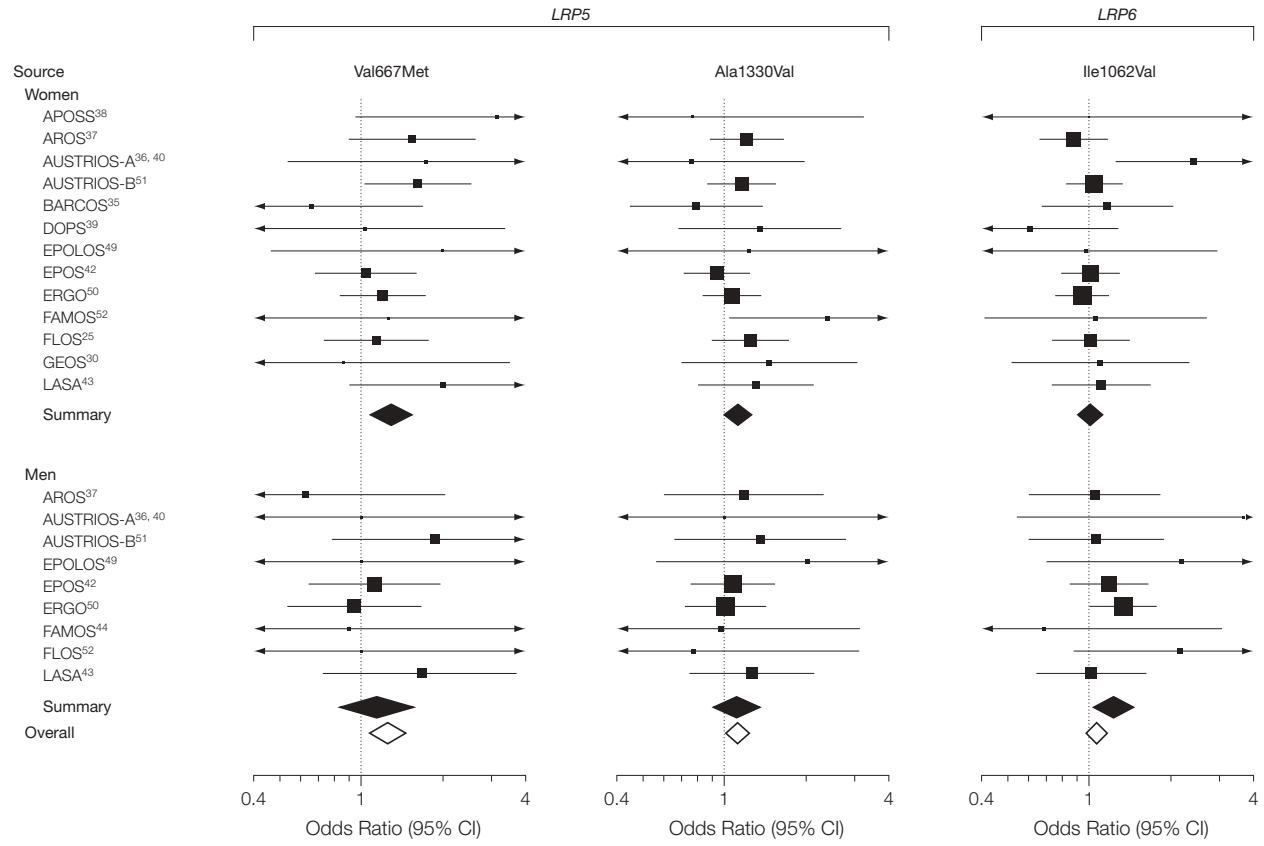
Sensitivity and Interaction Analyses. There was no evidence for a statisti-

Figure 3. Odds of Any Fracture, per Copy of the Risk Allele



Results based on inverse-variance random-effects analysis of individual-level data. Summary estimates of the odds ratios and their 95% confidence intervals (CIs) are given. The size of the data markers is proportional to the weight (inverse of the variance) of each study. AOS did not have available data on any fracture and therefore is not included in this analysis.

