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Identification of genetic and clinical risk factors for bone loss in Korean population

한국인의 골 소실에 영향을 미치는 유전적 및 임상적 요인의 발굴

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이 지 현

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Department of Medicine

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Abstract

Identification of genetic and clinical risk factors for bone loss in Korean population

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Background and Purpose: Excessive loss of bone mass is related to a higher risk of fragility fractures and mortality. However, few longitudinal studies have investigated the risk factors for bone mass loss. This study aimed to identify the clinical and genetic risk factors for bone mass loss in the Ansung cohort, from a Korean community-based prospective cohort study, during a period of 6 years.

Methods: We enrolled men aged 50 years and older (n=645) and postmenopausal women (n=683) who underwent bone mineral density (BMD) measurement twice using dual-energy X-ray absorptiometry between 2007 and 2014. A multiple linear regression was used to analyze the relationship between annualized hip BMD changes and covariates. Further, we analyzed a total of 2,614 single-nucleotide polymorphisms (SNPs) extracted from 23 candidate genes related to bone metabolism and previous genome-wide association studies. The Gene-Environment Interaction

and Phenotype cohort, which included people from a health check-up program, was then used to validate the related genetic variants.

Results: The rate of hip BMD loss was faster in women than in men with increasing age. The annualized hip BMD changes in men were positively correlated with the waist circumference (WC) (β=0.019, *P*<0.001), lean mass (LM) change (%/y) (β=0.274, *P*<0.001), alcohol intake (β=0.112, *P*=0.049), and increased red blood cell count (β=0.144, *P*=0.030) but were negatively correlated with the current smoking status (β=−0.122, *P*=0.028). In women aged 45–59 years, increasing WC (β=0.016, *P*=0.010) and LM changes (%/y) (β=0.452, *P*<0.001) were positively correlated with the annualized hip BMD change, whereas years since menopause ≤3 years (β=−0.311, *P*=0.004) was inversely correlated with the annualized hip BMD change. The annualized hip BMD change in women aged ≥60 years, significantly correlated with increasing age (β=−0.023, *P*=0.020), WC (β=0.011, *P*=0.018), LM change (%/y) (β=0.108, *P*=0.039), fat mass change (%/y) (β=0.039, *P*=0.001), alcohol intake (β=−0.245, *P*=0.021), and platelet count (β=−0.002, *P*=0.014). Rs4988300 of *LRP5* (β=0.127, *P*=0.007) and rs7325635 of *TNFSF11* (β=0.146, *P*=0.001) showed the best correlation with the annualized hip BMD change in men and women, respectively. In men, the rs2470688 variant in the intron of *PRKCB* was correlated with the annualized hip BMD change (*P*=0.009 and 0.003 in the Ansung and validation cohorts, respectively). However, after Benjamini-Hochberg adjustment, none of the SNPs were correlated with the annualized hip BMD change.

Conclusions: Low WC and LM loss were correlated with an increased risk of hip BMD loss in Korean men and women. Sex- and age-specific factors that impact on

the hip bone mass loss were also identified. None of the SNPs were correlated with the hip BMD loss after multiple comparisons. Early detection of the risk factors for BMD loss may result in the development of individualized osteoporosis and fracture prevention strategies. Further studies are necessary to better determine the risk of loss of bone mass in men and women.

Keywords: Osteoporosis, Bone density, Body composition, menopause

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List of Abbreviations

BMD: Bone mineral density

BMI: Body mass index

CI: Confidence interval

DXA: Dual-energy X-ray absorptiometry

FM: Fat mass

FN: Femur neck

GENIE: Gene-Environment Interaction and Phenotype

GWAS: Genome-wide association studies

GWS: Genome-wide significance

LM: Lean mass

LS: Lumbar spine

RANK: Receptor activator of nuclear factor-kappaB

RANKL: RANK ligand

RBC: Red blood cell

SNP: Single-nucleotide polymorphism

TH: Total hip

VDR: Vitamin D receptor

VIF: Variance inflation factor

WC: Waist circumference

YSM: Years since menopause

Introduction

Bone mass loss is a natural feature of the aging process, which results from disproportionate bone resorption relative to formation and it plays a role in the pathophysiology of osteoporosis. After achieving a peak bone mass, bone loss continues throughout life (1). A previous study revealed that loss of bone mass increases with age and it is more distinct in women than in men (2). Excessive loss of bone is related to a higher risk of fragility fractures (3) and death (4).

Osteoporosis is a common disease characterized by low bone density, degradation of the bone micro-architecture, and increased risk for fracture (5). The single best indicator of osteoporotic fractures is bone mineral density (BMD), which has been commonly used as a reference standard in the description of osteoporosis (6). Osteoporosis is defined as a BMD T-score of −2.5 or lower (7). Among Koreans aged ≥50 years, the prevalence of osteoporosis ranged from 7.3% to 12.9% in men and 24.0% to 38.0% in women (8, 9).

It is important to identify factors that will help screen high-risk individuals who are prone to bone loss, in order to reduce the risk of osteoporosis and osteoporotic fractures. A cost-effective risk assessment is required to classify high-risk individuals for appropriate care. Specifically, in those with osteopenia (−2.5 < T-score ≤−1), considering the risk factors for bone mass loss is important in determining the need for BMD re-assessment (7).

BMD and bone loss risk are strongly heritable (10). Several environmental factors influence bone formation and resorption over an individual's lifetime; nonetheless, a

previous study among twins and a family reported that BMD is a highly heritable trait, with heritability estimates ranging from 50% to 80% (11). Children of women with osteoporosis are more likely to have reduced BMD (12). In a study on bone loss involving postmenopausal female twins over a 5-year period, Makovey et al. showed that approximately 40% of variation in bone mass loss at the lumbar spine (LS) was determined by genetics (13). Estrogen deficiency after menopause is one of the most significant factors for bone loss in women, and a study among twins showed that the age for onset of menopause is genetically influenced (14).

Wnt signaling and the *RANKL/RANK/OPG* are two major signaling pathways that determine the bone mass (15, 16). Stimulation of bone resorption is a common component in the etiology of bone loss and fractures (17). Various loci have been found to be related to BMD and fractures in genome-wide association studies (GWAS). Initial studies identified several loci related to BMD, including *RANK*, *RANKL*, *OPG, ESR1*, *ZBTB40*, and *LRP5* (18-20). In a large candidate gene study based on GWAS data from five cohorts, nine genes (*ESR1*, *ITGA1*, *LRP4*, *LRP5*, *SPP1*, *SOST*, *TNFRSF11A*, *TNFSF11, TNFRSF11B)* were correlated with LS-BMD, whereas *LRP5*, *SPP1*, *SOST,* and *TNFRSF11A* were correlated with osteoporotic fractures (21). Nonetheless, the correlation between osteoporosis-related genetic loci and bone loss is not well understood.

Peak bone mass is likely to be genetically determined; however, bone loss is influenced by lifestyle factors such as cigarette smoking, alcohol intake, calcium intake, and physical activity (11). Weight loss is an important determinant of bone loss (22). Body weight is largely made up of fat mass (FM) and lean mass (LM)

(23). However, data regarding the correlation between body composition and bone mass loss are limited. The role of body composition in bone loss could be dependent on age, sex, and ethnicity (24). Aging is related to the loss of LM and strength, and in older people, age-related LM loss is followed by fat gain (25). LM loss, a well-known mechanical mediator of bone health, may potentially play a role in age-related changes in bone mass and quality. A previous study showed a correlation between LM and BMD at multiple skeletal sites in men and women; however, the participants were mostly Caucasian (26). Moreover, hematopoiesis and bone metabolism are linked (27). A previous study reported a 38% higher incidence of hip fracture in postmenopausal women with anemia (28). In both men and women, the highest platelet count within the normal range was significantly related to osteopenia and osteoporosis (29).

Changes in BMD have been shown to be a significant risk factor for fractures in both men and women, regardless of baseline BMD (1). However, there is little data on longitudinal BMD changes in Asian populations.

Thus, first improving modifiable factors is a good approach to avoid accelerated bone loss in individuals with osteoporosis who are at high risk for fracture. This present study aimed to identify the genetic and clinical risk factors for bone mass loss in a community-based prospective cohort of Korean men aged 50 years and older and postmenopausal women.

Materials and Methods

1. Study participants

1-1. Participants in the candidate gene association study

We selected participants from the Ansung cohort for the candidate gene association study, which is a large prospective, community-based, and epidemiologic cohort study. The Ansung project is part of the Korean Genome and Epidemiology Study, which is funded by the Korean government and it aims to identify the gene-environment variables and their interactions in patients with chronic diseases (30). Since 2001, a biennial review and survey have been conducted. The participants were between the ages of 40 and 69 years. All participants signed a written informed consent. Of the 5,018 participants, 3,233 underwent dual-energy X-ray absorptiometry (DXA) measurement on the fourth wave (2007–2008), and 2,596 participants on the seventh wave (2013–2014). On both the fourth and seventh waves, roughly 6 years after the baseline test, a total of 2,293 participants underwent DXA examinations.

