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Association Analyses of *RANKL/RANK/OPG* Gene Polymorphisms with Femoral Neck Compression Strength Index Variation in Caucasians

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Abstract

Femoral neck compression strength index (fCSI), a novel phenotypic parameter that integrates bone density, bone size, and body size, has significant potential to improve hip fracture risk assessment. The genetic factors underlying variations in fCSI, however, remain largely unknown. Given the important roles of the receptor activator of the nuclear factor- κ B ligand/receptor activator of the nuclear factor- κ B/osteoprotegerin (RANKL/RANK/OPG) pathway in the regulation of bone remodeling, we tested the associations between *RANKL/RANK/OPG* polymorphisms and variations in fCSI as well as its components (femoral neck bone mineral density [fBMD], femoral neck width [FNW], and weight). This was accomplished with a sample comprising 1873 subjects from 405 Caucasian nuclear families. Of the 37 total SNPs studied in these three genes, 3 SNPs, namely, rs12585014, rs7988338, and rs2148073, of *RANKL* were significantly associated with fCSI ($P = 0.0007$, 0.0007 , and 0.0005 , respectively) after conservative Bonferroni correction. Moreover, the three SNPs were approximately in complete linkage disequilibrium. Haplotype-based association tests corroborated the single-SNP results since haplotype 1 of block 1 of the *RANKL* gene achieved an even more significant association with fCSI ($P = 0.0003$) than any of the individual SNPs. However, we did not detect any significant associations of these genes with fBMD, FNW, or weight. In summary, our findings suggest that the *RANKL* gene may play an important role in variation in fCSI, independent of fBMD and non-fBMD components.

Keywords

Femoral neck compression strength index; Femoral neck bone mineral density; Femoral neck width; *RANKL/RANK/OPG* gene; Quantitative transmission disequilibrium test

Osteoporosis is a systemic skeletal disorder characterized by impaired bone strength and an elevated risk of osteoporotic fractures. Osteoporosis represents a major health problem throughout the world and the most serious consequence of osteoporosis is hip fracture, which has a high associated morbidity and mortality [1]. Currently, measurements of bone mineral density (BMD) are widely used to assess hip fracture risk [2,3]. However, BMD alone can only account for about 50–70% of total bone strength variation [4]. Among other risk factors of hip fracture, femoral neck width (FNW) and body weight have been repeatedly reported to be correlated with hip fracture risk [5–10]. Femoral neck compression strength index (fCSI) [11], a function of femoral neck BMD (fBMD), FNW, and weight based on structural engineering principles, may have the potential to improve hip fracture risk assessment. Karlamangla et al. [11] observed that a 1-standard-deviation (SD) decrease in fCSI was associated with an approximately 2.56-fold increase in hip fracture risk ($P < 0.0001$), while a 1-SD decrease in fBMD was associated with only a 1.96-fold increase in hip fracture ($P = 0.0013$).

Identification of genetic factors contributing to fCSI may help us understand the pathogenesis of osteoporosis and, also, help in the development of better diagnostic, prevention and treatment strategies. Thus far, little is known about the genetic determinants underlying fCSI.

The receptor activator of the nuclear factor- κ B ligand (RANKL)/receptor activator of the nuclear factor- κ B (RANK)/osteoprotegerin (OPG) pathway plays an important role in the regulation of bone remodeling and osteoclast differentiation and osteolysis [12]. Ligation of RANK with RANKL can promote formation, activation, and survival of multinucleated osteoclasts during normal bone remodeling and a variety of pathologic conditions [13]. OPG, a decoy receptor for RANKL, protects bones from excessive bone resorption via binding to RANKL and preventing it from binding to RANK [14]. The genes encoding these products are well accepted as candidate genes that influence the development of osteoporosis [15,16]. Nevertheless, most studies implicating these genes have focused on association analyses with BMD, often yielding inconsistent conclusions. For example, Koh et al. [17] observed a statistically significant association of *RANK* polymorphisms with BMD of proximal femur sites, while Kim et al. [18] did not find any association between *RANK* and fBMD. Similar inconsistencies exist in studies that investigated the relationship of *RANKL* or *OPG* to BMD [18–20]. In the current study, for the first time, we comparatively examined the influence of *RANKL/RANK/OPG* gene variants on fCSI, fBMD, FNW, and weight variation in a family-based cohort. The purpose of this study was to advance our knowledge about the role of the RANKL/RANK/OPG pathway in hip osteoporotic fracture risk and to help fill some gaps in our understanding of fCSI as a predictor of osteoporosis.

