WNT RECEPTORS, BONE MASS AND FRACTURES: GENE-WIDE ASSOCIATION ANALYSIS OF *LRP5* AND *LRP6* POLYMORPHISMS WITH REPLICATION

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ABSTRACT

Objectives. Genes explaining the susceptibility to osteoporosis have not been fully elucidated. Our objective was to explore the association of polymorphisms capturing common variations of the lipoprotein receptor related protein (LRP) 5 and 6 genes, encoding two Wnt receptors, with femoral neck bone mineral density (BMD) and osteoporotic fractures of the spine and the hip.

Design. Cross-sectional, case-control and replication genetic association study.

Methods. Thirty nine tagging and functional single nucleotide polymorphisms (SNP) were analyzed in a group of 1043 postmenopausal women and 394 women with hip fractures. The results were replicated in a different group of 342 women.

Results. Three SNPs of the *LRP6* gene were associated with BMD (nominal uncorrected p-values<0.05) in the discovery cohort. One showed a significant association after multiple test correction; two of them were also associated in the replication cohort, with a combined standardized mean difference of 0.51 (p=0.009) and 0.65 (p<0.0001) across rs11054704 and rs2302685 genotypes. In the discovery cohort, several *LRP5* SNPs were associated with vertebral fractures (odds ratio 0.67; p=0.01), with hip fractures (unadjusted odds ratios between 0.59 and 1.21, p=0.005-0.033, but not significant after multiple test- or age-adjustment), and with height and the projected femoral neck area, but not with BMD. Transcripts of *LRP5* and *LRP6* were similarly abundant in bone samples.

Conclusions. In this study we found common polymorphisms of *LRP5* associated with osteoporotic fractures, and polymorphisms of the *LRP6* gene associated with BMD, thus suggesting them as likely candidates to contribute explaining the hereditary influence on osteoporosis.

KEYWORDS: Osteoporosis, fractures, Wnt pathway, genetic association study.

INTRODUCTION

The Wnt family includes more than fifteen proteins which have been shown to play an important role in organ development and the homeostasis of several adult tissues, including bone. Wnt ligands stimulate the differentiation of pluripotential precursors towards the osteoblastic lineage, and have an anabolic effect on bone. Intracellular signals transducing Wnt effects are complex and include several pathways, the best known of which is the so called canonical pathway. It starts with the union of Wnt ligands to a membrane receptor complex constituted by a lipoprotein receptor related peptide (LRP) and a frizzled protein. There are at least 10 members of the frizzled family. There are also several LRP's, with positive or negative effects on Wnt signaling (1). The best known members of the LRP family are LRP5 and LRP6. The role of LRP5 was emphasized by the discovery of some patients with either high or low bone mass phenotypes, caused by activating and loss-of-function mutations, respectively, of *LRP5*. Furthermore, several candidate gene studies and some multicenter large-scale and genome-wide studies (GWAS) suggested that common *LRP5* polymorphisms may influence bone mineral density (BMD) in the general population. Nevertheless, recent animal studies have raised some doubts about the direct role of *LRP5* in bone (2). The role of *LRP6* has not been studied extensively, but both *LRP5* and *LRP6* appear to be necessary for skeletal homeostasis (3; 4). Moreover, some investigators suggested that *LRP6* polymorphisms influence BMD (5; 6), but the results were not confirmed in other studies (7; 8). Therefore, this is a controversial issue. Population differences and the particular polymorphisms included in those analyses may explain the discordant results. On the other hand, little is known about the relationship between *LRP5*/6 polymorphisms and hip fractures, which are the most devastating osteoporotic fractures. Therefore, the aim of this study was to use a gene-wide approach to

explore the association of common polymorphisms of *LRP5* and *LRP6* genes with femoral neck BMD and osteoporotic fractures of the spine and the hip.