Men aged <50 years (n=168), premenopausal women (n=262), participants taking anti-osteoporotic medication (n=169), those with cancer (n=15), and those without waist circumference (WC) (n=45), body composition (n=63), and genomic (n=242) data were excluded from this research. The final analysis included 1,328 participants (men, n=645; postmenopausal women, n=683) (**Figure 1**). The age of men ranged from 50 to 75 years, whereas that of postmenopausal women ranged from 45 to 76 years. Lifestyle and sociodemographic characteristics such as age, sex, drinking and

smoking habits, exercise habits, anti-osteoporotic medication use, menopause, hormone replacement therapy, history of diabetes, and hypertension, were all obtained through interviews.

1-2. Participants in the validation study

Participants were drawn from the Gene-Environment Interaction and Phenotype (GENIE) cohort, a part of the Health and Prevention Enhancement study of the Seoul National University Hospital Gangnam Center in Korea. The study was designed to investigate the correlation between genetic variability, environmental factors, lifestyle factors, metabolic diseases, and malignancies (31). The participants who visited the Seoul National University Hospital Gangnam Center for a health check-up since 2003 were included in the cohort. Individuals without BMD data (n=4,747), those who were lost to follow-up (n=1,803), those with a follow-up time of <5 or >7 years (n=588), and those with poor-quality genomic data (n=88) were excluded from the GENIE cohort. Participants aged <50 years (n=309), those with a history of anti-osteoporotic treatment (n=42), those with a malignancy (n=39), and those with no questionnaire data (n=15) were also excluded. Finally, 368 participants (100 men and 268 women) qualified for the validation study (**Figure 2**).

Figure 1. Flow diagram of study participants of Ansung cohort

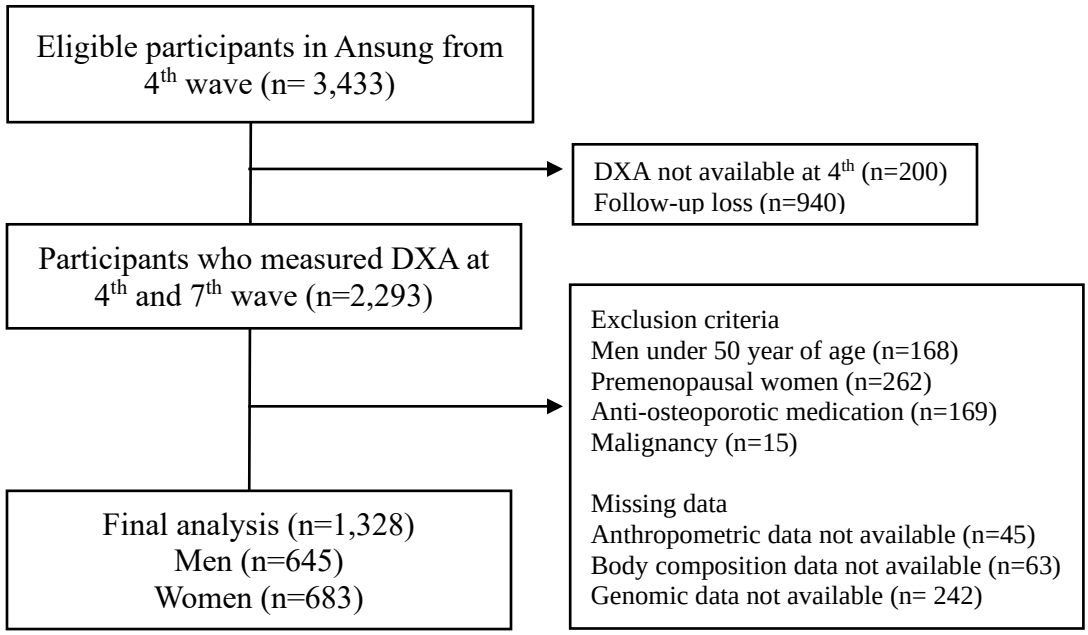
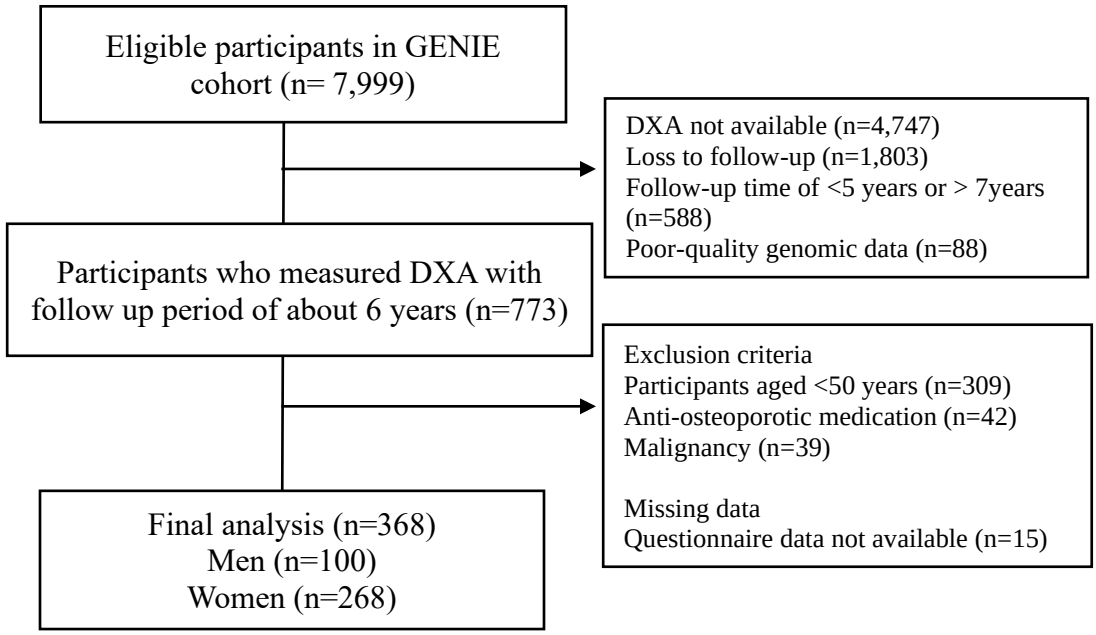


Figure 2. Flow diagram of study participants of GENIE cohort



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2. BMD measurements

DXA (Lunar Prodigy, Systems, Chalfont St. Giles, UK) was used to calculate the baseline BMD (g/cm^2) at the total hip (TH) and femur neck (FN), and Encore Software 11.0 (Encore Software Inc., Minneapolis, MN, USA) was used to analyze the data. A DXA technician followed the technical requirements outlined in the manufacturer's manual. To evaluate the reliability of DXA performance over time, the spine phantom BMD value was examined at least once a week. BMD values were kept within a tolerance of ±1.5% (33). According to the recommendation by the International Society for Clinical Densitometry (34), a suitable phantom for unit, radiation efficiency, tissue equivalent materials, and absorption coefficient were tested and calibrated. In the Ansung and GENIE cohorts, precision errors of BMD (coefficient of variation percentage) were 1.7%, 1.7%, and 1.8% for the LS, TH, and FN, respectively (35). Hip BMD loss was estimated from the fourth to the seventh wave, and the result was expressed as an annualized hip BMD change (to determine this, the difference between the repeat and baseline BMD values was divided by the baseline value and the interval (in years) between the two assessments).

3. Anthropometric and body composition measurements

The height and weight of the participants were assessed using the traditional procedure while dressed in light casual clothing. Body mass index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared

(kg/m^2). WC was calculated using the mid-point between the upper part of the iliac crest and the lowest ribs. LM and FM were assessed and calibrated using an InBody720 body composition analyzer (Biospace Co., Seoul, Korea) based on age, height, weight, level of exercise, and physique. LM and FM losses were measured from the fourth to the seventh wave and the results were expressed as the annualized percentage changes in LM and FM.

4. Vertebral fractures

The Vertebral heights were assessed for vertebral fractures, which were confirmed by lateral spine radiographs using the approach by Eastell et al. (36). The anterior to posterior, middle to posterior, and posterior to posterior above and below ratios were estimated. As described in our published paper, a vertebral fracture was defined if any of the aforementioned ratios were more than three standard deviations (SDs) below the normal mean for the vertebral level (37).

5. Covariate assessment

Alcohol intake and smoking status were categorized as never, former, and current. Former smokers were those who had smoked more than five packs of cigarettes in their lives or had quit smoking less than 6 months before baseline. Former drinkers were described as those who had consumed less than 5 grams of ethanol per day or had stopped 6 months prior to baseline. Regular exercise was defined as physical activity that was done on a regular basis until the body

perspired. Participants with diabetes mellitus had a glycated hemoglobin (HbA1c) level ≥6.5%, or an 8-hour fasting plasma glucose level ≥126 mg/dL (7.0 mmol/L) and were taking anti-diabetic medications, including insulin, at the time of the survey. Hypertension was described as systolic blood pressure greater than 140 mmHg, diastolic blood pressure greater than 90 mmHg, or use of anti-hypertensive medications. Menopause was regarded as the cessation of a menstrual cycle for a period of 12 months. Fasting blood sugar and HbA1c levels, red blood cell (RBC), white blood cell, and platelet counts were measured in a central laboratory using blood samples collected after an overnight fast.