Materials and Methods

Subjects

This study was approved by the Institutional Review Boards of all involved institutions. Signed informed-consent documents were obtained from all study participants before they entered the study. For each study subject, information on age, gender, medical history, and family history was acquired. The study design and sampling procedures were published previously [21]. Briefly, individuals with chronic diseases or conditions that might potentially affect bone mass, structure, or metabolism were excluded. All of the 1873 participants from 405 nuclear families were U.S. whites of European origin. Family size ranged from 3 to 12, and the average was 4.62. The sample yielded 1512 sib pairs and 2266 parent–offspring pairs in total.

Phenotype Measurement

fCSI is originally and generally derived from DXA measurements in the field. It is computed as follows:

$$\text{fCSI} = (\text{BMD} \times \text{FNW}) / \text{Weight} \quad (1)$$

where BMD refers to the projected (areal) BMD of the femoral neck examined by DXA. FNW was computed as femoral neck area (also by DXA) divided by the width of the neck box (standard width = 1.5 cm [22]). According to standard protocol, the neck box includes the entire width of the neck and is positioned at the neck's narrowest section [11]. BMD and bone area at the femoral neck were measured using a Hologic QDR 2000+ or 4500 DXA (Hologic Inc., Bedford, MA, USA) machine. Both machines were calibrated daily. The coefficients of variation (CV) values of the DXA measurements for BMD were 1.87% on

the Hologic 2000+ and 1.98% on the Hologic 4500. The CVs of femoral neck area measurements obtained on the Hologic 2000+ and 4500 were 2.70% and 2.78%, respectively. Approximately 92% of the subjects were measured with the Hologic 4500 scanner and members from the same nuclear family were measured on the same scanner. BMD data obtained from different machines were transformed to a compatible measurement using the transformation formula described by Genant [23]. Weight was measured with the subject in light indoor clothing, using a calibrated balance beam scale. Height was measured using a calibrated stadiometer. The CV values of weight and height measurements were 1.2% and 0.9%, respectively.

SNP Selection and Genotyping

Genomic DNA was extracted from whole blood using a commercial isolation kit (Gentra Systems, Minneapolis, MN, USA). DNA concentration was assessed by a DU530 UV/VIS spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA). The candidate SNPs were selected according to several widely used public databases such as dbSNP (<http://www.ncbi.nlm.nih.gov/SNP>) and HapMap (<http://www.hapmap.org>). A total of 41 SNPs in the three genes of interest (12 for *RANKL*, 19 for *RANK*, 10 for *OPG*) were selected on the basis of the following criteria: (1) validation status in Caucasians; (2) an average density of 1 SNP per 3–5 kb; (3) degree of heterozygosity, i.e., minor allele frequencies (MAF) >0.05; (4) functional relevance and importance; and (5) having been reported to dbSNP by various sources. According to the HapMap data and the linkage disequilibrium (LD) patterns ($r^2 \geq 0.8$) of these three genes, the 12 SNPs of *RANKL* captured variation of 35 SNPs in this region, the 19 SNPs of *RANK* captured 31 SNPs in this region, and the 10 SNPs of *OPG* captured 41 SNPs in this region, respectively. All of the SNPs were successfully genotyped using the high-throughput BeadArray SNP genotyping technology of Illumina Inc. (San Diego, CA, USA). According to Illumina's report, the average rate of missing genotype data was ~0.05% and the average genotyping error rate estimated through blind duplicating was <0.01%.

