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The association between genetic variants of *RUNX2*, *ADIPOQ* and vertebral fracture in Korean postmenopausal women

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Abstract Contrary to the traditional belief that obesity acts as a protective factor for bone, recent epidemiologic studies have shown that body fat might be a risk factor for osteoporosis and bone fracture. Accordingly, we evaluated the association between the phenotypes of osteoporosis or vertebral fracture and variants of obesity-related genes, peroxisome proliferator-activated receptor-gamma (*PPARG*), runt-related transcription factor 2 (*RUNX2*), leptin receptor (*LEPR*), and adiponectin (*ADIPOQ*). In total, 907 postmenopausal healthy women, aged 60–79 years, were included in this study. BMD and bio-markers of bone health and adiposity were measured. We genotyped for four single nucleotide polymorphisms (SNPs) from four genes (*PPARG*, *RUNX2*, *LEPR*, *ADIPOQ*). A general linear model for continuous dependent variables and a logistic regression model for categorical dependent variables were used to analyze the statistical differences among genotype groups. Compared with the TT subjects at rs7771980 in *RUNX2*, C-carrier (TC + CC) subjects had a lower vertebral fracture risk after adjusting for age, smoking, alcohol, total calorie intake, total energy expenditure, total calcium intake, total fat intake, weight, body fat. Odds ratio (OR) and 95 % interval (CI) for the vertebral fracture risk was 0.55 (95 % CI 0.32–0.94). After adjusting for multiple variables, the prevalence of vertebral fracture was highest in GG subjects at rs1501299 in *ADIPOQ* ($p = 0.0473$). A high calcium intake (>1000 mg/day) contributed to a high bone mineral density (BMD) in GT + TT subjects at rs1501299 in *ADIPOQ* (p for inter-action = 0.0295). Even if the mechanisms between obes-ity-related genes and bone health are not fully established, the results of our study revealed the association of certain SNPs from obesity-related genes with BMD or vertebral fracture risk in postmenopausal Korean women.

Keywords Polymorphisms · Runt-related transcription factor 2 (*RUNX2*) · Adiponectin (*ADIPOQ*) · Osteoporosis · Vertebral fracture

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Introduction

Although recent epidemiologic studies have shown that bone mineral density (BMD) is higher in overweight/obese subjects, several studies strongly have shown that body fat might be a risk factor of osteoporosis and bone fracture [1–3]. In addition, increasing evidence demonstrates that fat mass is negatively related to lower bone mineral density [4–6]. Zhao et al. [6] showed that when adjusted for the mechanical loading effect of body weight on bone mass, fat mass is negatively correlated with bone mass in both Chinese and Caucasians. Osteoblasts and adipocytes are derived from the same mesenchymal marrow stroma/stem cells (mMSC) [7]. For a mesenchymal stem cell to become

an osteoblast, several key factors, such as runt-related transcription factor 2 (*RUNX-2*) must be activated, and to achieve full adipocytic differentiation, peroxisome proliferator-activated receptor-γ (*PPARG*) are also required. Moreover, some adipokines, which are derived from adipocytes, such as leptin (*LEP*) and adiponectin (*ADIPOQ*), play a critical role in bone formation and bone resorption [8–11]. For this reason, we chose 4 SNPs: Peroxisome proliferator-activated receptor-gamma (*PPARG*), runt-related transcription factor 2 (*RUNX2*), leptin receptor (*LEPR*), and adiponectin (*ADIPOQ*), previously reported to show the association with the phenotype of obesity. We performed this study to evaluate the association between polymorphisms of the above-mentioned genes and BMD or vertebral fracture in Korean postmenopausal women. In addition, we examined the gene–nutrient interaction, between calcium intake and genes affecting osteoporosis or vertebral fracture.