6. Genotyping and quality control

Genomic DNA from the participants of the Ansung and GENIE cohorts was analyzed using Affymetrix Genome-Wide human single-nucleotide polymorphism (SNP) 5.0 arrays and the Affymetrix Axiom KORV1.1-96 Array (Affymetrix, Santa Clara, CA, USA), respectively, which was performed by DNA link Inc. (Seoul, Korea). SNPs related to low BMD and fractures have been identified in the previous GWAS and candidate gene association studies (18-21, 38-63). Thus, the following candidate genes were selected: *TNFSF11, TNFRSF11A, TNFRSF11B, LRP5, CTNNB1, DKK1, SFRP4, SOST, WNT4, WNT5B, WNT16, MEF2C, AXIN1, ESR1, RUNX2, SP7, SOX9, SOX6, VDR, FOXL1, BMP2, P2Rx7,* and *ZBTB40.* These genes are involved in bone metabolism pathways, including *RANKL*/*RANK*/*OPG*, *Wnt* signaling, estrogen

signaling, differentiation of mesenchymal stem cells, vitamin D signaling, Hedgehog signaling, *BMP* signaling, and other pathways (**Table 1**). Out of the 23 candidate genes (+/-5 kb) related to bone metabolism, a total of 1,973 SNPs were extracted. We also selected 641 SNPs correlated with the BMD and fractures at the genome-wide significance (GWS) level or suggestive of the GWS level in the previous studies (18-21, 38-50). Finally, the relationship between bone mass loss and the 2,614 SNPs was analyzed. Poor-quality SNPs were filtered out, which were identified as SNPs with a minor allele frequency <1%, Hardy-Weinberg equilibrium $P<10^{-6}$, and a low call rate (<95%). SNPs were obtained using the linkage disequilibrium threshold (r2) of 0.05. Genotype imputation was done using hg 18 as a reference.

Table 1. The selected candidate genes

No	Signaling pathway	Genes	Locus	Spine BMD	Hip BMD	Fracture	Mode of identification	Reference
1	*RANKL/RANK/OPG* pathway	*TNFSF11*	13q14	-	+	+	Candidate gene; GWAS; GWAS meta-analysis	(21, 51)
2		*TNFRSF11A*	18q21	+	+	+	Candidate gene; GWAS; GWAS meta-analysis	(21, 48, 51)
3		*TNFRSF11B*	8q24	+	+	+	Candidate gene; GWAS; GWAS meta-analysis	(21, 48, 52, 53)
4	*Wnt* signaling pathway	*LRP5*	11q13	+	+	+	Candidate gene; GWAS; GWAS meta-analysis	(46, 48, 53)
5		*CTNNB1*	3p22	-	+	+	GWAS meta-analysis	(46, 48, 53)
6		*DKK1*	10q21	+	+	+	GWAS meta-analysis	(46, 54)
7		*SFRP4*	7p14	+	+	-	GWAS meta-analysis	(46, 55)
8		*SOST*	17q21	-	-	+	Candidate gene; GWAS; GWAS meta-analysis	(21, 46, 53)
9		*WNT4*	1p36	+	+	+	GWAS meta-analysis	(18, 46)
10		*WNT5B*	12p13	+	+	-	GWAS meta-analysis	(46)
11		*WNT16*	7q31	+	+	+	GWAS meta-analysis	(45, 46)

12		*MEF2C*	5q14	-	+	-	GWAS meta-analysis	(48)
13		*AXIN1*	16p13	+	+	-	GWAS meta-analysis	(46)
14	Estrogen signaling pathway	*ESR1*	6q25	+	+	+	Candidate gene; GWAS	(21, 48, 52, 53, 56-58)
15	Mesenchymal stem cell differentiation	*RUNX2*	6p21	+	-	-	GWAS meta-analysis	(46)
16		*SP7*	12q13	+	-	-	GWAS	(19)
17		*SOX9*	17q24	-	+	-	GWAS meta-analysis	(46)
18		*SOX6*	11p15	+	+	-	GWAS meta-analysis	(44, 48, 53)
19	Vitamin D signaling pathway	*VDR*	12q13	+	+	-	Candidate gene; Association analysis of VDR polymorphism and BMD and bone loss	(56, 59)
20	Hedgehog signaling pathway	*FOXL1*	16q24	+	-	-	GWAS meta-analysis	(48)
21	*BMP* signaling pathway	*BMP2*	20p12	+	+	+	Genome-wide linkage analysis, Genome scan meta-analysis	(60, 61)
22	Other pathway	*P2Rx7*	12q24	+	+	+	Association analysis of P2RX7 polymorphism and BMD and bone loss	(62, 63)
23		*ZBTB40*	1p36	+	+	+	GWAS, GWAS meta-analysis	(46, 48, 53)

7. Statistical analyses

The data are presented as mean±SD or number (%). The baseline characteristics of the participants were compared using Student's t-test for continuous variables and the chi-square test for categorical variables. In the multiple linear regression analysis, women were classified into two age groups: 45–59 years and ≥60 years. Age-stratified LM and FM changes were examined using analysis of variance. Multicollinearity between covariates was assessed using variance inflation factors (VIFs). The VIFs were all less than 5. Lasso regression was used to select the variables. The PLINK software tool version 1.07 was used to analyze genome-wide association patterns, and Manhattan plots were created using R software version 3.2.2. (2015; The R Foundation for Statistical Computing, Vienna, Austria). The relationships were also analyzed after adjusting for multiple comparisons (64). The association between the annualized hip BMD change and SNPs was investigated using multiple linear regression analyses corrected for age, BMI, and TH-BMD. In the validation cohort study, genetic variants that reached the significance threshold (P<0.05) in the Ansung cohort were examined. Standardized differences between included and excluded datasets were analyzed using the "stddiff" package in R. Standardized difference >0.2 was regarded a significant imbalance (65). Statistical analyses were done using STATA 14.0 statistical package (StataCorp) and SPSS (version 20.0). A P value <0.05 was considered statistically significant.

Results

1. Baseline characteristics of the study participants

The baseline characteristics of all participants in the Ansung cohort are shown in **Table 2**. The mean age was 61.8±7.4 and 61.6±7.4 years in men and women, respectively. Osteoporosis was more common in postmenopausal women (22.1%) than in men (10.1%). Men had more LM than women, whereas women had more FM. Among the participants of the Ansung cohort, 56.8% of men and 64.8% of women were excluded. The excluded men were slightly younger and had a lower WC, BMI, LM, and BMD, whereas the excluded women had a longer year since menopause (YSM), as well as lower WC and RBC counts. Fractures were more prevalent among the included men (14.7%) than among the excluded men (10.7%). However, we analyzed the standardized difference between the two datasets and identified no significant imbalance except for YSM in women, implying that there was no selection bias.

Table 2. Baseline characteristics of total participants in Ansung cohort

Variables	Men				Women			
	Excluded	Included	*P*	Standardized difference	Excluded	Included	*P*	Standardized difference
N	848	645			1257	683		
Age (yr)	60.2±9.4	61.8±7.4	**<0.001**	0.182	61.2±9.3	61.6±7.4	0.221	0.056
Height (cm)	165.9±6.0	165.6±5.9	0.397	0.044	152.1±6.1	152.4±5.7	0.277	0.051
Weight (kg)	65.0±10.2	66.0±9.3	0.052	0.102	57.5±8.8	58.1±8.4	0.186	0.063
BMI (kg/cm^2)	23.6±3.2	24.0±2.9	**0.004**	0.151	24.8±3.3	25.0±3.3	0.342	0.045
WC (cm)	88.0±8.8	89.2±8.0	**0.005**	0.147	88.6±9.0	89.6±8.9	**0.011**	0.121
YSM (yr)	-	-			17.0±9.3	13.2±8.5	**<0.001**	**0.427**
Regular exercise	234 (27.7)	207 (32.1)	0.065	0.096	339 (27.1)	187 (27.4)	0.894	0.006
Current smoker, n (%)	340 (40.1)	240 (37.2)	0.257	0.059	31 (2.5)	8 (1.2)	0.052	0.097
Current drinker, n (%)	548 (64.6)	451 (69.9)	**0.031**	0.113	257 (20.4)	155 (22.7)	0.247	0.055
Fracture, n (%)	91 (10.7)	95 (14.7)	0.022	0.120	233 (18.5)	136 (19.9)	0.468	0.034
Hypertension, n (%)	301 (35.5)	245 (38.0)	0.323	0.052	501 (39.9)	309 (45.2)	**0.022**	0.109
Diabetes mellitus, n (%)	129 (15.2)	103 (16.0)	0.689	0.021	209 (16.6)	96 (14.1)	0.137	0.071
HRT, n (%)					11 (0.9)	8 (1.2)	0.527	0.029
Osteoporosis, n (%)	111 (13.1)	65 (10.1)	0.075	-0.094	305 (24.3)	151 (22.1)	0.313	-0.051
TH-BMD (g/cm^2)	0.961±0.161	0.983±0.127	**0.004**	0.153	0.856±0.157	0.859±0.126	0.651	0.021
TH-BMD T-score	0.2±1.2	0.3±1.0	0.013	0.090	-0.6±1.3	-0.6±1.1	0.823	0
FN-BMD (g/cm^2)	0.894±0.177	0.913±0.125	**0.021**	0.122	0.798±0.148	0.796±0.116	0.679	0.019
FN-BMD T-score	-0.4±1.2	-0.3±1.0	0.162	0.091	-0.8±1.2	-0.9±1.0	0.529	-0.091
Total fat mass (kg)	13.2±4.8	13.6±4.5	0.106	0.092	17.9±5.4	18.2±5.3	0.244	0.059
Total lean mass (kg)	48.9±6.8	49.5±6.1	**0.046**	0.104	37.3±4.7	37.5±4.3	0.361	0.044
White blood cell (x10^3/mm^3)	6.69±2.03	6.78±2.09	0.456	0.039	6.08±2.33	6.08±1.90	0.981	0.001
Red blood cell (x10^6/mm^3)	4.57±0.43	4.56±0.42	0.442	0.040	4.11±0.39	4.15±0.35	**0.014**	0.119
Platelet (x10^3/mm^3)	223.8±58.9	224.8±57.2	0.741	0.017	244.3±69.6	246.9±58.3	0.421	0.040