Statistical Analysis

PedCheck [24] was used to check Mendelian consistency of SNP genotype data, and inconsistent genotypes were removed. Then the error checking option in Merlin [25] was run to identify and disregard the genotypes flanking excessive recombinants, further reducing genotyping errors. Allele frequencies of all SNPs were calculated by allele counting. The PEDSTATS procedure embedded in Merlin was used to test the Hardy–Weinberg equilibrium. Four SNPs were removed due to the violation of any of the above rules. In total, 37 SNPs (10 for *RANKL*, 18 for *RANK*, 9 for *OPG*) were used for the following association analysis.

LD and haplotype analyses for the three candidate genes were based on 703 unrelated parents (340 males and 363 females ranging in age from 40.7 to 87.9 years) from the 405 nuclear families. Population haplotypes and their frequencies were inferred using PHASE v2.1.1 [26]. The GOLD program (<http://www.sph.umich.edu/csg/abecasis/GOLD/>) was used to define LD block structure and to chart pairwise $|D'|$ derived from haplotype data. HaploBlock-Finder (<http://cgi.uc.edu/cgi-bin/kzhang/haploBlockFinder.cgi/>) was used to identify block structures and select haplotype-tagging SNPs (htSNPs) for the three candidate genes. To infer haplotypes defined by the htSNPs within each block of each gene for all of the subjects among 405 families, we adopted the algorithm of integer linear programming (ILP) implemented in PedPhase V2.0 (<http://www.cs.ucr.edu/~jili/haplotyping.html>), which is based on LD assumption and is able to recover phase information at each marker locus with great speed and accuracy, even in the presence of 20% missing data [27].

Using the QTDT program (<http://www.sph.umich.edu/csg/abecasis/QTDT/>), we estimated the heritability of fCSI, fBMD, FNW, and weight. QTDT was also used to test the SNPs and haplotypes with estimated frequencies >5% for associations with all phenotypes in the entire sample. We adopted the orthogonal model implemented in QTDT for our association analyses. Within-family association tests were performed. *P*-values < 0.05 by QTDT were defined as nominally significant and were further subjected to Bonferroni correction to account for the multiple comparison problems. The experiment-level significance was set to 0.05/*N*, where *N* is the number of statistical tests. Specifically, the significance level for single SNP tests was 0.0014 (0.05/37). Similarly, 40 haplotypes were identified and the significance level for haplotype tests was 0.0013 (0.05/40). fCSI was calculated from raw values of fBMD, FNW, and weight. Stepwise linear regression was used to evaluate the effects of potential covariates (fBMD and FNW—age, sex, age², age² × sex, age × sex, height, and weight; fCSI and weight—age, sex, age², age² × sex, age × sex, and height), and only significant items (*P* < 0.05) were included as covariates to adjust the raw value for the subsequent association analyses. Age² was included in covariate screening based on the consideration that maybe the relationship between age and fCSI is more than a simple linear correlation. Such covariates have been widely considered in the field [28,29]. The distribution of the residuals of all the phenotypes was tested for normality by Kolmogorov–Smirnov test. The Box-Cox procedure was performed for phenotype residuals in nonnormal distribution. All of the above procedures were conducted using the software MINITAB (Minitab Inc., State College, PA, USA). Association analyses were also performed in the gender subgroups respectively to detect the potential sex-stratified bone effects of the three genes. The procedures were the same as those done in the entire samples.

Results

Heritability Calculation

Basic characteristics of the study sample are reported in Table 1. After adjusting for the significant covariates and the Box-Cox procedure, the residuals of all phenotypes of all subjects followed a normal distribution (*P* > 0.05). The narrow-sense heritability of fCSI, fBMD, FNW, and weight were estimated to be 47.6%, 62.2%, 35.5%, and 46.1%, respectively.

