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Genetic Profiling of Decreased Bone Mineral Density in an Independent Sample of Caucasian Women

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Abstract

Purpose: We aimed to examine the distribution of risk alleles in an independent sample, and to determine if such genetic components are associated with bone mineral density (BMD) variation in the sample.

Methods: Existing genotype data of 1205 women in the cross-sectional Genomic Wide scans for Female Osteoporosis Gene Study (GWSFO) were analyzed. Multi-loci genetic risk scores (GRSs) based on 62 BMD-associated single nucleotide polymorphisms (SNPs) were calculated. Regression analysis was employed to assess the association between GRSs and BMD. To examine the effect of SNPs clustered within key pathways associated with the development of osteoporosis, subtype weighted GRS specific to WNT signaling (6 SNPs), RANK-RANKL-OPG (3 SNPs), and mesenchymal stem differentiation (3 SNPs) were generated for analysis.

Results: The unweighted GRS ranged from 48 to 80. One third of the women carried 66% risk alleles. After adjusting for age, height, and body weight, each unit increase of weighted GRS was associated with a decrease in BMD of 0.097 at femur ($p < 0.0001$) and 0.110 ($p < 0.0001$) at lumbar spine. The weighted GRS accounted for only 3.17–4.52% of BMD variance. The WNT-signaling pathway GRS (6 SNPs) and the RANK-RANKL-OPG signaling pathway GRS (3 SNPs) both were significantly associated with decreased BMD at femur neck ($p = 0.0004$ and $p = 0.0063$, respectively) and lumbar spine ($p < 0.0001$ and $p = 0.0001$, respectively), while the mesenchymal stem cell differentiation pathways (3 SNPs) GRS were associated only with the lumbar spine BMD ($p = 0.045$).

Conclusions: A substantially large percentage of healthy Caucasian women have a high genetic risk of osteoporosis. Weighted GRS was significantly associated with decreased BMD. The contribution of subtype GRS to the BMD variation differs by specific biological pathway and skeletal regions.

Mini Abstract:

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Disclosures

Xiangxue Xiao, Darius Roohani, and Qing Wu declare that they have no conflict of interest.

Genetic risk of low bone mineral density in women remains unclear. This study found that a large percentage of Caucasian women have a high genetic risk of osteoporosis, and genetic risk scores are significantly associated with BMD variation in a bone healthy sample of Caucasian women.

Keywords

genetic risk score; bone mineral density; osteoporosis; Caucasian women

Introduction:

Osteoporosis is a bone disease characterized by low bone mass and increased risk of fracture [1-3]. Worldwide, osteoporosis causes more than 8.9 million fractures annually, resulting in a heavy social and economic burden [4]. In the United States alone, osteoporosis-related fracture incidence is expected to grow by almost 50%, to >3 million by 2025, resulting in a corresponding expenditure of \$25.3 billion each year [5]. More notably, of the estimated 10 million Americans with osteoporosis, about 80% are women. More than half of all Caucasian women age 50 years and older are estimated to have low bone mass [6].

To date, bone mineral density (BMD) remains the operational definition of osteoporosis and the single best predictor of osteoporotic fracture risk [7, 8]. However, bone loss is an irreversible condition during aging, and thus of osteoporosis; as a result, once low-BMD is detected and osteoporosis diagnosed, current treatment will only help to prevent the condition from worsening [9, 10]. Therefore, early detection of osteoporosis and preventive intervention can help offset the risk of bone fragility.