Figure 4. Odds of Vertebral Fracture, per Copy of the Risk Allele



Results based on inverse-variance random-effects analysis of individual-level data. Summary estimates of the odds ratios and their 95% confidence intervals (CIs) are given. The size of the data markers is proportional to the weight (inverse of the variance) of each study. AOS, FOS, GOOD, MrOS, and UFO did not have available data on vertebral fracture and therefore are not included in this analysis.

Table 4. Unadjusted Odds for Fracture Risk per Minor Allele for *LRP5* Val667Met, *LRP5* Ala1330Val, *LRP6* Ile1062Val, and *LRP5* Haplotype Alleles[a]

	Any Fracture			Vertebral Fractures (All Types)		
SNP, Subgroup	No.[b]	OR (95% CI)	*P* Value	No.[b]	OR (95% CI)	*P* Value
LRP5 Val667Met (per Met copy)						
Men	10 975	1.09 (0.93-1.27)	.28	4782	1.14 (0.84-1.57)	.40
Women	20 460	1.17 (1.06-1.30)	.003	15 706	1.29 (1.08-1.54)	.004
All	31 435	1.14 (1.05-1.24)	.002	20 488	1.26 (1.08-1.47)	.004
LRP5 Ala1330Val (per Val copy)						
Men	11 035	1.07 (0.97-1.17)	.20	4786	1.11 (0.91-1.36)	.30
Women	20 164	1.06 (1.00-1.14)	.06	15 310	1.12 (1.00-1.26)	.049
All	31 199	1.06 (1.01-1.12)	.02	20 096	1.12 (1.01-1.24)	.03
LRP6 Ile1062Val (per Val copy)						
Men	11 102	0.98 (0.90-1.07)	.71	4849	1.23 (1.04-1.46)	.02
Women	20 704	1.01 (0.94-1.09)	.69	15 838	1.01 (0.91-1.12)	.84
All	31 806	1.00 (0.95-1.06)	.95	20 687	1.07 (0.98-1.17)	.15
Haplotype (Val667Met-Ala1330Val)						
1 (Val667-Ala1330)	30 227	1 [Reference]	NA	19 737	1 [Reference]	NA
2 (Val667-Val1330)		1.06 (0.95-1.19)	.30		1.04 (0.91-1.19)	.59
3 (Met667-Val1330)		1.18 (1.02-1.37)	.02		1.28 (1.08-1.55)	.006

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.
[a] Results on single SNPs are based on inverse-variance random-effects analysis of individual-level data. Results on haplotypes are based on linear mixed models.
[b] Number of individuals in these analyses.

cally significant interaction of the *LRP5* variants with age for lumbar spine BMD, femoral neck BMD, any prevalent fracture, and any prevalent vertebral fracture. The same was true when interactions with menopausal status were assessed, with a single exception: each copy of the Val1330 allele was associated with an approximately 18-mg/cm^2 decrease in femoral neck BMD among premenopausal women but with only a 4-mg/cm^2 decrease among postmenopausal women ($P = .007$ for the Val1330 by menopausal status interaction).

COMMENT

In this large-scale multicenter collaborative study, we obtained evidence that genetic variation of the *LRP5* gene is associated with both BMD and fracture risk. The magnitude of the effects was modest but very consistent across studies. The effect size was 14 to 20 mg/cm^2 for lumbar spine and 8 to 11 mg/cm^2 at the femoral neck, which approximately corresponds to a 0.15-SD difference at both sites. Based on the general acceptance that a 1-SD reduction in bone mass doubles the fracture rate,[66] an increase of fracture risk of about 15% to 20% is expected. This is similar to the observed effects on fracture, although adjustment for BMD only partly reduced the increase in fracture risk. This could raise the possibility of effects on bone quality, bone dimension, or other nonskeletal determinants of fracture, but also could be due to error in measurement of BMD. Further work will be required to address this point.