Linkage Disequilibrium and Haplotype Analyses

Basic characteristics of the studied SNPs are summarized in Table 2. The gene and LD structures are shown in Fig. 1. Ten SNPs of the *RANKL* gene spanned ~36 kb, with an average density of 1 SNP per 3.6 kb. These 10 SNPs constituted two blocks that were 28.1 and 9.8 kb in length for blocks 1 and 2, respectively. Block 1 spanned from the promoter to intron 2. Block 2 ranged from intron 2 to the 3' untranslated region (3'-UTR). Three htSNPs (rs9525641, rs2148073, rs3742257) were identified by HaploBlockFinder to represent common haplotypes of the overall two blocks (Fig. 1a).

As indicated in Fig. 1b, 18 SNPs are spaced 3.4 kb apart on average and cover the full transcript length of *RANK*. Seven LD blocks were identified, ranging in size from 5.9 to 11.1 kb. Blocks 1 and 2 were located in intron 1. Block 3 ranged from intron 2 to intron 3, while block 4 spanned from intron 3 to intron 4. Block 5 extended from intron 7 to intron 9. SNP 15 could not be assigned to any block due to its low LD with any of the other SNPs, so it was designated alone as block 6. Block 7 encompassed the 3'-UTR. Accordingly, 14 htSNPs were selected to represent the haplotype diversity of *RANK*.

OPG is 29 kb in length. Nine SNPs are indicated by their locations in Fig. 1c. The average density was 1 SNP per 3.2 kb. Two blocks, that are 10.9 and 23.8 kb long, were identified.

Block 1 spanned from intron 2 to the 3'-UTR, while block 2 ranged from the promoter to intron 1. Five htSNPs were selected to represent both blocks.

Association Analyses

Table 3 reports the results of SNP-based association tests. Nominally significant associations were found between fCSI and five markers (SNPs 1, 2, 4, 6, and 7) of *RANKL*. Three of these (SNPs 1, 2, and 6) remained significant after conservative Bonferroni correction ($P = 0.0007$, 0.0007 , and 0.0005 , respectively). These three SNPs were approximately in complete LD (D' values between SNP 1 and SNP 2, between SNP 1 and SNP 6, and between SNP 2 and SNP 6 were 1, 0.981, and 0.981, respectively). Statistically significant associations were found between fBMD and SNPs 1, 2, and 7 of *RANKL* and SNP 10 of *RANK*. Also, there was a significant association between weight and SNP 16 of *RANK*. However, they were not significant after Bonferroni correction. The haplotype-based association results based on htSNPs were generally consistent with the results from SNP-based association analyses (Table 4). Nominally significant associations with fCSI were found for two major haplotypes of *RANKL* in block 1, namely, hap1 and hap2 (i.e., "A-C," with a frequency of 16.2%, $P = 0.0003$, and "A-G," with a frequency of 39.3%, $P = 0.0024$, respectively). Hap1 remained significant after Bonferroni correction. Nominally significant associations were observed between these two haplotypes and fBMD ($P = 0.0461$ and 0.0053 , respectively). One nominally significant association was also found between weight and block 3-hap4 of *RANK* (i.e., "G-T," with a frequency of 54.8%; $P = 0.0162$). No significant association was detected in the sex-stratified analyses. Information about phenotypes in subjects with the different genotypes and haplotypes are listed in Tables 5 and 6.