Prior twin studies have reported substantial heritability (50% to 85%) for BMD, indicating that up to 80% of BMD variance is determined by genetic factors [11-16]. Phenotypic risk factors can change over time, but genes remain constant. Hence, in addition to the established factors, uncovering the underlying genetic risk inherent in certain populations will help identify susceptible groups and hasten the implementation of preventive treatments [17]. With a mounting number of genome-wide association studies (GWAS) implemented, dozens of Single Nucleotide Polymorphisms (SNPs) have been found to be associated with BMD [18, 19]. However, what remains unclear is how these variants cause decreased BMD and osteoporosis. The allelic frequency of these discovered SNPs in the general population is featured with high variability and small effect size. Therefore, it can be anticipated that the contribution of any single gene or single SNP to osteoporosis prognosis is minimal [20]. This suggests that osteoporosis may be caused by cumulative effects of many genetic variants. Based on a recent well-powered genome-wide meta-analysis [21], we generated genetic risk scores (GRS), which is the sum of risk alleles at each locus, in order to quantify the genetic effect contributing to population BMD variation.

Although prior literature has summarized the overall genetic risk for osteoporosis and low BMD, most of this research focused on the diseased population, so the genetic risk of decreased BMD and osteoporosis in a healthy population remains unclear. Therefore, quantifying such genetic risk may help with early detection of osteoporosis and facilitate preventive intervention for this disease, especially in Caucasian women, the most vulnerable

group. The Genomic Wide Scans for Female Osteoporosis Gene Study (GWSFO) is a whole-genome genotyping study that has never been applied in any BMD-associated SNP-discovering analysis. To take advantage of this independent dataset, our aim was to examine the distribution of BMD related genetic variants in a cohort free of osteoporosis and to further estimate the magnitude of the association between weighted GRS and BMD in a healthy population.

Moreover, previous studies have provided new insights on disease pathogenesis by determining that the associated loci can be traced to certain novel molecular pathways. Because many BMD associated loci do indeed map to genes that fall within pathways related to the regulation of bone formation [22], we further generated subtype GRS for specific biologic pathways, including WNT signaling pathway (6 SNPs), RANK-RANKL-OPG pathway (3 SNPs) and mesenchymal stem cell differentiation function (3 SNPs), and then examined their association with normal BMD variation in a healthy population.

Subjects and Methods:

Data source

The GWSFO was used as the data source for this study. With approval of the institutional review board at University of Nevada, Las Vegas, GWSFO genotype and phenotype data was acquired from dbGaP (phs000390.v1.p1). Genotyped GWSFO data had 1617 individuals with SNP genotypes of the Affymetrix 6.0 (“million-SNP”) Array set (Affymetrix, Santa Clara, California). A total of 934,940 SNP markers with known chromosome locations, and SNP markers with minor allele frequencies greater than or equal to 0.01, were analyzed.

Study subjects

The GWSFO study includes unrelated Caucasian subjects over 18 years of age, all of whom were recruited from the Kansas City and the surrounding area. Eligible participants were European-origin Caucasians who were willing to participate in the study and agreed to an exam for bone densitometry and a blood draw. Written informed consent was obtained from each participant. Participants were selected regardless of BMD or other clinical phenotypes and were generally healthy. Detailed GWSFO study design and recruitment was described at dbGap (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000390.v1.p1). Our present study restricted subjects only to women, and a total of 1,209 women were included in this sample. After removing the outliers, 1205 people were remained for analysis. Participants’ gender were verified using gender auto-detection from the original data on chromosome X. The results showed 97% accuracy of gender in this sample.

BMD measurement

BMD values (g/cm^2) were measured by using a dual-energy X-ray absorptiometry (DXA) machine – the Hologic Discovery DXA System. The lumbar spine BMD was derived by combining BMD of Lumbar Spine₁₋₄. The hip total BMD combined BMD of the femoral neck, trochanter, and intertrochanteric region.

Assessment of Covariates

The information of age and lifestyle factors that are important for bone mass variation was ascertained in the confidential interview that included a standard medical questionnaire, which consisted of variables used in this study, such as age, gender, race, exercise, smoking, and alcohol consumption. Height was measured in centimeters, in the standing position, and weight was measured in kilograms and with a balance beam. Both were measured using a calibrated Health-O-Meter Professional height and weight scale, with all wearing with light-weight, indoor clothing and wearing no shoes.