Materials and methods

Study population

Nine hundred and seven healthy postmenopausal women, aged 60–79 years, who visited for a periodic health check-up under the program of the National Health Assessment in the women's hospital (Mizmedi Hospital, Seoul, Korea) were recruited for this study. We excluded participants with a history of co-morbid diseases known to affect bone metabolism: malignancy, inflammatory bowel disease, pituitary diseases, hyperthyroidism, primary hyperparathyroidism, renal failure, rheumatic disease, or adrenal disease. Participants who receiving medication such as glucocorticoids, hormone replacement therapy (HRT) or selective estrogen receptor modulator (SERM), bisphosphonate, or calcitonin over the past year were also excluded. In addition, we further excluded participants with vertebral fractures resulting from known accidental traumas.

Clinical and laboratory measurements

Study data included medical history, physical examination, information provided by a questionnaire, anthropometric measurements and laboratory measurements. Information on the medical history, current use of medications, weekly alcohol consumption and smoking status were obtained from all participants using a standard questionnaire. Trained nurses measured the height and weight with participants wearing lightweight hospital gowns and no shoes. Body mass index (BMI) was calculated as the weight (kg) divided by the square of the height (m). Body fat was

measured using In body 3.0. The waist circumference (WC) at the level of the umbilicus was measured while standing by a single examiner. The blood pressure (BP) was measured with a standard mercury sphygmomanometer in seated participants after a 5-min rest.

A venous blood sample was drawn from an antecubital vein in the morning after more than 12 h of fasting. The serum levels of fasting plasma glucose (mg/dl), triglyceride levels (mg/dl), high density lipoprotein (HDL) cholesterol (mg/dl), low density lipoprotein (LDL) cholesterol (mg/dl) and other biochemical markers were measured. Metabolic syndrome was defined by the criteria recommended by the National Cholesterol Education Program's Adult Treatment Panel III (NCEP III) guidelines [12]. Bone parameters included BMD (g/cm^2) in the hip and spine, history of vertebral fractures, urine deoxypyridinoline (DPD), and serum total osteocalcin (OC). Lateral radiographs of the thoracic and lumbar spine demonstrating the presence of vertebral fractures were interpreted by radiographic morphometry using Genant's semi-quantitative method [13]. Two radiologists independently evaluated every radiograph for vertebral fracture. If the two evaluations did not agree, a third radiologist discussed the results with other examiners and a diagnosis was made. BMD determined at the lumbar spine (L2–L4) was assessed using dual energy X-ray absorptiometry (DXA) (Norland, XR-46, USA).

Nutritional assessment was performed by dietitians using the 24 h diet recall method [14]. From these data, we calculated the energy expenditure, total calorie intake, calcium intake, and fat intake. This study was conducted upon approval of all research procedures by the Mizmedi Hospital institutional review board. Written informed consent for the genetics study was obtained from all participants. Questions about alcohol consumption included frequency of alcohol consumption on a weekly basis and usual consumption amount on a daily basis (≥20 g/day). Participants who reported smoking were considered current smokers.

Genotyping and selection of SNPs

DNA was isolated from the blood and purified for polymerase chain reaction (PCR) analysis using the QIAamp DNA Mini Kit (Qiagen Inc., Chatsworth, CA, USA). Genotyping was carried out using the TaqMan platform (Applied Biosystems, ABI prism 7900). SNPs were chosen for genotyping if they fit one or more of the following criteria: SNPs within the chosen loci that have previously been reported in the literature to show an association with the phenotype of interest were chosen for genotyping. SNPs that may cause functional protein changes (such as non-synonymous amino acid changes, or SNPs that reside on splice sites) were considered for genotyping. In

addition, SNPs residing in known or putative transcription factor binding sites and having the potential to alter gene expression were considered for genotyping. Finally, SNPs with good minor allele frequency (>1 %) were preferentially chosen for genotyping. From these criteria, we finally selected four SNPs from different genes to be investigated (Table 2).

Statistical analysis

Statistical analyses were performed using SAS software for Windows (v.9.1, Cary, NC, USA).