Discussion

In the present study, we examined 405 nuclear families, using the QTDT approach, to test for associations between *RANKL/RANK/OPG* polymorphisms and variations in fCSI and its components (fBMD, FNW, and weight). For *RANKL*, we found that SNPs 1, 2, and 6 (namely, rs12585014, rs7988338, and rs2148073) were significantly associated with fCSI even after conservative Bonferroni correction. Both SNP1 and SNP2 are located in the promoter region of *RANKL*. According to FASTSNP (<http://fastsnp.ibms.sinica.edu.tw/pages/inputCandidateGeneSearch.jsp>), an online bioinformatics tools for SNPs, polymorphisms of these two SNPs may result in different transcription rates, which is an intriguing concept that warrants confirmation in an appropriate experimental model. SNP 6 in intron2 of *RANKL* was also found to be significantly associated with fCSI variation ($P = 0.0005$), an observation that has never been reported previously. All three of these SNPs belong to block 1 of *RANKL*, and haplotype analyses showed that hap1 in block 1 was also significantly associated with fCSI variation, corroborating our single-marker analyses. In contrast, no significant association was found between *RANKL* polymorphisms and fBMD, FNW, or weight in either single SNP tests or haplotype tests. We did not find any significant association between any phenotype and *RANK* or *OPG*, nor did we find significant association in the sex-stratified analyses. We even tested the associations between the three studied genes and five femoral neck geometric variables [30,31]: buckling ratio (BR), an index of bone structural instability; cross-sectional area (CSA), an indicator of bone axial compression strength; cortical thickness (CT); endocortical diameter (ED), an estimate of endosteal width; and section modulus (Z), an index of bone bending strength [21]. Nevertheless, no significant association was detected (data not shown).

The roles of the three studied genes in hip bone phenotype variations have been intensively investigated in recent years, with BMD being the primary phenotype [18,20,32–37];

however, much inconsistency exists. For example, Mencej et al. [37] reported that the 693G > C polymorphism of *RANKL* was associated with fBMD in postmenopausal women, while no association between *RANKL* polymorphism and fBMD was detected in Kim and coworkers' study [18]. For *OPG*, Moffett et al. [36] found that women with the C/C (Lys3Asn polymorphism) genotype had a higher risk of femoral neck fracture hip fracture than those with the G/G genotype. However, Zhao et al. [33] did not find any association between this very variant and fBMD in postmenopausal women. Similar inconsistencies exist in studies for *RANK* [17,18].

In this study, several *RANKL* polymorphisms were significantly associated with fCSI after Bonferroni correction. Since the two SNPs showing a strong association with fCSI, SNP1 (rs12585014) and SNP2 (rs7988338), are both located in the promoter region of *RANKL*, we speculate that the significant association between *RANKL* and fCSI may be directly related to the regulation of *RANKL* gene expression. The effects of *RANKL* variation on fCSI may not be affected by gender since we did not detect any significant association in the sex-stratified analyses. Moreover, *RANKL* variants may not relate to the components of fCSI or femoral neck geometric variables individually since no significant association was found in the corresponding analyses. Previous genomewide linkage studies have suggested the importance of Chromosome13q, the genomic region harboring *RANKL*, to fCSI variation. For example, suggestive linkage between Chromosome13q and hip bone geometric indexes in Caucasian subjects was found in the Framingham study [38]. Similarly, Chromosome 13q showed at least three putative loci related to obesity [39]. Moreover, Eghbali-Fatourehchi et al. [40] reported that upregulation of *RANKL* in bone marrow cells can promote bone resorption, providing biological support for a functional role of *RANKL* in bone remodeling. Many studies showed that fBMD, femoral neck geometric parameters, and weight are highly correlated with each other [41–43]. Common genetic factors and molecular pathways may contribute to their variations in human population. Similar to metabolic syndrome (Mets), which was defined by multiple concurrent diseases including abdominal obesity, insulin resistance, dyslipidemia, and elevated blood pressure [44], one phenotype alone may not be able to predict hip fractures well. Thus, fCSI, which combined fBMD with FNW and weight, may help to investigate the genetic factors that may have a common contribution to a clustering of hip fracture-related phenotypes, maximizing the chance to identify hip fracture-related genetic factors.

Several limitations of the current study must be addressed. First, BMD values and femoral neck area obtained by DXA can provide only a two-dimensional approximation of the true three-dimensional structure of bone. Three-dimensional bone structure can be measured by volumetric quantitative computed tomography or magnetic resonance imaging [45], but these two methods are not feasible for large-scale investigations due to the high cost, long analysis time, and relatively high radiation exposure. Second, FNW was computed as the femoral neck area divided by the width of the neck box, presumed to be a constant of 1.5 cm. The constant is only an approximation. Consequently, our approach toward measuring FNW represents only an approximation. Nevertheless, Alonso et al. [46] found good correlations between automated measurements similar to those utilized in the current study and more cumbersome and time-consuming manual measurements. We are therefore confident in the phenotypic parameters utilized in the current study between manual and automated measurements.