We defined heavy drinking by using over 10 drinks per week, the highest level of the origin GWSFO classification. It was not feasible to define heavy drinking using an exact definition from the Substance Abuse and Mental Health Service Administration as the original GWSFO study does not have such information. Following the American Heart Association (AHA) guidelines, smoking status in this study was categorized as “never”, “former”, and “current”.

Genotyping

Genotype data performed on blood samples was acquired through dbGaP. Genotype imputation was conducted at the Sanger Imputation Service. Haplotype Reference Consortium (HRC) reference panel, a most comprehensive imputation reference panel, as well as Positional Burrows-Wheeler Transform (PBWT) imputing algorithm, were used for the genotype imputation in order to ensure high imputation quality. In the large-scale GWAS meta-analysis a total of 63 SNPs was reported to be associated with BMD [19]. Because rs344081 was associated with BMD only in man [19], only 62 SNPs were included in the imputation and analysis. Imputation quality was excellent, with mean $R^2 = 0.99$.

Genetic risk score

A genetic risk score (GRS) is a standardized metric derived from the number of risk alleles and their effect size for each patient. This metric allows the composite assessment of genetic risk in complex traits. Before conducting the GRS calculation, we performed linkage disequilibrium (LD) pruning in case any LD existed between SNPs. None of the 62 SNPs was removed after pruning. Both unweighted and weighted GRS were then calculated with algorithms described by Estrada and colleagues [19]. Briefly, the unweighted multi-locus genetic risk score (UW-GRS) was generated by summing the number of risk-conferring alleles that each individual had (0, 1, or 2) across all loci. The overall weighted GRS for both femoral neck (FN-GRS) and lumbar spine (LS-GRS) were calculated separately for each individual by summing the number of risk alleles at each locus multiplied by their effect size, which are regression coefficients related to hip total and lumbar spine BMD, from the referenced literature [21].

With the intention of determining if GRS of WNT signaling (6 SNPs), RANK-RANKL-OPG (3 SNPs), and mesenchymal stem cell differentiation function (3 SNPs) was associated with BMD for the femur and lumbar spine, subset GRS regarding loci clustered within each pathway were calculated [21]. Specific gene and loci for each subset GRS are listed in supplementary table 1.

Statistical analysis

Data are presented as means $\pm$ SD, or as numbers and percentages. Bivariate association between BMD and a number of variables, such as smoking, drinking, and exercise status, was assessed for statistical significance using the student t-test method. In subgroup analysis, age was categorized into three groups (<35, 35-55, >55 years). Analysis of variance (ANOVA) was used to compare mean values of both GRS and BMD respectively by age groups. Bonferroni correction was applied to the multiple testing. UW-GRS was used to examine the distribution of the number of risk alleles in the study sample. Separate statistical models were employed to assess the association of BMD with different sets of GRS for each skeletal region. A model-selection analysis was conducted to determine which variables should be selected. The quadratic term of age, the interaction term of age and other variables (GRS etc.), and other biological plausible terms were included in turn and examined using *F*-test. However, the results showed that no quadratic term or the interaction term was significant in the model for BMD on the hip and lumbar spine in the healthy sample of Caucasian women. Therefore, the generic regression model was formulated as follow: $BMD = \beta_0 + \beta_1 * Age + \beta_2 * Age^2 + \beta_3 * Height + \beta_4 * Weight + \beta_5 * GRS_i$

Where β_5 are the expected change in dependent variable for a one-unit change in independent variables when the other covariates are held fixed. The same modeling approach was used by introducing various weighted GRS (*i*), including overall GRS (FN-GRS, LS-GRS), WNT signaling GRS (6 SNPs), RANK-RANKL-OPG GRS (3 SNPs), and mesenchymal stem cell differential GRS (3 SNPs). The strength of the effect of each individual independent variable and its relative importance to the dependent variable were further examined by using standard regression and the Lindeman, Merenda and Gold method (“lmg” method) [23], respectively. We also conducted sensitivity analysis by excluding subjects who were current smokers or heavy alcohol drinkers.