BMI, body mass index; WC, waist circumference; YSM, Years since menopause; HRT, hormone replacement therapy; TH, total hip; FN, femur neck.

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2. Annualized hip BMD changes

The annualized hip BMD changes (%/y) were normally distributed (**Figure 3**). Hip BMD loss was faster in postmenopausal women than in men. **Figure 4** shows the annualized hip BMD changes (%/y) of the participants by age group. The mean annualized hip BMD changes were −0.1±0.7%/y in men and −0.6±0.8%/y in women. Hip BMD loss in men increased with advancing age (*P* for trend <0.001). In contrast, women showed a sharp decline in hip BMD after menopause, which further accelerated after 70 years of age (*P* for trend=0.005).

3. Annualized LM and FM changes

According to age, LM loss (%/y) increased in men aged ≥65 years than in those aged <65 years. Compared to women aged <60 years, LM loss increased in women aged ≥60 years (**Figure 5**). Change in FM of men in all age groups did not show significant differences. Increase in FM was significantly greater in women aged 45–49 and 55–59 years than in women aged 70–76 years. It is notable that the increase in FM reduced in women after 70 years of age (**Figure 6**).

4. Clinical risk factors affecting hip BMD change

The annualized hip BMD change in men was positively correlated with WC (β=0.019, *P*<0.001), LM change (%/y) (β=0.274, *P*<0.001), alcohol intake (β=0.112, *P*=0.049), and increased RBC count (β=0.144, *P*=0.030) but was negatively correlated with current smoking status (β=−0.122, *P*=0.028). In postmenopausal

women aged 45–59 years, WC (β=0.016, *P*=0.010) and LM change (%/y) (β=0.452, *P*<0.001) were positively correlated with the annualized hip BMD change, whereas YSM ≤3 years (β=−0.311, *P*=0.004) was inversely correlated with annualized hip BMD change. The annualized hip BMD change in postmenopausal women aged ≥60 years was significantly correlated with increasing age (β=−0.023, *P*=0.020), WC (β=0.011, *P*=0.018), LM change (%/y) (β=0.108, *P*=0.039), FM change (%/y) (β=0.039, *P*=0.001), alcohol intake (β=−0.245, *P*=0.021), and platelet count (β=−0.002, *P*=0.014) (**Table 3**).

Figure 3. Annualized hip BMD changes among men and women

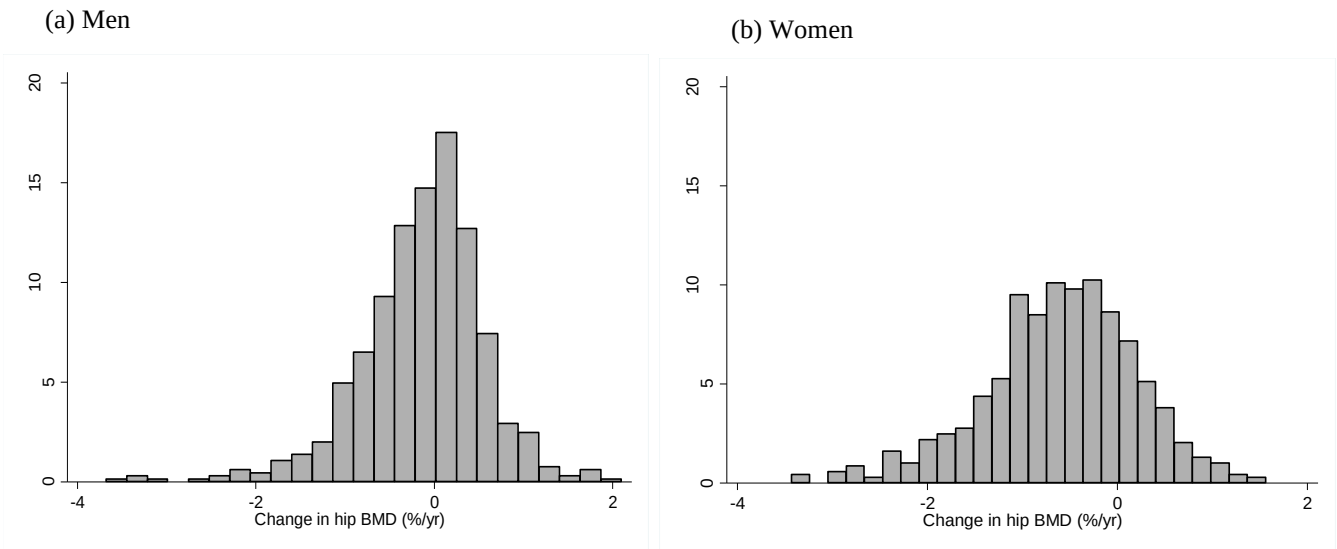

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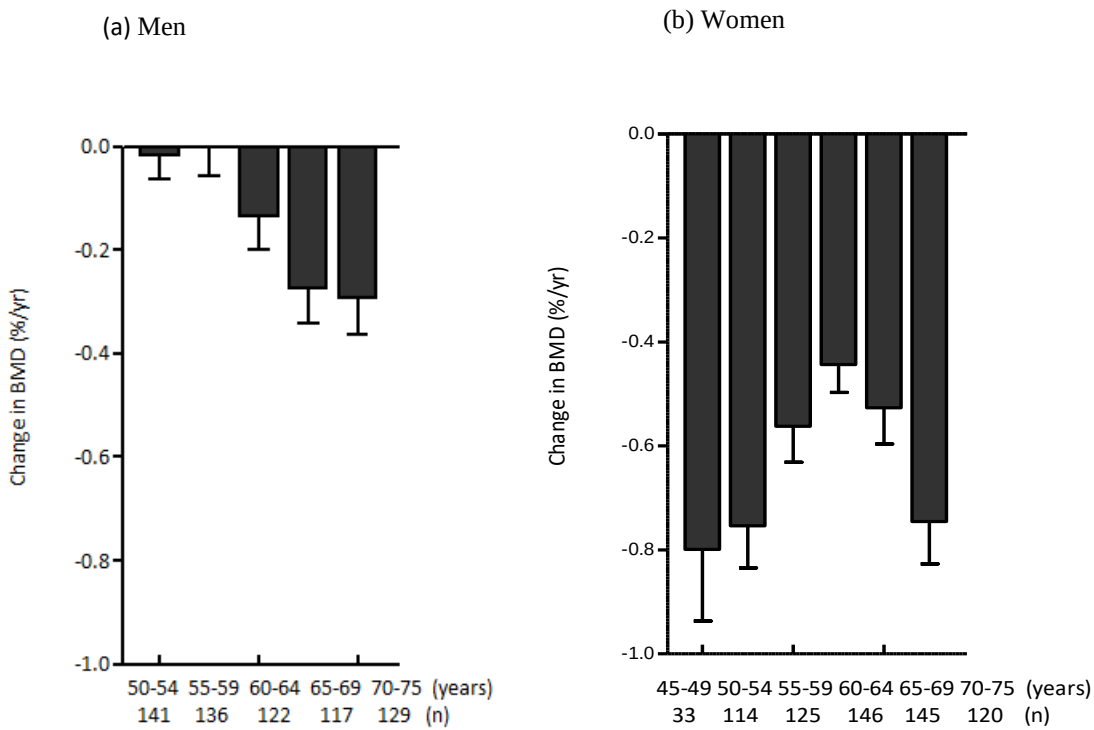
Figure 4. Age-stratified annualized hip BMD changes among men and women