MATERIALS AND METHODS

Subjects.- To study the association of genetic polymorphisms with BMD and vertebral fractures we performed a cross-sectional study including 1043 postmenopausal women over 50 years of age (age 51-90) living in Cantabria, a region in Northern Spain with a population of 550.000. They included volunteers recruited by advertisements and women sent to our clinic because of osteoporosis concerns ("Santander group") and women taking part in a population-based cohort study on the epidemiology of osteoporosis ("Camargo cohort") (9). Both groups belong to the same geographical region. Femoral neck BMD was measured by DXA using a Hologic QDR 4500 densitometer. The projected neck area in the DXA output was also analyzed. Since the densitometer uses a fixed length for femoral neck assessment, the projected area is a function of the neck diameter. The presence of vertebral fractures was defined as a loss of vertebral body height higher than 20% in lateral X-rays (available in 637 women) assessed by an experienced reader, prior to genotyping.

To study the association with hip fractures, we recruited a convenience sample of unselected patients admitted to hospital because a hip fracture, 60 to 90 years of age (n=394). Those with fractures due to high-impact trauma (such as traffic accidents and falls from a height) were excluded. Control subjects (n=529) included women from the Santander and Camargo groups within the same age range but without osteoporotic fractures.

Women with present or past diseases (cancer, rheumatoid arthritis, malabsorption, severe systemic diseases, etc) or treatments (corticosteroids, anticonvulsants, hormone replacement therapy, etc.) known to affect bone metabolism, or with non-Spanish ancestors were excluded.

The study protocol was approved by the Institutional Committee of Ethics in Clinical Research, and informed consent was obtained from study subjects or their representatives.

The results about genetic polymorphisms and BMD were replicated in a cohort of women attending a menopause clinic in Valencia, a region in Eastern Spain. After applying similar exclusion criteria, the cohort included 342 Caucasian postmenopausal women aged 41-69 years. BMD was measured by DXA using either a Lunar or a Norland densitometer. The results from different densitometers were standardized as proposed by Lu et al. (10).

Genotyping.- The Hapmap database was explored to identify SNPs of the *LRP5* and *LRP6* genes in the Caucasian population. Then, tag-SNPs were selected using the algorithms available in Haploview with the "aggressive tagging" option (11). Minor allele frequency (MAF) of 0.1 and r^2 0.8 were used as criteria. In addition, we included some SNPs with potential regulatory function as assessed by the bioinformatics suite Pupa (12; 13). Therefore, 31 SNPs of the *LRP5* gene and 11 of the *LRP6* gene were finally selected. DNA was isolated from peripheral blood or buccal swabs by using column-based commercial methods and quantified with the Qubit procedure (Invitrogen, Carlsbad, CA, USA). Then alleles at each locus were analyzed by a mass-array Sequenom platform at the Centro Nacional de Genotipado (Santiago de Compostela, Spain). In a sub-sample of study participants, the rs3736228 polymorphism of the *LRP5* gene was analyzed by using a Taqman assay (Applied Biosystems). Polymorphisms associated with BMD in the discovery cohort were analyzed in the replication cohort by using the same procedure (mass-array) for genotyping at Unidad Central de Investigación (Facultad de Medicina, Valencia, Spain). Replicate samples were included to confirm the consistency of results.

Gene expression.- Trabecular bone samples were obtained from the femoral heads of patients subjected to hip replacement because of severe osteoarthritis. RNA was isolated with Trizol (Invitrogen), and further purified by using a column adsorption procedure (Qiagen, Hilden,

Germany). Aliquots of RNA (250 ng) were reverse-transcribed with the Superscript III kit (Invitrogen) and quantified by real-time PCR in an ABI7300 apparatus (Applied Biosystems, Foster City, CA), using specific primers and FAM-labelled probes for *LRP5* and *LRP6* (Taqman gene expression assays, Applied Biosystems). The results were then normalized to the expression of the housekeeping gene TATA box protein (TBP) and an universal reference RNA (Stratagene), by the $2^{-\Delta\Delta Ct}$ method (14).