Results

Sample characteristics

The characteristics of 1205 participants are shown in Table 1. The subjects have an average age of 49 ± 13 years. The percentage of current smoker and heavy drinker were 8.6% and 2.7%, respectively. Using the criteria that osteoporosis was defined as T-score -2.5 at femoral neck, the prevalence of osteoporosis in this sample was 0.6%. The normally distributed unweighted GRS had a median of 63 (range from 48 to 80) (Supplementary Figure 1). Approximately 10% of individuals had unweighted-GRS fewer than 57; another 10% had more than 69 risk genotypes. The mean and range for the subtype unweighted GRS were: 7.8 (2 to 13) for WNT signaling pathway (6 SNPs); 2.6 (0 to 6) for RANK-RANKL-OPG signaling (3 SNPs); and 3.2 (0 to 6) for mesenchymal stem cell (3 SNPs).

The GRS and BMD of each skeletal region were further analyzed by smoking status, alcohol consumption, exercises status, and age group. As shown in Table 2, groups that smoked, or did not exercise had slight higher GRS. Younger group and non-smokers group had higher BMD of both hip and spine. We applied the Bonferroni correction to control the error rate for the multiple tests, the critical *p* level for significance was set to 0.003 after adjusting for

the 16 tests. The mean BMD of both the femur and the lumbar spine was significantly different among the three age groups ($p < 0.0001$). No significant difference in either FN-GRS or LS-GRS was found among the age groups. Significant linear correlation between weighted GRS and BMD were observed at both skeletal sites (Supplementary Figure 2).

GRS and BMD

The Multiple linear regression indicated that, along with age, the overall weighted GRS were significantly associated with lower BMD at both hip ($p < 0.0001$) and lumbar spine ($p < 0.0001$). For every one-unit increase of FN-GRS or LS-GRS, the BMD at femur and lumbar spine decreased for 0.097 g/cm^2 and 0.110 g/cm^2 , respectively. From the standardized regression analysis, compared with age, overall GRS contributed more to lowering BMD at lumbar spine. Body weight was positively associated with BMD at both skeletal sites. Height was significantly associated with lumbar spine BMD, but not with hip total BMD (Table 3). The four covariates (age, height, weight, and FN_GRS/LS_GRS) together explained ~33.61% and ~24.25% of the variance in hip total BMD and lumbar spine BMD, respectively. More notably, relative importance analysis showed that FN_GRS and LS_GRS accounted for 3.17% and 4.52% of the total explained variance, respectively.

To examine if association between BMD and GRS varies in different age stages, we conducted subgroup analysis to examine the effect of overall weighted GRS on BMD for each age group. After adjusting for height and body weight, the coefficient of weighted overall GRS in the regression model was -0.119 for group of age < 35 years ($p < 0.01$), -0.090 for group of age between 35 to 55 years ($p < 0.0001$), and -0.095 for group of age > 55 years ($p < 0.0001$). These results indicate that the effect of GRS to the normal variation of BMD was relatively constant for different age groups.

To examine the association between BMD and each subtype GRS particularly related to certain biologic pathways, separated multiple linear regressions were conducted for each subtype GRS and each estimate was adjusted for age, height, and body weight. As showed in Table 4, the WNT signaling GRS (6 SNPs) were significantly associated with lower BMD at either femur or lumbar spine. For every one-unit increase of WNT signaling GRS (6 SNPs), the BMD of the femur and the lumbar spine decreased for 0.111 g/cm^2 and 0.128 g/cm^2 , respectively. GRS of the mesenchymal stem cell differentiation function (3 SNPs) were negatively associated with lumbar spine BMD ($p = 0.045$), but not with hip total BMD ($p = 0.99$). For every one-unit increase of RANK-RANKL-OPG signaling (3 SNPs) and mesenchymal stem cell GRS (3 SNPs), the BMD at lumbar spine decreased 0.145 g/cm^2 and 0.138 g/cm^2 , respectively.