Statistical results were presented as the mean ± standard deviation or median for continuous variables, while categorical variables were presented using frequencies or percentages. Significance was declared at a two-sided 0.05 level, unless otherwise specified. In order to adjust for age, smoking, alcohol, total calorie intake, total energy expenditure, total calcium intake, total fat intake, body weight, body fat percentage, we made use of a general linear model for continuous dependent variables and a logistic regression model for categorical dependent variables (risk of vertebral fracture and metabolic syndrome). A genotype for each SNP was coded as a three-category variable: 1 = homozygote for allele 1; 2 = heterozygote; 3 = homozygote for allele 2 (for example, A/A, A/G, G/G, respectively). The genotype frequencies of all polymorphisms studied were tested for Hardy–Weinberg equilibrium. We used an additive model to compare each of the three genotype groups. However, some of the analysis was performed in a "dominant model" (analyzing by combining heterozygote group and rare allele homozygote group together) if MAF (minor allele frequency) is too low (<0.1).

Results

Table 1 displays baseline characteristics between fracture ($n = 189$) and no fracture groups ($n = 711$) among 907 subjects (7 were missed for measurement of fracture). At baseline, mean age of fracture and no fracture group is 67.1 ± 7.3 and 64.8 ± 4.7 years old, respectively. The mean age was higher in the fracture group compared to the no fracture group ($p < 0.001$). BMI between the two groups was not significantly different. However, body fat percentage and waist circumference were much higher in the fracture group compared to the no fracture group ($p = 0.0383$, 0.082 respectively). Among other metabolic variables, serum HDL cholesterol in the fracture group was lower than in the no fracture group ($p = 0.011$). Bone mineral density in all sites of the fracture group is lower than the no fracture group.

Genomic data of four SNPs from different genes (*RUNX2*, *PPARG*, *ADIPOQ*, and *LEPR*) are shown in Table 2. Because rs7771980 in *RUNX2* gene had a small MAF (minor allele frequency) of 0.081, we combined the TC and CC group and analyzed them together. As rs8179183 (K656N) in the *LEPR* gene also had a small MAF of 0.079, we combined the GC and CC group as well. All genotype data satisfied the Hardy–Weinberg Equilibrium test ($p > 0.05$) (Table 2).

In rs7771980(*RUNX2*), the risk of vertebral fracture in the TC + CC genotype group was 45 % lower than in the

Table 1 Baseline characteristics in the fracture and no fracture groups

variables	fracture	Non-fracture	p value
Number (%)	189 (21 %)	711 (79 %)	
Age (years)	67.10 ± 7.33	64.81 ± 4.74	<0.001
Body fat (%)	35.47 ± 4.78	34.55 ± 4.83	0.0383
BMI (kg/m^2)	24.58 ± 2.95	24.12 ± 2.78	0.9465
Waist (cm)	90.68 ± 7.37	88.41 ± 8.10	0.0082
Systolic BP	133.34 ± 17.20	130.92 ± 15.90	0.9198
TG (mg/dl)	129.44 ± 78.82	126.16 ± 69.11	0.4489
HDL (mg/dl)	52.14 ± 11.99	53.38 ± 13.74	0.0110
Glucose (mg/dl)	86.62 ± 19.14	87.47 ± 18.54	0.3104
Met SD	77 (33.61 %)	239 (40.74 %)	0.7481
LBMD (g/cm^3)	0.81 ± 0.21	0.84 ± 0.14	0.0468
Femoral neck	0.64 ± 0.13	0.68 ± 0.12	<0.001
Trochanter	0.51 ± 0.10	0.55 ± 0.09	<0.001
Ward	0.40 ± 0.10	0.45 ± 0.12	<0.001
Calcium intake	708.49 ± 243.40	703.61 ± 231.62	0.4302
Current smoke	5 (2.65 %)	10 (1.41 %)	0.8347
Alcohol drink	41 (21.69 %)	126 (17.73 %)	0.2629

BMI body mass index, *BP* blood pressure, *TG* triglyceride, *HDL* high density lipoprotein, *Met SD* metabolic syndrome, *LBMD* lumbar bone mineral density