Data are presented as Mean ± SEM.
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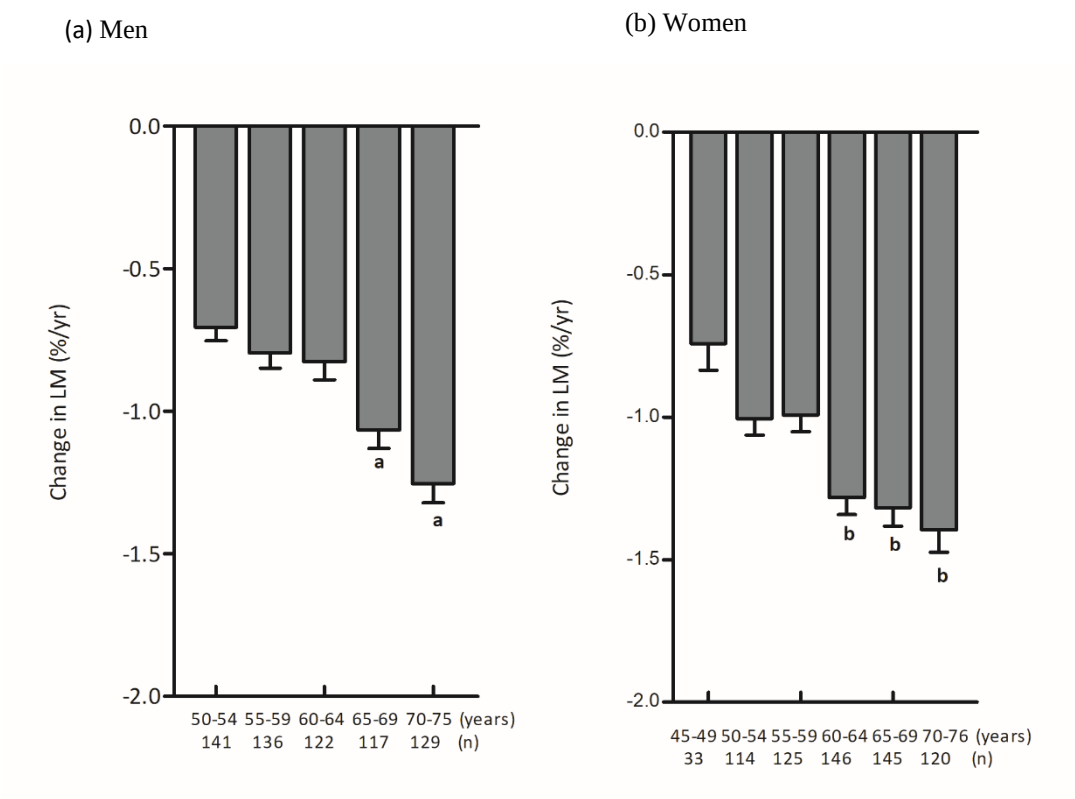
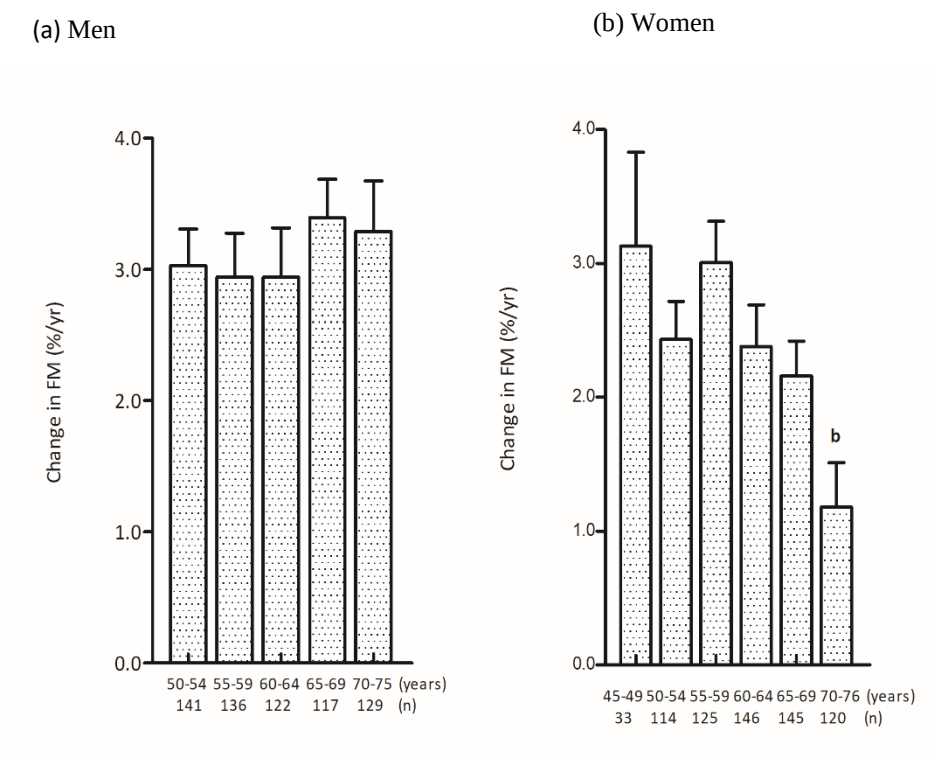
Figure 5. Age-stratified annualized LM changes among men and women



Data are presented as Mean ± SEM
a, P<0.05 vs aged 50-54, 55-59, 60-64 years in men; b, P<0.05 vs aged 45-49, 50-54, 55-59 years in women after ANOVA with Tukey test

Figure 6. Age-stratified annualized FM changes among men and women



Data are presented as Mean ± SEM
b, $P<0.05$ vs aged 45-49, 55-59 years in women after ANOVA with Tukey test

Table 3. Multiple linear regression analysis of annualized hip BMD change in men and women

Variables	Men (n=645)		Women aged 45-59yrs (n=272)		Women aged over 60yrs (n=411)	
	β (SE)	*P*	β (SE)	*P*	β (SE)	*P*
Age (yr)	−0.004 (0.004)	0.327	0.010 (0.015)	0.501	−0.023 (0.010)	**0.020**
Waist circumference (cm)	0.019 (0.004)	**<0.001**	0.016 (0.006)	**0.010**	0.011 (0.005)	**0.018**
LM change (%/yr)	0.274 (0.040)	**<0.001**	0.452 (0.077)	**<0.001**	0.108 (0.052)	**0.039**
FM change (%/yr)	0.013 (0.007)	0.066	0.011 (0.014)	0.446	0.039 (0.011)	**0.001**
TH-BMD (g/cm^2)	0.366 (0.221)	0.098	−0.769 (0.426)	0.072	−0.181 (0.380)	0.634
Current smoking	−0.122 (0.055)	**0.028**	0.208 (0.542)	0.702	−0.111 (0.323)	0.732
Alcohol intake	0.112 (0.057)	**0.049**	−0.071 (0.098)	0.471	−0.245 (0.106)	0.021
Regular exercise	0.005 (0.056)	0.929	−0.088 (0.098)	0.369	0.011 (0.094)	0.906
RBC ($x10^{\wedge 6}/mm^3$)	0.144 (0.066)	**0.030**	−0.084 (0.143)	0.555	0.148 (0.112)	0.188
Platelet ($x10^{\wedge 3}/mm^3$)	−0.0004 (0.0005)	0.427	0.001 (0.001)	0.527	−0.002 (0.001)	**0.014**
YSM ≤3 years	N/A		−0.311 (0.107)	**0.004**	N/A	

The dependent variable was annualized total hip BMD change, and the multiple linear regression analysis was adjusted for age, waist circumference, LM change (%/yr), FM change (%/yr), TH-BMD, current smoking, alcohol intake, regular exercise, RBC, and YSM ≤ 3 years (in women). LM, lean mass; FM, fat mass; YSM, Years since menopause; TH, total hip. $P<0.05$

5. Baseline characteristics of the candidate gene association study cohort and validation cohort

The baseline characteristics of the candidate gene association study and validation cohorts (Ansung cohort for candidate gene association study; GENIE cohort for validation) are shown in **Table 4**. In the candidate gene association study cohort, the average age of the study population was 61.7 years, and 51.4% of the participants were women. Osteoporosis was more common in both men and women in the Ansung cohort than in the validation cohort. The validation cohort was younger and had a higher proportion of women than the Ansung cohort, with an average age of 57.2 years and 72.8% of them being women. Men in the validation cohort had a lower WC and LM, and a higher FM than those in the Ansung cohort. Women in the validation cohort had a lower BMI, WC, LM, and FM than those in the candidate gene association study cohort. However, TH-BMD was higher in women in the validation cohort than in those in the candidate gene association study cohort (**Table 4**).