Sensitivity analysis was conducted to further examine the robustness of the above findings by excluding excessive alcohol users and tobacco users. The overall GRS and WNT signaling pathway GRS (6 SNPs) remain associated with lower BMD significantly at both skeleton sites. For every one-unit increase of the overall weighted GRS, hip total BMD decreased by 0.1 g/cm^2 , whereas lumbar spine BMD decreased by 0.109 g/cm^2 . Mesenchymal stem cell differentiation GRS (3 SNPs) was not associated with lower lumbar spine BMD after excessive alcohol users and current smokers were excluded (Table 5).

Discussion

In our present study, we constructed five sets of related GRS based on well-powered GWAS meta-analysis [20], and examined their association with BMD in a sample of healthy Caucasian women. We demonstrated that, after adjusting for age, body weight, and height, a higher GRS was significantly associated with lower BMD at both the femur and the lumbar spine. Considering the nature of the healthy sample for this study, our findings strongly indicated the ability of genetic profiling in explaining normal bone mass variation.

Susceptibility of low BMD was demonstrated as being highly heritable [24]. Based on the 63 BMD-lowering SNPs identified from large-scale GWAS meta-analysis, prior studies have demonstrated the significant association between GRS and low BMD [25]. Our results were consistent with findings from prior studies [20], as the weighted GRS were significantly associated with decreased BMD. As well, subtype GRS was significantly associated with decreased BMD at multiple skeletal sites. Notably, our standardized regression analysis demonstrated that the standardized coefficient of overall weighted GRS is comparable with that of age. Therefore, besides the significant role aging plays in normal BMD change, GRS, which was considered genetic causation of pathological change of BMD, shared almost the same importance as age to normal BMD variation. In addition, our subgroup analysis indicated GRS played an important role throughout the whole lifetime of individuals, with relatively constant effect on decreasing BMD in three age stages (<35, 35-55, >55 years). Likely, during the bone tissue accumulating stage at a young age, individuals with higher GRS may have less or slower bone mass increases compared to people with lower genetic risk of bone fragility; whereas in the BMD declining stage at old age, higher GRS may indicate greater bone loss.

Activities of osteocytes, osteoclasts, and osteoblasts are controlled by certain biological pathways, and they together regulate the process of bone turnover continuously. Canonical WNT signaling controls osteoblastic bone formation, while RANK-RANKL-OPG pathway controls osteoclastic bone resorption [26-31]. Prior study has provided strong statistical evidence of the significant clustering of the BMD-associated loci in the three key biological pathways (WNT-signaling, RANK-RANKL-OPG, and mesenchymal stem cell differentiation) by applying the Gene Relationships Across Implicated Loci text-mining algorithm [20]. To understand the polygenic nature of BMD and the critical role of those biological pathways influencing BMD in the healthy Caucasian sample, we calculated GRS for WNT signaling pathway (6 SNPs), RANK-RANKL-OPG signaling pathway (3 SNPs), and mesenchymal stem cell (3 SNPs). The results showed that association between most subtype GRS and decreased BMD was significant. Both GRS of WNT signaling and RANK-RANKL-OPG pathways were negatively associated with BMD. GRS of mesenchymal stem cell was found to have negative association with total spine BMD. Overall, our findings regarding subtype GRS were consistent with prior studies involving different samples [21]. In a study by Warrington et al, although their algorithm used to calculate the GRS was adding up the risk alleles of each loci without considering the different strength of each SNP, associations between those three biology pathways specified GRS in adolescents were still observed [21]. Similarly, association between several bone metabolisms related subtype GRS and Z-score in the pediatric field were also observed by

Mitchell et al [32]. Due to different study methodology and population setting, varied magnitude of association between GRS and BMD has been observed. While the pathway-specific GRSs produced significant and meaningful results, these should be interpreted with caution. The reason for this is the possibility that major contributing SNPs are outside the pathway chosen to be studied, and the occurrence of considerable cross talk occurs between overlapping pathways. More studies regarding pathway-specific GRSs of osteoporosis are warranted.