Statistical analysis.- Haplotypic blocks were estimated by the Gabriel method, implemented in Haploview (11). The departure from Hardy-Weinberg equilibrium was tested with Plink software (15). The association of alleles with BMD was studied at the single-locus level, assuming additive and recessive models, with Plink. The presence of population stratification was explored with STRUCTURE software, running a dataset with five markers located in different chromosomes (16). BMD results in different cohorts were combined by computing the Hedges' standardized weighted mean difference with MIX software (17; 18). The significance threshold after multiple test correction for each gene was estimated by considering the effective number of independent marker loci, as proposed by Li and Ji, using the single nucleotide spectral decomposition software (SNPSpD), developed by Nyholt (19).Uncorrected nominal p-values are shown, unless otherwise indicated. p-values <0.05 were considered as statistically significant. The study power (estimated with QUANTO software, available at http://hydra.usc.edu/gxe/) was higher than 89% to find genetic effects explaining at least 1% of BMD variance. Power to detect a fracture odds ratio of 1.4 or higher was 80% and 54%, for hip fractures and vertebral fractures, respectively, assuming an additive model and a minor allele frequency of 0.2.

RESULTS

Bone mineral density

Four SNPs of the *LRP5* gene were excluded due to inaccurate separation or low calling rate. The genotyping rate in the remaining set (table 1) was 97.4%. The allelic frequencies were similar to those reported in other Caucasian populations and there was no evidence for departure from the Hardy-Weinberg equilibrium. Only one SNP was associated with a nominal p-value less than 0.05 (0.02, well above the significance 0.003 threshold after multiple test correction) (table 1). No significant hidden population stratification was detected running the STRUCTURE software. The characteristics of women included in the study are shown in table 2.

The rs4988321 polymorphism of the *LRP5* gene showed a marginal association with age-adjusted BMD (p=0.0488) (figure 1), that did not reach the multiple test-adjusted threshold of significance (estimated as p=0.003). Three SNPs of the *LRP6* gene were also associated with BMD: rs11054704 (additive p=0.035; recessive p= 0.016), rs2302685 (additive p=0.058; recessive p=0.0039), and rs10845493 (additive p=0.54; recessive p=0.036) (figure 2). Only the p-value for the rs2302685 polymorphism was below the multiple test-adjusted threshold for significance (estimated as 0.006 for the *LRP6* SNP set).

We selected those three SNPs for replication in a different cohort (Valencia). In this cohort, rs10845493 was not associated with BMD, but the association was replicated for rs11054704 (p= 0.016, additive model) and almost reached statistical significance for rs2302685 (p=0.057, additive model). The combined standardized weighted mean differences between women with opposite genotypes were 0.51 standard deviations (95% confidence interval 0.13-0.89) and 0.65 standard deviations (95% confidence interval 0.35-0.96) for rs11054704 and rs2302685 loci, respectively (table 3).

Fractures

The association of genetic polymorphisms with fractures was analyzed in 637 women with spine X-rays available (140 with osteoporotic vertebral fractures and 497 controls without fractures). Several polymorphisms of the *LRP5* gene tended to be associated with fractures, with nominal p-values <0.05, but they did not reach the multiple test threshold for significance (0.003). Loci rs312788 and rs160607 showed the most significant association The age-adjusted odds ratio was 0.67 in both cases (p=0.009 and 0.01, respectively; figure 3). The rs3736228 polymorphism could not be included in the multiplex reaction along other polymorphisms and was genotyped later in a single assay. It also tended to be associated with fractures in our population (OR 1.5; 95% confidence interval 1.1-2.2; p=0.025).

Since *LRP5* polymorphisms showed a trend for association with vertebral fractures, but not with BMD, we explored their relationship with body size. After excluding women with vertebral fractures (which can cause a loss of height by themselves), several *LRP5* polymorphisms were associated with height (uncorrected p-values 0.002-0.04; figure 3). Likewise, they were associated with the projected femoral neck area, as measured from the densitometer output, even after controlling for height (p-values=0.0014-0.048, figure 3).

Several *LRP5* polymorphisms also tended to be associated with hip fractures (uncorrected p-values <0.05), including some found associated with vertebral fractures (rs4988300 and rs160607). However, the hip fracture group was somewhat older than the control group. After adjusting the results introducing age as a covariate, similar odds ratios were observed, but with larger confidence intervals and no longer statistically significant (table 4).

SNPs of the *LRP6* gene were not significantly associated with either vertebral or hip fractures.

The incomplete assessment of fractures in the Valencia cohort did not allow replicating fracture association data.