In summary, using a relatively large sample of nuclear families, we investigated the association of *RANKL/RANK/OPG* gene polymorphisms with fCSI and its three components. Our findings suggest that polymorphisms of *RANKL* play important roles in determination of fCSI, a trait that warrants further study since it is a good and independent

predictor of hip fracture risk, except for fBMD and FNW. Further statistical and functional studies are necessary to replicate and confirm our findings.

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Fig. 1. Genomic structures and linkage disequilibrium (LD) structures of *RANKL*, *RANK*, and *OPG*. The analyzed SNPs are marked in the sequence by their locations. SNP IDs of each gene correspond to those in Table 2. The htSNPs are in boldface. Block size is indicated. The color darkens as D' between SNPs increases. **a** *RANKL*; **b** *RANK*; **c** *OPG*

Table 1

Basic characteristics of the study subjects

Trait	Male		Female	
	<i>N</i>	Mean $\pm$ SD	<i>N</i>	Mean $\pm$ SD
Height (m)	680	1.78 $\pm$ 0.07	1039	1.64 $\pm$ 0.06
Weight (kg)	684	88.96 $\pm$ 15.66	1043	71.54 $\pm$ 16.07
Age (yr)	747	48.93 $\pm$ 17.24	1118	46.69 $\pm$ 26.83
fBMD (g/cm ²)	698	0.86 $\pm$ 0.15	1049	0.81 $\pm$ 0.14
FNK_Area (cm ²)	684	5.90 $\pm$ 0.48	1041	5.02 $\pm$ 0.38
FNW (cm)	684	3.93 $\pm$ 0.32	1041	3.35 $\pm$ 0.25
fCSI (g/kg $\times$ m)	622	3.89 $\pm$ 0.75	967	3.90 $\pm$ 0.79

fBMD femoral neck bone mineral density, *FNK* femoral neck, *FNW* femoral neck width, *fCSI* femoral neck compression strength index

Table 2

Basic characteristics of all SNPs for *RANKL*, *RANK*, and *OPG*

SNP ^a	Name ^a	Position	Alleles ^b	MAF	Region
<i>RANKL</i>					
1	rs12585014	42038559	G/A	0.1805	Promoter
2	rs7988338	42042842	G/A	0.1803	Promoter
3	rs9525641	42046024	A/G	0.4211	Intron1
4	rs2277438	42053168	A/G	0.1808	Intron1
5	rs9525645	42057925	A/G	0.1840	Intron2
6	rs2148073	42061799	G/C	0.1821	Intron2
7	rs2200287	42066660	G/A	0.3978	Intron2
8	rs3742257	42071198	G/A	0.4839	Intron2
9	rs1054016	42080002	C/A	0.3949	Exon5
10	rs346578	42080987	G/A	0.0614	3'-UTR
<i>RANK</i>					
1	rs4941125	58148664	A/G	0.3132	Intron1
2	rs7235803	58151359	A/G	0.3372	Intron1
3	rs4436867	58154624	C/A	0.2363	Intron1
4	rs8086340	58157958	C/G	0.4436	Intron1
5	rs12956925	58164620	G/A	0.1691	Intron1
6	rs3826619	58166730	G/A	0.1055	Intron2
7	rs11664594	58169186	T/A	0.3480	Intron3
8	rs3826620	58172484	C/A	0.2811	Intron3
9	rs12969194	58175045	T/A	0.2702	Intron4
10	rs4303637	58182743	A/G	0.3250	Intron7
11	rs17069904	58183929	G/A	0.0970	Intron7
12	rs6567274	58188780	C/A	0.3455	Intron9
13	rs12959396	58190289	A/C	0.4766	Intron9
14	rs4426449	58193797	G/A	0.3447	Intron9
15	rs9646629	58202179	G/C	0.3572	Intron9
16	rs884205	58205837	C/A	0.2628	3'-UTR