Table 4. Baseline characteristics of the candidate gene association study (Ansung cohort) and validation study (GENIE cohort)

Variables	Candidate gene association Study (Ansung cohort)		Validation study (GENIE cohort)	
	Men	Women	Men	Women
N	645	683	100	268
Age (yr)	61.8±7.4	61.6±7.4	60.7±7.3	55.8±5.2*
Height (cm)	165.6±5.9	152.4±5.7	169.2±5.5*	158.1±4.7*
Weight (kg)	66.0±9.3	58.1±8.4	68.1±8.2*	56.1±6.6*
BMI (kg/cm^2)	24.0±2.9	25.0±3.3	23.8±2.5	22.5±2.6*
WC (cm)	89.2±8.0	89.6±8.9	85.8±11.1*	81.2±8.7*
TH-BMD (g/cm^2)	0.983±0.127	0.859±0.126	0.997±0.125	0.920±0.116*
TH-BMD T-score	0.3±1.0	-0.6±1.1	0.4±1.0	-0.3±1.0*
FN-BMD (g/cm^2)	0.913±0.125	0.796±0.116	0.912±0.128	0.866±0.109*
FN-BMD T-score	-0.3±0.9	-1.0±1.0	-0.3±1.0	-0.4±0.9*
Osteoporosis, n (%)	65 (10.1)	151 (22.1)	2 (2.0)*	5 (1.9)*
Total fat mass (kg)	13.6±4.5	18.2±5.3	15.4±4.0*	16.8±4.2*
Total lean mass (kg)	49.5±6.1	37.5±4.3	29.5±3.6*	20.6±2.4*
Regular exercise	207 (32.1)	187 (27.4)	41 (41.0)*	89 (33.2)
Current smoker, n (%)	240 (37.2)	8 (1.2)	23 (23.0)*	12 (4.5)*
Current drinker, n (%)	451 (69.9)	155 (22.7)	31 (32.0)*	49 (18.3)
Hypertension, n (%)	245 (38.0)	309 (45.2)	29 (29.0)	39 (14.6)*
Diabetes mellitus, n (%)	103 (16.0)	96 (14.1)	9 (9.0)	9 (3.4)*
WBC (x10^^{3}/mm^3)	6.78±2.09	6.07±1.90	5.65±1.63*	4.85±1.39*
RBC (x10^^{6}/mm^3)	4.56±0.42	4.24±0.46	4.75±0.62*	4.37±0.50*
Platelet (x10^3/mm3)	224.8±57.5	246.9±58.3	229.9±58.9	251.4±59.9

BMI, body mass index; WC, waist circumference; BMD, bone mineral density; TH, total hip; FN, femur neck; WBC, White blood cell; RBC, Red blood cell.
P^*<0.05 between candidate gene association study and validation set.

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6. Genetic risk factors affecting hip BMD change

6-1. Genetic risk factors affecting hip BMD change in Ansung cohort

The relationship between the genetic risk factors and annualized hip BMD change was investigated using multiple linear regression analyses. After correcting for age, BMI, and TH-BMD, the annualized hip BMD change was correlated with *WLS*, *RUNX2*, *ESR1,* and *LRP5* in men and with *WLS*, *WNT4, CTNNB1, ESR1, TNFRSF11B, SOX6, WNT5B, VDR, TNFSF11,* and *TNFRSF11A* in women ($P<0.05$) (**Table 5**). Although the relationships were not significant after adjusting for multiple comparisons, rs4988300 for *LRP5* ($\beta=0.127$, $P=0.007$) and rs7325635 for *TNFSF11* ($\beta=0.146$, $P=0.001$) were best relevant to the annualized hip BMD change in men and women, respectively. They were statistically significant by applying the LASSO algorithm, despite the fact that the correlations were not significant after adjusting for multiple comparisons. **Figure 7** shows Manhattan plots for the annualized hip BMD change in men and women.

6-2. Genetic risk factors affecting hip BMD change in the GENIE cohort

In the GENIE cohort, 12 variants in men were further investigated. Among them, one SNP showed an association with the annualized hip BMD change in the validation study ($P<0.05$). In men, the rs2470688 variant in the intron of *PRKCB* was related to the annualized hip BMD change ($P=0.009$ and 0.003 in the Ansung and validation cohorts, respectively). Men with the TT genotype of rs2470688 variant near *PRKCB* showed a lower hip BMD loss than those with the TC or CC genotype

(0.03±0.65, −0.20±0.73, −0.16±0.66 *P*=0.036, *P*=0.004, respectively) (**Figure 8**). There was no association noted between the rs2470688 variants and vertebral fracture. In women, 14 variants were evaluated in the validation cohort; however, none were significant.

Table 5. SNP lists with a p-value less than 0.05 for annualized hip BMD change

(a) Men

CHR	SNP	BP	Closest gene	Candidate gene association study		Validation study	
				β	*P* *	β	*P* *
1	rs2566754	68413826	*WLS*	-0.089	0.038	NA	
3	rs784288	170453925	*MECOM*	-0.110	0.012	-0.018	0.825
6	rs6931664	151899287	*C6orf97*	0.101	0.012	-0.06	0.425
6	rs6930053	45596736	*RUNX2*	-0.081	0.034	NA	
6	rs9479188	152430137	*ESR1*	0.087	0.032	NA	
10	rs1896367	53739192	*PRKG1-AS1*	-0.088	0.03	NA	
11	rs4988300	67845407	*LRP5*	0.126	0.008	NA	
14	rs10137819	100464694	*MEG8 SNORD113-3*	0.148	0.02	-0.078	0.459
16	rs2470688	24024978	*PRKCB LOC105371141*	0.099	0.009	0.199	0.003
18	rs7227401	20192656	*OSBPL1A*	0.123	0.032	NA	
19	rs10403583	43983611	*LGALS4*	-0.141	0.015	NA	
21	rs2244352	39679843	*GET1 GET1-SH3BGR*	0.108	0.017	NA	

P *, adjusted for age, BMI, and baseline TH-BMD.

(b) Women

CHR	SNP	BP	Closest gene	Candidate gene association study		Validation study	
				β	*P* *	β	*P* *
1	rs2566784	68375323	*WLS*	0.127	0.006	NA	
1	rs1046310	22316474	*WNT4*	-0.162	0.041	0.098	0.172
3	rs436448	41096255	*CTNNB1*	0.124	0.0007	-0.032	0.482
3	rs12492719	41220735	*CTNNB1*	-0.235	0.007	-0.129	0.125
6	rs1115582	152462823	*ESR1*	-0.313	0.004	-0.060	0.414
8	rs1485286	120019849	*TNFRSF11B*	-0.120	0.009	NA	
11	rs297335	16353636	*SOX6*	-0.116	0.019	0.079	0.101
12	rs2107523	1526622	*WNT5B*	-0.109	0.017	NA	
12	rs2107301	46541837	*VDR*	0.115	0.019	NA	
13	rs7325635	42043319	*TNFSF11*	0.150	0.0007	0.054	0.239
16	rs11648832	7721461	*RBFOX1*	0.152	0.001	NA	
17	rs7212466	27868497	*MYO1D*	0.172	0.038	0.044	0.528
18	rs17069904	58183929	*TNFRSF11A*	0.117	0.048	-0.008	0.881
20	rs6070709	57072494	*SLM02*	0.136	0.028	0.088	0.156

P *, adjusted for age, BMI, and baseline TH-BMD.

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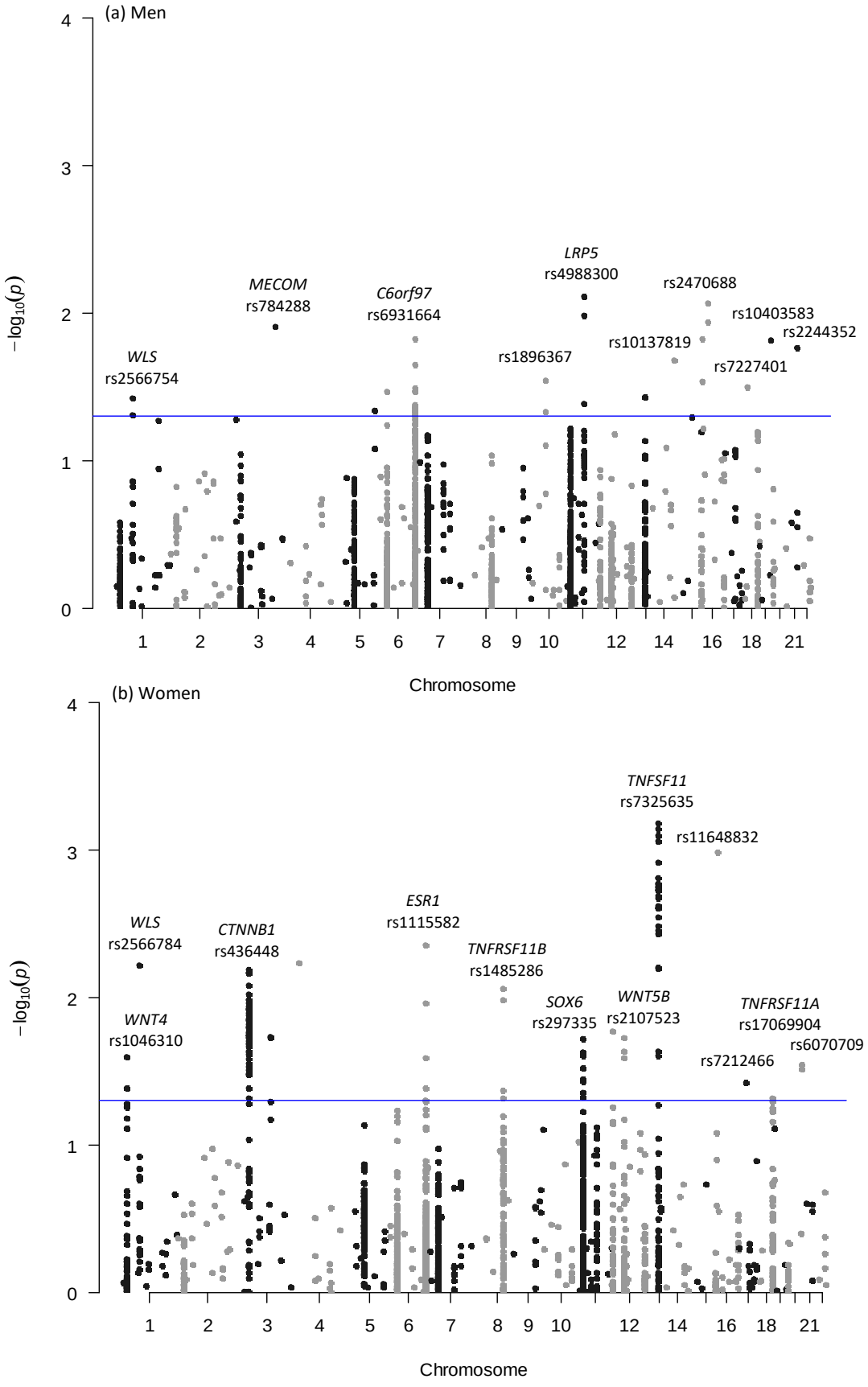