Table 2 Genomic data according to candidate SNPs

Gene Symbol	RUNX2	PPARG	ADIPOQ	LEPR
SNP Name		IVS5 + 357G/A	276G > T	K656N
rs number	rs7771980	rs2938392	rs1501299	rs8179183
Major	T	A	G	G
Minor	C	G	T	C
Freq Minor	0.081	0.453	0.310	0.079
Genotype (%)	TT (84.53)	AA (30.06)	GT (47.34)	GG (84.93)
	TC (14.68)	AG (49.77)	GT (43.91)	GC (14.17)
	CC (0.79)	GG (20.17)	TT (8.75)	CC (0.9)
HWE (p value)	0.52	0.74	0.26	0.21

SNP single nucleotide polymorphism, *MAF* minor allele frequency, *HWE* Hardy–Weinberg equilibrium

TT group (OR 0.55; 95 % CI 0.32–0.94) after adjusting for age, smoking, alcohol, total calorie intake, total energy expenditure, total calcium intake, total fat intake, body weight and body fat percentage (Table 3; Fig. 1). There was no statistical difference in the bone mineral density of all sites and bone marker (urine DPD and serum OC) between the two genotype groups. In rs2938392 (*PPARG*), the femoral neck BMD in the AA group, AG group, and GG group was 0.68 ± 0.16 g/cm², 0.67 ± 0.10 g/cm², and 0.66 ± 0.10 g/cm², has a tendency to decrease according to the genotype ($p = 0.056$). However, the lumbar spine BMD and that of other femur sites (trochanter, ward) showed no difference between the two groups. The risk of vertebral fracture and bone markers among the three genotype groups did not differ. In rs1501299 (*ADIPOQ*), all BMD data (lumbar, femoral neck, trochanter, ward) did not show any difference among the three genotype groups, but the risk of vertebral fracture had a significant difference. The prevalence of vertebral fracture was 24.06 % in the GG group, 18.99 % in the GT group, and 15.38 % in the TT group, showing a gradual decrease according to the ordered genotype group (OR 0.76; 95 % CI 0.58–0.99) (Table 3; Fig. 1).

In rs8179183 (*LEPR*), all BMD data (lumbar, femoral neck, trochanter and ward BMD, fracture and bone markers) failed to show any difference among the three genotype groups.

Gene–nutrient interaction analysis

In addition, we investigated gene–nutrient interaction. We defined high calcium intake as over 1000 mg of calcium intake a day. The lumbar spine BMD (g/cm²) did not significantly differ between high and low calcium intake in the GG group of rs1501299 of *ADIPOQ* gene. However, in the GT + TT group, the lumbar spine BMD of the high calcium intake group was higher than the low calcium intake group ($p = 0.0264$). There was an interaction between the *ADIPOQ* gene genotype group and calcium intake ($p = 0.0295$, Fig. 2). Other genomic variants such as rs293892 (*PPARG*), 7771980 (*RUNX2*), and rs8179183 (*LEPR*) did not interact with the calcium intake group (data not shown).

Discussion

We demonstrated that variants of obesity-related genes (*ADIPOQ* and *LEPR*) are related to vertebral fracture, suggestive of a genetic-mechanical linkage between obesity and osteoporosis. As *RUNX2* is a key molecule for osteoblast development, some studies already have been published regarding genomic association [15, 16]. Bustamante et al. [15] showed that the −1025 T/C polymorphism (rs7771980) in promoter 2 of *RUNX2* is related to lumbar spine and femoral neck bone mineral density (BMD) in a cohort of 821 Spanish postmenopausal women. In our study, we investigated the association between the genotype group in rs7771980 and not only BMD but also vertebral fracture which showed significant difference (Fig. 1). The most commonly studied variant of *PPARG* in terms of obesity, insulin resistance, and metabolic syndrome is rs1801282 (Pro12Ala) [28-34]. However, Rhee et al. [17] failed to demonstrate the association between genotype variants of rs1801282 (Pro12Ala) and BMD in all sites. Instead, we selected the rs2938392 (Intro 2) SNP, which is one of the candidate SNPs from a large-scale whole genome wide association study [18]. Compared with a Caucasian population (C: 0.349, T: 0.651 Hapmap), the genomic frequency of our subjects is 0.453 of the G(C) and 0.547 of the A(T) allele individually (Table 2). Although we failed to demonstrate an association between genotype variants of rs2938392 and vertebral fracture, lumbar spine BMD is weakly associated with genotype variants of rs2938392 ($p < 0.01$). Two SNPs—T45G (rs224176) and G276T (rs1501299), have been extensively analyzed and associated with obesity. These two SNPs have also been chosen to demonstrate an association between the genetic variants of *AIPOQ* and bone mineral density [19, 20]. Because rs2241766 has no hapmap data, we selected rs1501299 as a final candidate SNP of *ADIPOQ*. Interestingly, our data demonstrate that variants of rs1501299 (*ADIPOQ*) are not associated with BMD in all sites, but are associated with vertebral fracture (Table 3). Although leptin is a key hormone, which regulates appetite and osteoblast differentiation, polymorphism association