Gene expression

Gene transcripts were measured in 17 femoral bone samples of patients with osteoarthritis undergoing hip replacement surgery. Transcripts of *LRP5* and *LRP6* were similarly abundant: 1.5 (range 0.1-4.9) and 1.2 (range 0.1-5.7) arbitrary units, respectively (not significantly different).

DISCUSSION

Rare cases of loss-of-function or gain-of-function mutations of *LRP5* are associated with marked decreases or increases, respectively, of bone mass (20-23). Although the skeletal impact of common allelic variants is less clear, several polymorphisms of the *LRP5* gene have been associated with bone mass and fractures, in candidate gene studies (6; 24; 25) and in some, but not all genome-wide studies (26; 27). The nonsynonymous rs3736228 polymorphism has been the most widely studied SNP in candidate gene studies (25; 28) and was identified as a quantitative trait locus in the Rotterdam cohort GWAS. It showed a stronger association with spine BMD than with femoral neck BMD (26). In a meta-analysis including 10 eligible studies with 16705 individuals, Tran et al. estimated that the difference in femoral neck BMD across rs3736288 genotypes was 0.011 g/cm^2, roughly equivalent to 0.1 standard deviations (25). Our study was not powered to detect such a small difference. The LRP5 polymorphism was also associated with fractures in the GWAS and in a large multicenter study (29). In the present study we confirmed the trend for association with vertebral fractures, but we were not able to confirm the association with BMD. Several factors may explain the differences between studies, including age and menopausal status of subjects. In fact, the association with fractures but not with BMD might suggest an effect of *LRP5* alleles on other factors influencing bone strength, such as bone tissue quality or bone geometry. In the present study some *LRP5* polymorphisms were associated with body height and femoral neck size. This is in line with

previously published results (30), and suggests that *LRP5* may influence skeletal development and growth, and consequently the peak bone mass attained in early adulthood, as also suggested by studies showing an association of *LRP5* polymorphisms with BMD in children and young adults (30-33). However, *LRP5* SNPs associated with fractures did not coincide with those associated with height or femoral neck projected area. Therefore, further studies are needed to clarify the mechanism explaining the association of *LRP5* variants with fractures.

LRP5 and LRP6 are known to bind Wnt ligands, but their relative importance in bone tissue is unclear. Whereas several mutations of the *LRP5* gene have been associated with abnormal human bone phenotypes (20; 21; 23; 34; 35), only a pedigree with a *LRP6* mutation causing metabolic syndrome, coronary heart disease and osteoporosis has been reported (36). Studies with knock-out mice suggest that both *LRP5* and *LRP6* are needed for the normal skeletal homeostasis (3; 4). In the present study, we showed that *LRP5* and *LRP6* are expressed in similar amounts in human bone. However, unlike *LRP5* polymorphisms, the association of genetic variants of *LRP6* with bone mass has been rarely studied, and controversial results have been reported. Van Meurs et al first published that the nonsynonymous rs2302685 polymorphism (Ile1062Val) of the *LRP6* gene was associated with fragility fractures in men of the Rotterdam cohort (6). However, the results were not confirmed in a multicenter study (29) and no evidence for association with SNPS in the *LRP6* region was found in a meta-analysis of data directly obtained or imputed from GWAS results (37). However, Sims et al reported an association of genetic variants of *LRP6*, and specifically rs11054704, with BMD in postmenopausal women (5). Trying to clarify the possible influence of *LRP6* variants on bone mass, we performed a gene-wide analysis including tagging *LRP6* SNPs in a defined population of postmenopausal women. There is no doubt that exploring the association of several SNPs with a phenotype inflate type I error (ie, the possibility of false positive associations). However, there is no general consensus about the best way to control it without

compromising study power, particularly in hypothesis-driven candidate gene studies with several SNPs in linkage disequilibrium. Here we used the method proposed by Nyholt, which takes into consideration the linkage between the genotyped loci (19). The replication in independent cohorts may be the best way to confirm that the results are not just a chance finding. In the present study we found an association of *LRP6* SNPs with BMD, with some p-values below the multiple test-adjusted threshold for significance. Furthermore, in the replication cohort we found a consistent association of two *LRP6* polymorphisms with BMD. Thus, our results support the hypothesis that allelic variants of *LRP6* are associated with BMD in postmenopausal women.