Figure 7. Manhattan plots for the candidate gene association study on the annualized hip BMD change in men and women. The blue line represents the threshold (− log10(5 × 10–2)).
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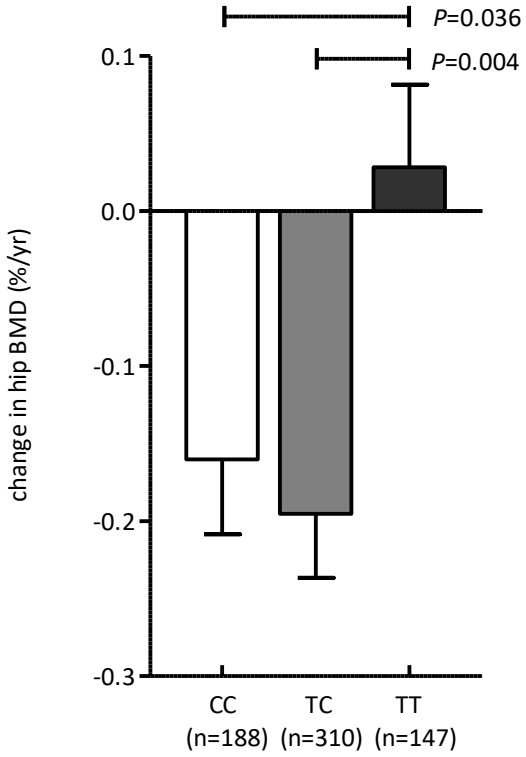
Figure 8. Annualized hip BMD changes according to the rs2470688 variants



Discussion

In this Korean community-based prospective cohort study, the hip BMD loss in men increased with low WC, loss of LM, and current smoking status, and decreased with alcohol intake and an increase in RBC count. Low WC, loss of LM, and YSM ≤3 years were related to hip BMD loss in women aged 45–59 years. Increasing age, low WC, LM and FM losses, alcohol intake, and increased platelet count were correlated with hip BMD loss in women aged 60 years and older. Using a candidate gene association study, we discovered the association between annualized hip BMD change and the genes related to bone metabolism (*WLS, RUNX2, ESR1,* and *LRP5* in men and *WLS, WNT4, CTNNB1, ESR1, TNFRSF11B, SOX6, WNT5B, VDR, TNFSF11*, and *TNFRSF11A* in women). In men and postmenopausal women, rs4988300 of *LRP5* and rs7326535 of *TNFSF11* had the strongest association with hip BMD change, respectively. In men, the rs2470688 variant in the intron of *PRKCB* was correlated with the annualized hip BMD change in the candidate gene association and validation cohort studies. However, the relationships between the SNPs and annualized hip BMD change were not significant after adjusting for multiple comparisons.

According to age, there were disparities in hip BMD loss between the sexes. The decline in BMD was more dramatic in postmenopausal women than in men with increasing age. Bone loss occurs later in men than in women, owing to a higher degree of sex steroids by the age of 65 and 70 years (66). In men, bone loss is persistent and accelerated with the natural aging process. It is mostly due to reduced bone formation (67). Accelerated bone loss in postmenopausal women begins between the ages of 45 and 49 years after the onset of menopause. It is caused by estrogen deficiency and leads to an imbalance in bone resorption compared to formation (68). In women aged ≥55 years, the rate of bone loss declined by the age of 65−69 years. This is an age-related bone mass loss that occurs in men. Subsequently, the rate of bone loss accelerated at the age of 70 years, and was similar to that observed at the age of 45−49 years in this study. In our study, loss of LM and attenuated gain of FM may be correlated with increased bone loss in women after 70 years of age. Similarly, Berger et al. showed this second period of accelerated bone loss in elderly women (69).

Weight reduction is correlated with bone loss and is influenced by age, sex, and adiposity (22). Nonetheless, the relative contribution of LM and FM to bone mass is still debated, and both LM and FM affect BMD, depending on the measured skeletal site, parameter of bone mass, and menopause (70). Some studies have shown that the impact of FM on BMD in postmenopausal women is more significant than that of LM (71, 72). Furthermore, the results have been inconsistent, with some studies suggesting that LM, rather than FM, is more closely correlated with BMD (24, 73). Our findings indicate that LM loss is a major predictor of hip BMD loss in both men and postmenopausal women and that FM loss is a significant factor for bone mass loss in women aged 60 years and older. FM had a greater impact on BMD in postmenopausal women aged 60 years and older than in those aged <60 year. After 60 years of age, the decrease in LM was accelerated in both men and women, and FM continued to expand until 75 years of age (74). Although previous research on the association between WC and bone mass has yielded inconsistent results (75, 76), we discovered that WC is inversely related to bone loss. The level of sex hormone-binding globulin is lower in obese people, whereas the levels of free sex steroids, leptin, and insulin are higher. Sex steroids, leptin, and insulin have all been shown to increase bone mass (77). Furthermore, the aromatization of androgens to estrogens in adipose tissues is accelerated by increased fat, which is particularly important for the bone health of postmenopausal women (71).

In addition, we discovered a negative correlation between smoking and hip BMD loss in men. Smoking raises the risk of osteoporosis by lowering sex steroids, causing earlier menopause in women, and promoting bone resorption (78). Current alcohol intake status in women was correlated with hip BMD loss. Due to the suppression of osteoblastic activity, excessive alcohol intake has been shown to have negative effects on bone mass (79). However, Tucker et al. showed that men who consumed 1−2 drinks per day exhibited a 3.4%–4.5% higher hip BMD than nondrinkers and showed that these effects diminished with higher intake (80). In this study, hip BMD loss in men who currently consumed alcohol was observed to be lower. It is likely that nutrients derived from moderate alcohol intake may improve bone health (80). Furthermore, our study revealed an association between hip BMD loss and RBC count. The findings of the MrOS report on hip bone loss and anemia in men are consistent with our results (27). It is possible that the deterioration of bone affects the environment that supports hematopoiesis,

resulting in anemia (27). Anemia is related to physical disability and decreased muscle strength (81), which are known to be correlated with bone loss (82). Elevated platelet count was correlated with hip BMD loss in women aged 60 years and older. Osteoporosis and activated platelets are linked by chronic inflammation (83). Increased oxidative stress, which contributes to platelet activation, is caused by pro-inflammatory cytokines, including TNF-a and IL-6. It is possible that osteoclastic bone resorption is promoted by platelet activation factor (84).

We discovered a relationship between BMD-associated SNPs including *LRP5, TNFRSF11A*, *TNFSF11*, *TNFRSF11B,* and *ESR1* and bone loss using the candidate gene approach (47, 85). *WLS* and *LRP5* in men and *CTNNB1, WNT4*, and *WNT5B*, and *WLS* in women, which are related to the *Wnt* signaling pathway, were correlated with bone loss. Additionally, *LRP5* was significantly correlated with BMD (48, 86). Some bone loss-related SNPs, such as the *RANKL/RANK/OPG* pathway, were previously known to be involved in bone metabolism. *RANKL/TNFSF11* was closely related to BMD in previous studies, consistent with our findings (48, 85, 87). Rs7325635 of *TNFSF11* was linked to a lower risk of hip BMD loss. Furthermore, *RANKL* is a potent regulator of osteoclast formation and function and may be essential in mediating accelerated bone resorption after menopause (16). *TNFSF11* genetic variants interact with *OPG/TNFRSF11B*, which affects BMD in postmenopausal Korean women (88). The correlations of *RANK /TNFRSF11A* with BMD (19, 48, 85) and fracture (18) were reported in several studies, and a previous GWAS revealed the correlation of *TNFRSF11B* genetic variant with BMD and osteoporotic fracture risk (18). *ESR1* is another candidate gene for bone mass loss. Genetic variants of *ESR1* are related to BMD (48). However, this association was not found in another study (89). We identified the relationship between genetic variants of *ESR1* and bone loss in both men and women. *RUNK2* in men and *SOX6* in women was correlated with hip BMD loss. During endochondral bone formation, *RUNX2* and *SOX6* play a critical role (47, 48). In a previous meta-analysis, *SOX6* polymorphisms were identified to have an association with FN-BMD (48).