The present study has some limitations. First, the study was conducted on secondary data. Due to lack of menopausal information, we were unable to adjust for the effect of menopause related BMD variation in the study sample. Second, because the original GWSFO was a cross-sectional study, a casual effect cannot be determined due to the nature of the study design. However, it is biologically plausible to assume that increased GRS leads to decreased BMD not that decreased BMD causes increased GRS.

In summary, we examined the association between GRSs and BMD in a bone healthy sample, and our findings suggest the large percentage of Caucasian women have high genetic risk profiling, which may be the cause of high risk of bone fragility in their later life, as high GRS is associated with decreased BMD. Considering the high prevalence of osteoporosis among Caucasian women, examination of genetic profiling in this population may help early detection and prevention of osteoporosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

The data/analyses presented in the current publication are based on the use of study data downloaded from the dbGaP web site, under phs000390 (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000390.v1.p1). The research and analysis described in the current publication was supported by a grant from the National Institute of General Medical Sciences (5 U54 GM 104944). The funding sponsor was not involved in the analysis design, genotype imputation, data analysis, interpretation of the analysis results or in the preparation, review and/or approval of this manuscript.

Abbreviations

GRS	genetic risk score
BMD	bone mineral density
SNP	single nucleotide polymorphism
GWAS	genome-wide association study
dbGaP	the database of Genotypes and Phenotypes
GWSFO	Genomic Wide Scans for Female Osteoporosis Gene Study

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Table 1.

Basic characteristics of the GWSFO sample used in the analysis (n=1205)

		Femoral neck GRS		Lumbar spine GRS		Hip total BMD		Spine total BMD	
		Mean $\pm$ SD	p-value	Mean $\pm$ SD	p-value	Mean $\pm$ SD	p-value	Mean $\pm$ SD	p-value
Smoking	Yes	3.070 $\pm$ 0.260	0.17	3.225 $\pm$ 0.285	0.69	0.953 $\pm$ 0.138	0.23	1.015 $\pm$ 0.141	0.40
	No	3.049 $\pm$ 0.251		3.218 $\pm$ 0.270		0.964 $\pm$ 0.140		1.022 $\pm$ 0.143	
Alcohol consumption	Yes	3.058 $\pm$ 0.258	0.62	3.218 $\pm$ 0.277	0.55	0.964 $\pm$ 0.138	0.06	1.023 $\pm$ 0.141	0.06
	No	3.048 $\pm$ 0.238		3.231 $\pm$ 0.265		0.944 $\pm$ 0.144		1.003 $\pm$ 0.145	
Exercise	Yes	3.051 $\pm$ 0.253	0.21	3.215 $\pm$ 0.273	0.15	0.959 $\pm$ 0.140	0.37	1.017 $\pm$ 0.141	0.38
	No	3.073 $\pm$ 0.258		3.242 $\pm$ 0.282		0.967 $\pm$ 0.137		1.026 $\pm$ 0.144	
Age (years)	35	3.075 $\pm$ 0.246	0.49	3.247 $\pm$ 0.260	0.30	1.015 $\pm$ 0.125	<.0001	1.043 $\pm$ 0.112	<.0001
	35-55	3.051 $\pm$ 0.252		3.213 $\pm$ 0.276		0.974 $\pm$ 0.135		1.036 $\pm$ 0.138	
	55	3.052 $\pm$ 0.262		3.219 $\pm$ 0.280		0.905 $\pm$ 0.137		0.977 $\pm$ 0.155	

Table 2.