Several previous reports have suggested that the association between genetic variation of the *LRP5* gene and BMD might be stronger in men compared with women.[22,25] We could not find such a sex difference. In fact, for fractures we found a slightly stronger effect for women as compared with men, although power was lower to detect effects for men.

LRP5 may be involved in the establishment of peak bone mass[6] and to a lesser extent involved in bone loss. Bone mineral density is substantially affected by age-related bone loss at older ages, so differences in BMD between LRP5 genotype groups might become smaller with age.[25] In our study there was no clear influence of age on the magnitude of the association between *LRP5* variants and BMD or fracture. For femoral neck BMD, differences between the Ala1330Val genotypes were larger in premenopausal women compared with postmenopausal women, which could indicate that the effect of *LRP5* variants is largely seen on peak bone mass. However, this was not observed for lumbar spine BMD and the Ala1330Val variant or with the Val667Met polymorphism for any of the outcomes. Even with such large-scale evidence, the presence or absence of interaction effects should be interpreted very cautiously.

The 2 polymorphisms in *LRP5* are each strongly associated with BMD. Although these polymorphisms are in strong linkage disequilibrium, the risk alleles were separated in 2 haplotypes: haplotype 2, carrying the common Val667 and the Val1330 risk allele, and haplotype 3, carrying risk alleles for both Met667 and Val1330. Haplotypes 2 and 3 were both associated with BMD while haplotype 3 was more strongly associated, which suggests that both variants have distinct effects. However, we cannot exclude that the polymorphisms are in linkage disequilibrium with 1 or more other causative polymorphisms rather than having an effect themselves.

The 2 studied *LRP5* variants are situated in different domains of the protein. The Val667Met polymorphism is localized at the top of the third propeller module in the receptor extracellular domain. This domain is thought to be involved in binding of the Wnt-inhibitor Dkk1, so perhaps binding efficacy of this inhibitor is changed in the Met667 variant. The Ala1330Val polymorphism lies within a second low-density lipoprotein (LDL) receptor domain of LRP5. The function of this region in LRP5 is unknown, but similar domains in the LDL receptor domain interact with the propeller domains.[67] Therefore, variations in the LDL receptor domains, such as Ala1330Val, may still alter protein function. Indeed, a recent report showed in vitro that Wnt-signaling capacity of the *LRP5* Val1330 variant was decreased compared to the Ala1330 variant.[25]

The strengths of our consortium analysis include the very large sample size, consistency across cohorts, lack of publication bias within the consortium due to its prospective design, and analysis of individual-level data, which allows standardized statistical analyses across participating teams.

In particular, we focused on validation of genotyping to minimize genotyping errors and aimed at standardized definitions for the outcomes. Limitations arise due to ascertainment of fractures, which differed across participating studies. This could introduce some unavoidable heterogeneity in the analyses. Another potential limitation is due to missing data in some cohorts. In addition, our results might not pertain to Asian and/or African populations, since we only examined white populations.

Our findings demonstrate that the modest effects of common genetic variations in complex diseases can be effectively addressed through large consortia and coordinated, standardized analysis. Such effects might be missed by smaller and potentially underpowered individual studies. This prospective collaborative study with individual level-data of 37 534 participants shows an effect of *LRP5* genetic variation on both BMD and risk of fracture. While some other common variants have been associated previously with osteoporosis phenotypes with large-scale evidence,[17-19] this may be the first time that an association in this field crosses the threshold of genome-wide statistical significance ($P < 10^{-7}$). Given the large number of polymorphisms that can be tested in the human genome, it has been argued that to fully account for all these possible comparisons (regardless of whether all of them are made), a very conservative threshold is needed.[62-64] Although the magnitude of the effect was

modest, the effect was very consistent in different populations and independent of sex or age. This suggests a role for LRP5 in determining BMD and fracture risk throughout life in the general population. Although any single marker explains only a small portion of the phenotype risk, identification of several such osteoporosis risk variants may eventually help in improving clinical prediction. Single genetic risk variants such as *LRP5* variants may also offer useful insights about mechanisms and pathways that may be useful in drug development.

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