Furthermore, vitamin D interacts with vitamin D receptors (*VDR*) and is a potent effector of calcium homeostasis. In this study, a *VDR* genetic variant was correlated with hip BMD loss in women. The

VDR genetic variants have various effects on BMD in women (90). We also discovered a correlation between rs436448 and hip BMD loss in women, with *CTNNB1* being the closest gene. This result was consistent with the finding of a previous research on the correlation of *CTNNB*1 with FN-BMD (48). *CTNNB1* regulates osteoclast differentiation as a downstream effector of the canonical *Wnt* signaling pathway (91). In this study, rs784288 in the *MECOM* gene was correlated with hip BMD loss in men. The *MECOM* gene is related to hematopoietic differentiation and was correlated with fractures at the GWS level in a previous meta-analysis (50). In men, rs2470688 in *PRKCB* was related to hip BMD loss and the association was confirmed in the previous hip trochanter BMD GWAS (β=−0.084, *P*=2.43E−06) (49).

The strengths of this research lie in its large prospective cohort design, long duration of follow-up, and evaluation of BMD with data on possible confounding factors. To our knowledge, this research is novel because it is the first candidate gene association study to identify both clinical and genetic risk factors for hip BMD loss. Our research offers a comprehensive analysis of how BMD changes according to age and sex, with a significant clinical implication. In addition to rapid bone loss after menopause, we showed a second stage of rapid hip bone loss in elderly women. The elderly population may be more susceptible to hip fractures as a result of the second decline. This analysis, however, has several limitations. First, as our cohort comprised Korean men aged 50 years and older and postmenopausal women, the results may not be applicable to the general population. Ethnic differences may influence peak bone mass and bone mass loss. Compared to Caucasian women, LS-BMD loss was more rapid in Asian women (92). Research outcomes may be different in other ethnic groups. Second, women in the candidate gene association study were older and had a higher BMI and lower TH-BMD than those in the validation cohort. When evaluating the linear association of genetic variants with the annualized hip BMD change in both cohorts, we corrected for age, BMI, and TH-BMD to minimize these variations. However, when interpreting the results, the differences in baseline characteristics between cohorts should be taken into account. Third, after adjusting for multiple comparisons, the correlations between SNPs and annualized hip BMD change were not significant. The insignificant association between bone loss and genetic variants may be due to interactions

between environmental and genetic factors. It is possible that obesity plays a significant role in this gene-bone loss interaction. Obese and non-obese people may have different associations with bone loss and genetic variants. Furthermore, environmental factors, such as exercise, alcohol intake, cigarette smoking, and diet can influence the correlations of genetic variants with disease (93). While we found that rs4988300 of *LRP5* in men and rs7326535 of *TNFSF11* in women were best corelated to hip BMD loss in our cohort, other markers could be related to bone mass loss in other groups. Fourth, due to the limited data available from the questionnaires, calcium and vitamin D intake could not be taken into account. In addition, the correlation between bone mass loss and bone metabolism-associated factors, such as serum parathyroid hormone, calcium, 25-hydroxyvitamin D, and bone turnover markers, could not be investigated. Fifth, the number of participants was relatively small for a longitudinal study for osteoporosis. However, there is little evidence on BMD changes in the Asian population, and in this respect, this study had the opportunity to provide important information.

We were able to draw the following conclusions based on the findings of this study: (1) hip BMD loss in men is correlated with WC, loss of LM, current smoking status, alcohol intake, and RBC count; (2) hip BMD loss in women aged 45–59 years is correlated with WC, loss of LM, and YSM ≤3 years; and (3) hip BMD loss in women aged ≥60 years is correlated with age, WC, losses of LM and FM, alcohol intake, and increase in platelet count. After multiple testing adjustments, there was no correlation between any of the SNPs with hip BMD loss. Although the finding is not novel, the data represent an original contribution to the literature. To the best of our knowledge, this is the first study to demonstrate the association between RBC, platelet count, and longitudinal bone loss, and hematopoietic cell counts would be simple and inexpensive adjuncts to assess bone health. Identifying modifiable factors may aid in the development of an osteoporosis and fracture prevention plan for individuals who are at a higher risk of fracture. Further research is necessary to better determine the risk for bone mass loss in men and women.

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국문 초록

한국인의 골 소실에 영향을 미치는 유전적 및 임상적 요인의 발굴

이 지 현

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배경 및 목적: 과다한 골 소실은 취약성 골절 및 사망 위험의 증가와 관련이 있다. 그러나 골 소실 관련 위험인자에 대한 종단연구는 많지 않다. 본 연구는 6년간 한국인 전향적 코호트를 통해 골 소실 관련 유전적 및 임상적 요인을 알아보고자 한다.

방법: 한국의 지역사회 기반 전향적 코호트인 안성 코호트 대상자 중 2007년부터 2014년까지 2회의 이중에너지 X-선 흡수 계측법을 통하여 골밀도를 측정한 50세 이상 남성 645명과 폐경 후 여성 683명을 연구 대상으로 포함하였다. 공변량과 연간 대퇴골 전체 골밀도 변화 간 연관성은 다중 선형 회귀분석을 통해 분석되었다. 골대사와 관련된 23개의 후보 유전자와 이전의 전장유전체 연관분석에서 확인된 총 2,614개의 단일염기다형성(Single nucleotide polymorphism)을 분석하였다. 관련된 유전자 변이는 건강검진 프로그램에 참여한 대상자를 포함한 유전자-환경 상호작용 및 표현형 코호트에서 검증하였다.

결과: 대퇴골 전체 골밀도 소실률은 나이가 증가함에 따라 여성에서 남성보다 더 빠르다. 남성의 연간 대퇴골 전체 골밀도 변화는 허리둘레(β=0.019, *P*<0.001), 연간 근육량 변화(β=0.274, *P*<0.001), 현재 음주력(β=0.112, *P*=0.049), 적혈구 수의 증가(β=0.144, *P*=0.030)와 양의 연관성이 있었으며, 현재 흡연력(β=−0.122, *P*=0.028)과 음의 연관성이

있었다. 45~59세 여성에서 허리둘레(β=0.016, *P*=0.010) 및 연간 근육량 변화(β=0.452, *P*<0.001)는 연간 대퇴골 전체 골밀도 변화와 양의 연관성이 있었으며, 폐경 후 3년 이내인 경우(β=−0.311, *P*=0.004)는 음의 연관성이 있었다. 60세 이상 여성에서 연간 대퇴골 전체 골밀도 변화는 나이(β=−0.023, *P*=0.020), 허리둘레(β=0.011, *P*=0.018), 연간 근육량 변화(β=0.108, *P*=0.039), 연간 지방량 변화(β=0.039, *P*=0.001), 현재 음주력(β=−0.245, *P*=0.021), 혈소판 수의 증가(β=−0.002, *P*=0.014)와 통계적으로 유의한 연관성이 있었다. *LRP5* rs4988300 (β=0.127, *P*=0.007)과 *TNFSF11* rs7325635 (β=0.146, *P*=0.001)은 각각 남성 및 폐경 후 여성에서 연간 대퇴골 전체 골밀도 변화와 가장 유의한 단일염기다형성 변이였다. 남성에서 *PRKCB* 유전자의 인트론 내 rs2470688 변이와 연간 대퇴골 전체 골밀도 변화와 연관성이 있었다. 그러나 Benjamini-Hochberg 보정 후에는 연간 대퇴골 전체 골밀도 변화와 단일염기다형성 변이 간 연관성이 없었다.

결론: 본 연구에서 허리둘레 및 근육량의 감소는 한국인 남녀 모두에서 대퇴골 전체 골밀도의 소실 위험이 증가하는 것과 관련이 있었다. 대퇴골 전체 골밀도 소실에 성별 또는 연령별 영향을 미치는 요인도 확인되었다. 다중 비교 후 대퇴골 전체 골밀도 소실과 관련된 단일염기다형성 변이는 없었다. 골 소실의 위험 요소를 조기에 발견하면 개별화된 골다공증 및 골절 예방전략이 개발될 수 있다. 남성과 여성의 골 소실 위험을 더 잘 예측하기 위한 추가 연구가 향후 필요하다.

주 요 어: 골다공증, 골밀도, 신체조성, 폐경

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