Baseline description of weighted GRS and BMD at different skeletal sites stratified by smoking status, alcohol drinking, exercise, and age groups: results of t-test, analysis of variance (ANOVA) (N=1205)

Variable	Mean $\pm$ SD
Age (years)	48.71 $\pm$ 12.96
Height (cm)	163.39 $\pm$ 6.35
Weight (kg)	70.59 $\pm$ 16.21
Current smoker, n (%)	103 (8.55)
Alcohol consumption with 10/week, n (%)	33 (2.74)
BMD at lumbar spine (g/cm ²)	1.02 $\pm$ 0.14
BMD at femur neck (g/cm ²)	0.96 $\pm$ 0.14
Genetic risk score (unweighted)	
Overall	63.18 $\pm$ 4.96
WNT signaling	7.78 $\pm$ 1.77
RANK-RANKL-OPG	2.61 $\pm$ 1.15
Mesenchymal stem cell	3.22 $\pm$ 1.18

Table 3.

Association between Overall Genetic Risk Score (GRS) and BMD: Results of Multiple Linear Regression Analysis (N=1205)

BMD sites and covariates	Regression Coefficient (SE)	p	Standardized Regression	Relative importance* (%)
Hip total BMD				
Age	−0.004 (0.0003)	<0.0001	−0.355	11.50
Height	0.000 (0.0005)	0.43	0.020	1.45
Weight	0.004 (0.0002)	<0.0001	0.431	17.49
Femoral neck GRS (Overall)	−0.097 (0.0129)	<0.0001	−0.176	3.17
Spine total BMD				
Age	−0.002 (0.0003)	<0.0001	−0.216	4.53
Height	0.002 (0.0006)	0.001	0.084	2.28
Weight	0.003 (0.0002)	<0.0001	0.359	12.92
Lumbar spine GRS (Overall)	−0.110 (0.013)	<0.0001	−0.214	4.52

* Note: the model overall can explain 33.61% ($r^2=0.3361$) and 24.25% ($r^2=0.2425$) of the total variance of A and lumbar spine BMD.

Table 4.

Association between Subtype Genetic Risk Score (GRS) and BMD: Results of Multiple Linear Regression Analysis (N=1205)

BMD sites	Genetic risk score	Regression Coefficient (SE)	P- value	Standardized Regression
Hip total BMD				
	WNT signaling	-0.111 (0.03)	0.0004	-0.086
	RANK-RANKL-OPG signaling	-0.145 (0.066)	0.0063	-0.066
	Mesenchymal stem cell	-0.001(0.076)	0.99	-0.000
Spine total BMD				
	WNT signaling	-0.128 (0.029)	<0.0001	-0.113
	RANK-RANKL-OPG signaling	-0.138 (0.036)	0.0001	-0.099
	Mesenchymal stem cell	-0.138 (0.069)	0.045	-0.052

* Separated multiple regression was conducted for each subtype GRS, each estimate was adjusted for age, height, and weight.

Table 5.

Association between Subtype Genetic Risk Score (GRS) and BMD after excluding Excessive alcohol or tobacco users (N=1076)

BMD sites	Genetic risk score	Regression Coefficient (SE)	P- value	Standardized Regression
Hip total				
	Overall	-0.100 (0.014)	<0.0001	-0.182
	WNT signaling	-0.118(0.033)	0.0003	-0.092
	RANK-RANKL-OPG signaling	-0.142 (0.056)	0.012	-0.064
	Mesenchymal stem cell	0.022 (0.080)	0.787	0.007
Lumbar spine				
	Overall	-0.109 (0.014)	<0.0001	-0.211
	WNT signaling	-0.149 (0.031)	<0.0001	-0.132
	RANK-RANKL-OPG signaling	-0.128 (0.038)	0.0007	-0.093
	Mesenchymal stem cell	-0.129 (0.072)	0.07	-0.049

* Separated multiple regression was conducted for each subtype GRS, each estimate was adjusted for age, height, and weight.