Table 3 The association between genotype group of obesity-related genes and bone phenotype

variables	RUNX2 (rs7771980)		PPARG (rs2938392)			ADIPOQ (rs1501299)			LEPR (rs8179183)	
	TT	TC+CC	AA	AG	GG	GG	GT	TT	GG	GC+CC
Number (%)	760 (84.53)	137 (15.47)	270 (30.06)	447 (49.71)	181 (20.15)	428 (47.35)	397 (43.91)	79 (0.08)	767 (84.93)	136 (15.07)
LBMD(g/cm³)	**0.84 ± 0.16**	**0.81 ± 0.14$**	0.82 ± 0.14	0.84 ± 0.17	0.83 ± 0.14	0.83 ± 0.14	0.84 ± 0.18	0.83 ± 0.12	0.83 ± 0.14	0.85 ± 0.15
Femur neck BMD	0.67 ± 0.12	0.66 ± 0.09	**0.68 ± 0.16**	**0.67 ± 0.10**	**0.66 ± 0.10$**	0.67 ± 0.14	0.67 ± 0.09	0.68 ± 0.13	0.67 ± 0.12	0.67 ± 0.10
Trochanter BMD	0.54 ± 0.09	0.53 ± 0.08	0.54 ± 0.10	0.54 ± 0.09	0.54 ± 0.09	0.54 ± 0.10	0.54 ± 0.08	0.56 ± 0.10	0.54 ± 0.09	0.55 ± 0.09
Ward BMD	0.45 ± 0.12	0.43 ± 0.10	0.45 ± 0.15	0.45 ± 0.11	0.43 ± 0.10	0.45 ± 0.14	0.44 ± 0.09	0.45 ± 0.13	0.44 ± 0.12	0.44 ± 0.11
Fracture n (%)	168 (22.22)	19 (14.18)*	56 (21.05)	93 (20.90)	40 (22.22)	102 (24.06)	75 (18.99)	12 (15.38)*	166 (21.81)	22 (16.30)$
Osteocalcin	21.33 ± 7.94	21.32 ± 8.64	21.15 ± 7.92	21.14 ± 8.31	22.15 ± 7.64	21.45 ± 7.80	21.05 ± 8.03	22.12 ± 9.25	21.47 ± 8.28	20.67 ± 6.48
Urine DPD	9.48 ± 3.23	9.90 ± 4.20	9.91 ± 3.62	9.27 ± 3.12	9.78 ± 3.77	9.70 ± 3.45	9.50 ± 3.52	9.21 ± 2.69	9.52 ± 3.46	9.85 ± 3.17

LBMD lumbar bone mineral density, DPD deoxypyridinoline
* $p < 0.05$, $ $p < 0.1$: all data are adjusted for age, smoking, alcohol, total calorie intake, total energy expenditure, total calcium intake, total fat intake, weight, body percent fat
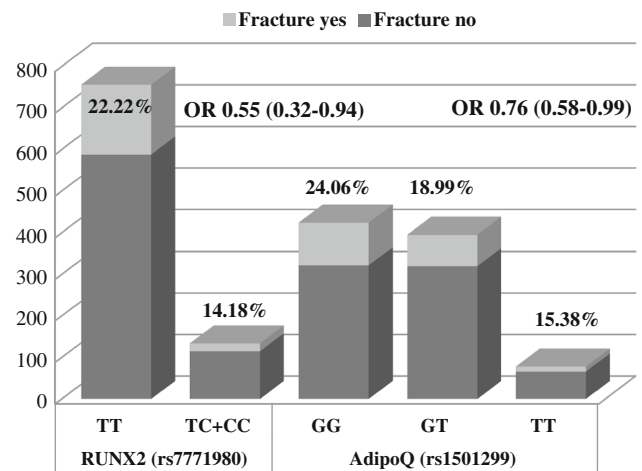