The non-synonymous polymorphism rs2302865 is located on exon 14 of the *LRP6* gene and causes an isoleucine/valine change. The rs11054704 polymorphism is located in intron 15, just 2.1 kb downstream of rs2302865, and both belong to the same haplotypic block. The molecular mechanisms involved in the association of these SNPs with BMD remain to be elucidated. Nevertheless, according to the Fast SNP bioinformatic tool (http://fastsnp.ibms.sinica.edu.tw), the region including rs11054704 may act as an intronic enhancer and there may be allelic differences in the binding affinity for some transcription factors (CEBP/ß, CdxA). On the other hand, different rs2302685 alleles not only induce an aminoacid change which may impair the activity of *LRP6*, but they may also have splicing regulatory consequences. Therefore, considered together, these results strongly suggest that these polymorphisms, or other linked variants located in the same region of the *LRP6* gene, are indeed associated with individual differences in BMD. Our data suggest that in women with the least frequent homozygous genotype BMD is 0.5 standard deviations lower than in those with the most frequent genotype. Such a difference is likely to have an important influence at the individual level, but it may be less important at the population level, because the risk genotype is present in only 2-3% of

women. The explanation for the negative results in other studies is unclear (7; 29), but it might be related to differences in the populations studied or to cohort heterogeneity.

This study has several limitations. We estimated that the number of subjects included in Cantabria cohort resulted in 89% power to detect a genetic effect explaining 1% of the BMD variance. However, the sample size limited the statistical power to detect smaller effects, particularly in the replication cohort, which was relatively small and without enough numbers of fractures for replication (only clinical vertebral fractures were recorded because X-rays were not routinely obtained). We were not able to demonstrate a parallel association of *LRP6* polymorphisms with fractures, which may be related to the small number of homozygotes for the risk allele and the moderate size of our fracture subgroup, which limited the power of the study when the minor allele frequencies were small. We do not have anthropometric data of patients with hip fractures. Therefore we could not include some potentially important factors, such as body weight, as covariates.

In summary, our data are in line with previously published studies showing an association of *LRP5* polymorphisms with osteoporotic fractures and strongly suggest that some genetic variants of *LRP6* are associated with bone mineral density in postmenopausal women, pointing towards both Wnt co-receptors as osteoporosis candidate genes.

Table 1. Polymorphisms genotyped.

Gene	Chrom	SNP	Position	Minor allele	Major allele	MAF	P (HWE)
LRP5	11	rs4988330	67837575	T	C	0.08	0.43
LRP5	11	rs7116604	67841050	A	G	0.13	0.83
LRP5	11	rs312014	67841538	C	G	0.39	0.35
LRP5	11	rs4988331	67841909	T	C	0.08	0.65
LRP5	11	rs4988300	67845407	G	T	0.48	0.81
LRP5	11	rs3781600	67849913	C	G	0.11	0.20
LRP5	11	rs312024	67851007	A	G	0.31	0.62
LRP5	11	rs314779	67854402	G	T	0.31	0.35
LRP5	11	rs606989	67858576	T	C	0.09	0.06
LRP5	11	rs314756	67868248	G	A	0.07	1
LRP5	11	rs3781596	67870578	C	G	0.14	0.69
LRP5	11	rs643981	67872340	T	C	0.44	0.88
LRP5	11	rs312786	67876553	T	G	0.29	0.64
LRP5	11	rs312788	67878871	G	T	0.44	0.85
LRP5	11	rs160607	67886182	A	G	0.43	0.73
LRP5	11	rs11826287	67903237	C	T	0.19	0.26
LRP5	11	rs671191	67906557	C	T	0.38	0.02
LRP5	11	rs4930573	67920032	G	C	0.32	0.87
LRP5	11	rs587397	67923999	G	C	0.09	0.65
LRP5	11	rs4988321	67930765	A	G	0.08	1
LRP5	11	rs2306862	67934086	T	C	0.20	0.55
LRP5	11	rs923346	67938951	C	T	0.20	0.60
LRP5	11	rs1784235	67942076	C	T	0.28	0.76
LRP5	11	rs556442	67949266	G	A	0.39	0.47
LRP5	11	rs12417014	67957583	T	C	0.10	0.13
LRP5	11	rs3781579	67966294	G	A	0.16	0.09
LRP5	11	rs632605	67972530	A	G	0.07	1
LRP6	12	rs2075241	12182746	C	G	0.14	0.05
LRP6	12	rs718403	12188223	T	C	0.24	0.43
LRP6	12	rs11054704	12191036	A	G	0.13	0.45
LRP6	12	rs2302685	12193165	C	T	0.16	0.53