SNP ^a	Name ^a	Position	Alleles ^b	MAF	Region
17	rs2957127	58210345	G/A	0.4507	3'-UTR
18	rs3017365	58214321	G/A	0.4908	3'-UTR
<i>OPG</i>					
1	rs11573959	120003443	G/A	0.1801	3'-UTR
2	rs6469783	120008446	G/A	0.1267	Intron3
3	rs4876869	120010458	A/G	0.4536	Intron2
4	rs1564858	120014347	G/A	0.1233	Intron2
5	rs1872426	120018937	C/A	0.4802	Intron1
6	rs7464496	120024023	A/G	0.4789	Intron1
7	rs2073618	120033233	C/G	0.4786	Exon1
8	rs4242592	120038156	C/A	0.4410	Promoter
9	rs10505349	120042699	G/C	0.2026	Promoter

UTR untranslated region

^aThe hSNPs are in boldface italics

^bThe second allele is the minor allele

Table 3

Single-SNP analysis for all phenotypes

SNP ^a	Name	<i>P</i> -value ^b (fCSI)	<i>P</i> -value ^b (fBMD)	<i>P</i> -value ^b (weight)
<i>RANKL</i>				
1	rs12585014	<i>0.0007</i> *	0.0483	0.0959
2	rs7988338	<i>0.0007</i> *	0.0488	0.0513
4	rs2277438	<i>0.0043</i>	0.1424	0.1529
6	rs2148073	<i>0.0005</i> *	0.1007	0.0616
7	rs2200287	<i>0.0023</i>	<i>0.0059</i>	0.2275
<i>RANK</i>				
10	rs4303637	0.0983	<i>0.0300</i>	0.7026
16	rs884205	0.1430	0.6763	<i>0.0249</i>

Note: Only SNPs nominally significant for any one phenotype are listed here

^aThe IDs of SNPs correspond to Table 2

^b*P*-values < 0.05 are in boldface italics.

* Significant *P*-values after Bonferroni correction. The significance threshold is $P < 0.0014$ (0.05/37)

Table 4

Haplotype analysis for all phenotypes

Block	Component SNPs ^a	htSNPs ^a	Type	Haplotype frequency	<i>P</i> -value ^b (FCSI)	<i>P</i> -value ^b (FBMD)	<i>P</i> -value ^b (FNNW)	<i>P</i> -value ^b (weight)
<i>RANKL</i>								
1	1-7	3, 6	AC	0.1624	0.0003 *	0.0461	0.0714	0.1474
			AG	0.3933	0.0024	0.0053	0.5986	0.4056
<i>RANK</i>								
3	6-7	6, 7	GT	0.5484	0.5528	0.4334	0.0162	0.6459

Note: Only haplotypes nominally significant for any one phenotype are listed here

^aThe IDs of SNPs and htSNPs correspond to Table 2

^b*P*-values < 0.05 are in boldface italics.

* Significant *P*-values after Bonferroni correction. The significance threshold is $P < 0.0013$ (0.05/40)