Fig. 1 Odds ratio of osteoporotic fracture according to variants of RUNX(rs7771980) and ADIPOQ(rs1501299); adjusted by age, smoke, alcohol, total calorie intake, total energy expenditure, total calcium intake, total fat intake, weight, body percent fat
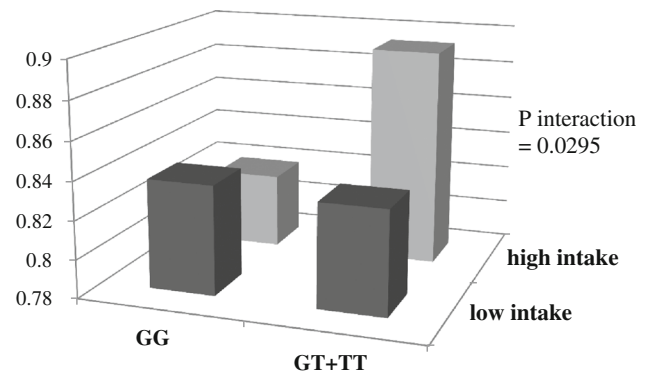

Fig. 2 ADIPOQ Calcium interaction to lumbar BMD (g/cm²); adjusted by age, smoke, alcohol, total calorie intake, total energy expenditure, total calcium intake, total fat intake, wt, body percent fat

studies regarding the leptin gene (LEP) or its receptor gene (LEPR) have not been widely investigated. A few studies have been published to demonstrate an association between bone mineral density and variants of LEPR—mostly focused on rs1137101 (Gln223Arg) [21–23]. Because preliminary data showed that only rs8179183 (K656N) is associated with metabolic syndrome among some candidate SNPs of LEP and LEPR, we finally chose rs8179183 to be analyzed. This SNP of rs8179183 (LEPR) is a novel SNP, which has never been studied. Numerous studies have indicated that some nutrients, including calcium, vitamin D, vitamin K, soybean, and fat intake, affect BMD and vertebral fracture [24–32]. As those studies did not always provide conclusive results, we hypothesized a nutrient individual effect on bone mineral density might differ according to their genotype. Our data demonstrated that calcium intake does not affect BMD in the GG genotype

group of rs1501299 (*ADIPOQ*), but BMD in the high calcium group is much higher than in the low calcium intake group in the GT + TT group of rs1501299 (*ADIPOQ*) (*p* interaction = 0.0295). The mechanism as to how calcium intake affects BMD via adiponectin gene expression remains unclear. Sun et al. [33] demonstrated that 1α,25-hydroxy vitamin D drives inflammatory cytokine expression; therefore, suppression of 1α,25-hydroxy vitamin D by dietary calcium inhibits adipocyte-derived inflammation associated with obesity.

There are possible limitations of a potential risk of reporting false positive due to the issue of multiple testing in the cross-section study. And we couldn't observe the association between an expression of gene and serum marker (for example, an association between an expression of *ADIPOQ* and level of adiponectin in serum).

In conclusion, some SNPs (*RUNX2*, *ADIPOQ*) were associated with fracture risk. Common genomic features of these genes in two phenotypes—fat and bone—give us the rationale to develop co-treatment drugs or nutrients to prevent obesity and osteoporosis simultaneously. Further, calcium intake is associated differently according to genotype of certain SNPs of the *ADIPOQ* gene. This study will be helpful for personalized treatment of osteoporosis and prevent of osteoporotic fracture.

Conflict of interest All authors state that they have no conflicts of interest.

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