LRP6	12	rs12833575	12216108	G	A	0.06	0.68
LRP6	12	rs2417085	12222642	C	T	0.47	0.42
LRP6	12	rs10845493	12223463	T	C	0.15	0.52
LRP6	12	rs10082834	12225273	C	G	0.11	0.48
LRP6	12	rs17374170	12255678	C	T	0.08	1
LRP6	12	rs17302049	12256592	G	A	0.17	0.81
LRP6	12	rs1181334	12258855	T	G	0.18	1

Table 2. Characteristics of study subjects (mean and SD or percentages).

Cohort/Phenotype	Santander/BMD-vertebral fractures (n=608)	Camargo/BMD-vertebral fractures (n=435)	Hip fractures (n=394)	Valencia/BMD (n= 342)
Age, yr	69±7	64±9	80±7	52±5
Weight, Kg	66±10	70±12	-	66±10
Height, cm	155±6	155±6	-	158±6
BMI, kg/m^2	27.6±4.1	28.9±4.8	-	26.5±4.3
Age at menopause, yr	50±5	49±5	-	48±4
Vertebral Fractures*, %	36	8	-	-
Arm/forearm fractures, %	11	8.4	16	9
BMD**, g/cm^2	0.671±0.112	0.720±0.117	-	0.804±0.115
Smoking, %	4	12	3	26
Calcium intake***, mg/day	659±397	680±358	620±376	-

*: data from 637 women with x-rays

**: Hologic DXA in Cantabria and standardized (Lunar or Norland) in Valencia

*** From dairy products

Table 3. Association of *LRP6* genotypes with BMD in the discovery and replication cohorts and combined estimate of effect. P-values for age-adjusted additive models in each cohort and standardized weighted mean difference (SWMD) between opposite homozygotes (in standard deviation units).

Locus/cohort	BMD g/cm^2				
rs11054704	AA	AG	GG	SWMD (GG-AA)	p
Cantabria	0.626±0.180 (19)	0.681±0.101 (201)	0.688±0.117 (782)		0.035
Valencia	0.757±0.103 (8)	0.782±0.108 (90)	0.813±0.116 (222)		0.016
Combined				0.51	0.009
rs2302685	CC	CT	TT	SWMD (TT-CC)	p
Cantabria	0.626±0.154 (30)	0.687±0.104 (254)	0.687±0.117 (724)		0.058
Valencia	0.770±0.090 (13)	0.788±0.112 (91)	0.812±0.119 (187)		0.057
Combined				0.65	<0.0001

Table 4. Association of *LRP5* polymorphisms with hip fractures. Odds ratio (OR), limits of the 95% confidence interval (L95,U95) and p-values for additive models in the unadjusted and age-adjusted analysis.