Table 5

Information about phenotypes in subjects with the different genotypes

SNP ^a	Type	N	Mean ± SD ^b				
				Weight (kg)	fBMD (g/cm ²)	FNW (cm)	fCSI (g/kg × m)
RANKL							
rs12585014	AA	43	82.38 ± 18.37	0.90 ± 0.13	3.63 ± 0.41	4.06 ± 0.69	
	AG	474	78.45 ± 18.00	0.83 ± 0.14	3.56 ± 0.39	3.86 ± 0.76	
	GG	1034	77.70 ± 17.55	0.83 ± 0.15	3.58 ± 0.40	3.90 ± 0.78	
rs7988338	AA	43	82.38 ± 18.37	0.90 ± 0.13	3.63 ± 0.41	4.06 ± 0.69	
	AG	473	78.40 ± 17.99	0.83 ± 0.14	3.56 ± 0.39	3.86 ± 0.76	
	GG	1034	77.70 ± 17.55	0.83 ± 0.15	3.58 ± 0.40	3.90 ± 0.78	
rs2277438	AA	1029	77.68 ± 17.51	0.83 ± 0.14	3.58 ± 0.40	3.91 ± 0.78	
	AG	475	78.44 ± 18.03	0.83 ± 0.14	3.56 ± 0.39	3.86 ± 0.76	
	GG	42	81.82 ± 18.22	0.90 ± 0.13	3.62 ± 0.41	4.07 ± 0.70	
rs2148073	CC	42	81.82 ± 18.22	0.90 ± 0.13	3.62 ± 0.41	4.07 ± 0.70	
	CG	480	78.50 ± 18.12	0.83 ± 0.14	3.56 ± 0.40	3.85 ± 0.76	
	GG	1027	77.74 ± 17.50	0.83 ± 0.14	3.58 ± 0.41	3.90 ± 0.78	
rs2200287	AA	218	78.21 ± 17.65	0.82 ± 0.13	3.58 ± 0.41	3.85 ± 0.77	
	AG	798	77.66 ± 17.34	0.83 ± 0.14	3.59 ± 0.41	3.92 ± 0.77	
	GG	535	78.60 ± 18.33	0.83 ± 0.15	3.56 ± 0.39	3.88 ± 0.77	
RANK							
rs4303637	AA	710	77.93 ± 17.60	0.83 ± 0.15	3.58 ± 0.42	3.93 ± 0.81	
	AG	667	77.96 ± 17.88	0.83 ± 0.14	3.57 ± 0.39	3.87 ± 0.74	
	GG	169	78.57 ± 17.60	0.82 ± 0.13	3.56 ± 0.36	3.83 ± 0.75	
rs884205	AA	113	80.68 ± 17.17	0.83 ± 0.11	3.56 ± 0.36	3.77 ± 0.74	
	AC	585	77.91 ± 18.01	0.83 ± 0.15	3.57 ± 0.38	3.89 ± 0.76	
	CC	845	77.73 ± 17.59	0.83 ± 0.14	3.58 ± 0.42	3.91 ± 0.79	

^aOnly SNPs nominally significant for any one phenotype are listed here

^bOnly raw values are listed. Raw values were adjusted by potential covariates (as described under Statistical Analysis) before association analysis

Table 6

Information about phenotypes in subjects with the different haplotypes

Block ^a	Haplotype name ^b	Type	Haplotype frequency	Mean ± SD ^c			
				Weight (kg)	IBMD (g/cm ²)	FNW (cm)	fCSI (g/kg × m)
RANKL							
Block 1	hap1	AC	0.1624	78.66 ± 18.02	0.84 ± 0.14	3.57 ± 0.39	3.90 ± 0.75
	hap2	AG	0.3933	77.85 ± 17.49	0.83 ± 0.14	3.58 ± 0.40	3.89 ± 0.77
	hap4	GG	0.4315	77.84 ± 17.84	0.83 ± 0.14	3.57 ± 0.40	3.90 ± 0.78
RANK							
Block 3	hap2	AT	0.0952	76.97 ± 16.99	0.84 ± 0.14	3.62 ± 0.44	4.04 ± 0.77
	hap3	GA	0.3415	78.17 ± 17.73	0.83 ± 0.14	3.57 ± 0.40	3.90 ± 0.77
	hap4	GT	0.5484	78.03 ± 17.73	0.82 ± 0.14	3.56 ± 0.40	3.86 ± 0.77

^a Only blocks in which haplotypes were nominally significant for any one phenotype are listed here. Haplotypes with a frequency <5% are not listed since they were not involved in association analysis

^b Subject with this haplotype

^c Only raw values are listed here. Raw values were adjusted by potential covariates (as described under Statistical Analysis) before association analysis