		Undjusted				Age-adjusted			
SNP	**Allele**	**OR**	**L95**	**U95**	**P**	**OR**	**L95**	**U95**	**P**
rs4988330	T	0.84	0.59	1.20	0.36	1.02	0.65	1.59	0.91
rs7116604	A	0.90	0.67	1.19	0.47	1.15	0.80	1.64	0.43
rs312014	C	1.01	0.84	1.22	0.86	0.88	0.70	1.11	0.30
rs4988331	T	0.59	0.41	0.85	**0.005**	0.71	0.46	1.10	0.13
rs4988300	G	1.25	1.04	1.50	**0.017**	1.12	0.89	1.40	0.32
rs3781600	C	0.98	0.73	1.31	0.91	1.14	0.79	1.64	0.46
rs312024	A	1.16	0.95	1.41	0.13	0.95	0.74	1.21	0.69
rs314779	G	0.90	0.74	1.10	0.31	0.90	0.70	1.14	0.40
rs606989	T	0.89	0.65	1.22	0.48	1.10	0.74	1.61	0.62
rs314756	G	0.87	0.60	1.26	0.49	0.81	0.52	1.27	0.36
rs3781596	C	0.74	0.56	0.97	**0.033**	0.93	0.66	1.31	0.70
rs643981	T	0.85	0.71	1.02	0.09	0.87	0.69	1.09	0.24
rs312786	T	0.93	0.76	1.14	0.52	0.83	0.65	1.07	0.16
rs312788	G	0.84	0.70	1.02	0.08	0.86	0.69	1.08	0.19
rs160607	A	0.81	0.67	0.97	**0.025**	0.83	0.67	1.04	0.12
rs11826287	C	0.96	0.76	1.22	0.78	0.90	0.67	1.19	0.47
rs671191	C	0.86	0.71	1.03	0.11	0.91	0.73	1.14	0.43
rs4930573	G	1.00	0.81	1.22	0.99	0.88	0.69	1.13	0.34
rs587397	G	0.84	0.60	1.17	0.31	0.81	0.53	1.22	0.31
rs4988321	A	1.10	0.78	1.56	0.55	1.12	0.73	1.71	0.58
rs2306862	T	1.01	0.80	1.28	0.88	0.93	0.69	1.24	0.62
rs923346	C	1.02	0.80	1.29	0.86	0.94	0.70	1.25	0.68
rs1784235	C	0.97	0.79	1.20	0.84	0.89	0.68	1.15	0.37
rs556442	G	0.94	0.77	1.14	0.54	0.99	0.77	1.26	0.95
rs12417014	T	0.88	0.64	1.21	0.44	0.99	0.66	1.47	0.97
rs3781579	G	0.97	0.76	1.25	0.86	0.96	0.70	1.31	0.81
rs632605	A	0.80	0.56	1.16	0.24	1.03	0.65	1.62	0.89

FIGURE LEGENDS

Figure 1.-Association of *LRP5* polymorphisms with femoral neck BMD. Nominal p-values under an age-adjusted recessive model.

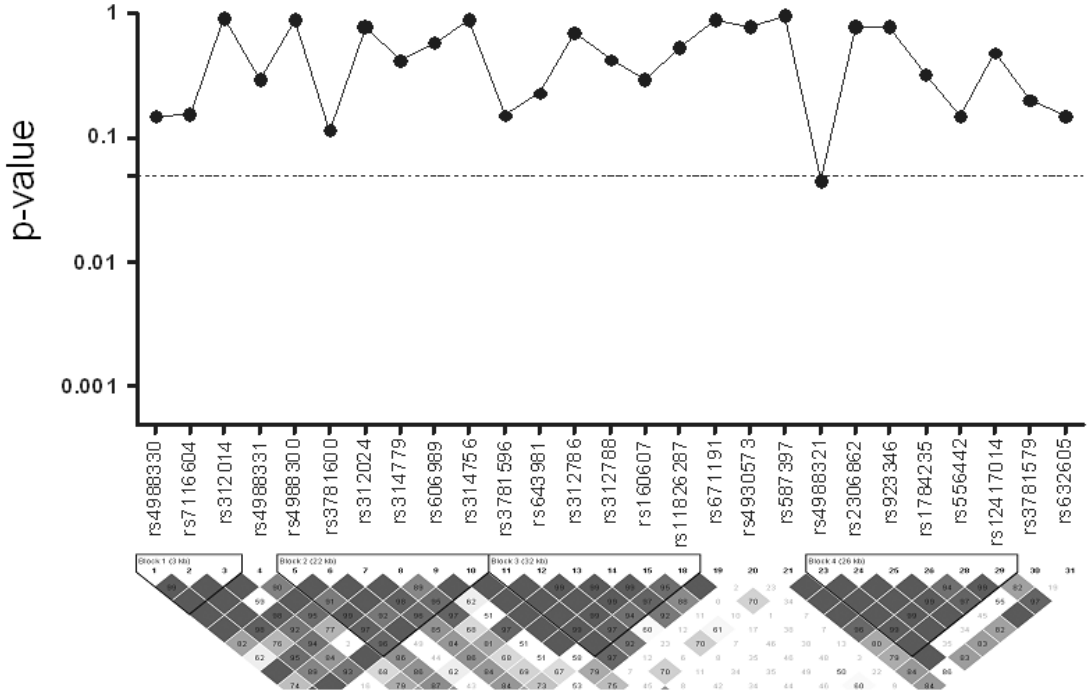


Figure 2.- Association of *LRP6* polymorphisms with femoral neck BMD. Nominal p-values under an age-adjusted recessive model.

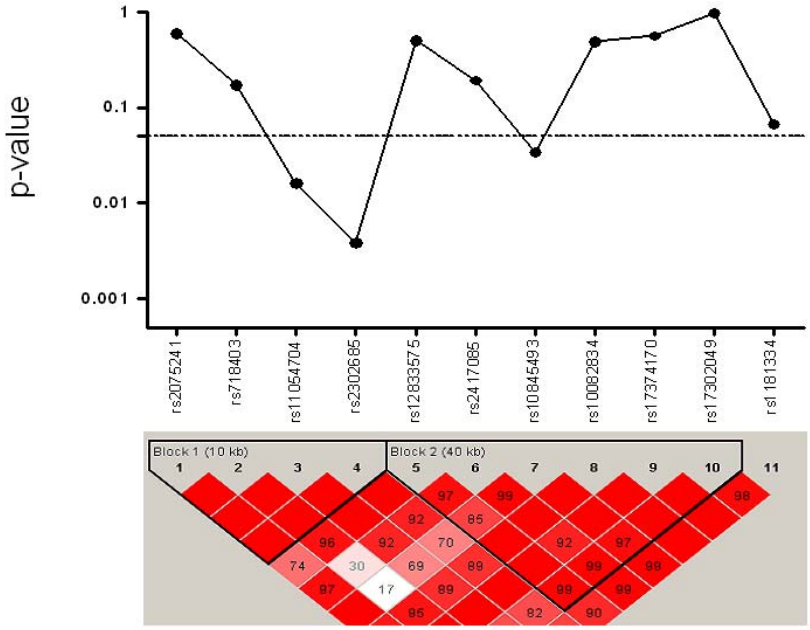
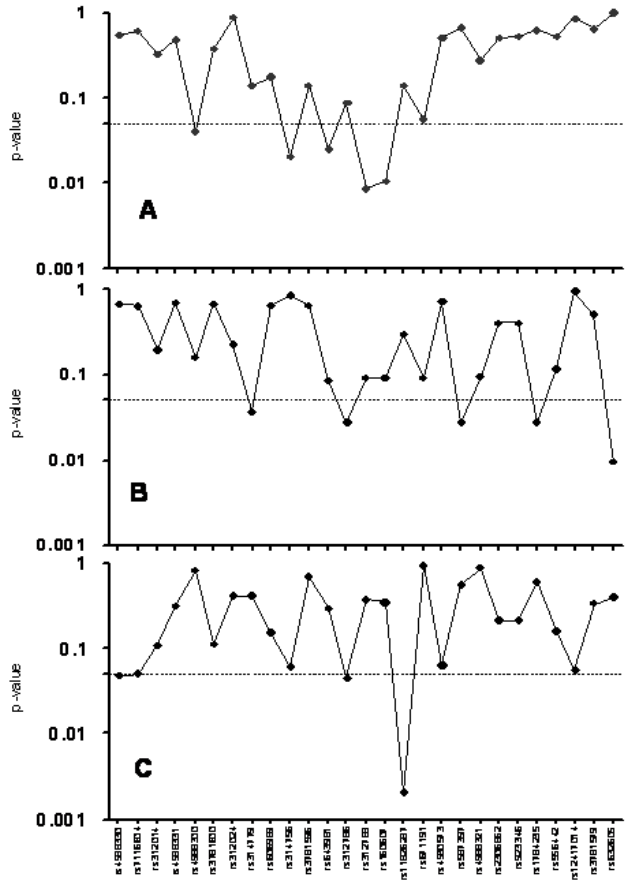


Figure 3.- Association of *LRP5* polymorphisms with osteoporotic vertebral fractures (A), femoral neck projected area (B) and height (C). Nominal p-values under an additive age-adjusted model.



Conflicts of interest
Authors do not have conflicts of interest relevant to this paper

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