

Associations of LRP5 and MTHFR Gene Variants With Osteoarthritis Prevalence in General Elderly Women: A Japanese Cohort Survey Randomly Sampled From a Basic Resident Registry

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Abstract

Osteoarthritis (OA) is a common and degenerative joint disorder in the elderly. A greater importance of understanding the relationship between genetic factors and OA prevalence has emerged with population aging. We therefore investigated the associations of several bone disease-related genetic variants with the prevalence of OA and osteoporosis in Japanese elderly women from the Obuse study cohort, which was randomly sampled from a basic town resident registry. In total, 206 female participants (mean \pm standard deviation age: 69.7 \pm 11.0 years) who completed OA, bone mineral density, and genotype assessments were included. The number of patients diagnosed as having knee/hip OA and osteoporosis was 59 (28.6%) and 30 (14.6%), respectively. Fisher's exact testing revealed significant relationships between the minor T allele of *LDL receptor related protein 5 (LRP5)* rs3736228 and the prevalences of knee/hip OA and osteoporosis. The respective odds ratios (ORs) of the TT genotype for knee/hip OA and osteoporosis were 7.28 (95% confidence interval [CI] 2.22–28.08) and 5.24 (95% CI 0.95–26.98). An additional subgroup analysis for knee OA revealed that the frequency of the common C allele of *methylenetetrahydrofolate reductase (MTHFR)* rs1801133 had a statistically significant protective association with the prevalence of knee OA (OR 0.58, 95% CI 0.35–0.97). In sum, the present study demonstrated significant associations of *LRP5* rs3736228 and *MTHFR* rs1801133 with knee/hip OA and osteoporosis prevalences and knee OA prevalence, respectively, in Japanese elderly women. These results will help further the understanding of OA pathogenesis and related genetic risk factors.

Introduction

Osteoarthritis (OA) is a common degenerative joint disorder occurring with age whose pathophysiology remains incompletely understood. At present, almost all non-surgical treatment options for OA are limited to analgesis and improving joint movement, with no fundamental cures¹. Osteoporosis is a widespread metabolic skeletal disease characterized by diminished bone mineral density (BMD) or bone strength, which increases the risk of fractures. Although several effective medications exist², both osteoporosis and OA are becoming major worldwide health concerns with population aging and rising health-care costs. Therefore, understanding the genetic risk factors for these disorders has emerged as an important issue for disease prevention and therapeutic management.

Many studies on the association of genetic factors with OA and osteoporosis have been reported to date. In the present day, the relationships among genetic variants and related disorders are generally investigated by genome-wide association studies (GWAS). Regarding the prevalence of OA and osteoporosis, 256 and 22 records, respectively, were found in the GWAS catalog (<https://www.ebi.ac.uk/gwas/>)³. Several gene polymorphisms appear to affect OA as well as osteoporosis; indeed, we very recently uncovered a novel association between a homocysteine metabolism-related *methylenetetrahydrofolate reductase (MTHFR)* C677T variant (rs1801133), which was reportedly related to osteoporosis, with the progression of spinal OA [under review].

We have recently established a new population-based epidemiological study of Japanese people that employs random sampling from the basic resident registry of Obuse, a rural town in Japan^{4,5}. The Obuse study contains detailed medical information on the community-dwelling elderly population with minimized selection bias, which allows for examination of a cohort representative of the general population. The present study aimed to investigate the associations of several reported bone disease-related genetic variants, including *MTHFR* rs1801133, with the prevalence of OA and osteoporosis in elderly women sampled from the Obuse study cohort. Significant associations were seen for *LDL receptor related protein 5 (LRP5)* rs3736228 and *MTHFR* rs1801133 with the prevalences of knee/hip OA and osteoporosis and knee OA, respectively, in Japanese elderly women.

Methods

The protocol of this study was approved by the investigational review board of Shinshu University Hospital, Japan (approval number: 2792). The research procedure was carried out in accordance with the ethical guidelines of the 2013 Declaration of Helsinki. Written informed consent was provided by all participants prior to the initiation of the study.

Study subjects

The Obuse study was launched in October 2014 for epidemiological data collection until June 2017. The study randomly sampled 1297 male and female individuals from 5352 members of the resident population between 50–89 years of age in the basic resident registry of Obuse town (Nagano Prefecture, Japan) as a joint collaboration with the cooperating town office. In total, 203 male and 212 female participants provided written informed consent and were enrolled in the Obuse study. The present investigation included 206 female subjects who completed assessments of knee and hip OA, BMD measurements of the total hips and lumbar spine, and genotype determination of the gene variants of interest.

Assessment of OA and osteoporosis

OA of the knee and hip was assessed by radiographic examination. The degree of degeneration was evaluated in accordance with the Kellgren–Lawrence (KL) grading system⁶. Radiographs were examined by 2 experienced orthopaedic surgeons (H.S. and Y.N.). The subjects with the worst KL grading of ≥ 3 in either side of the knees or hips or who had undergone arthroplasty for OA were judged as OA patients. BMD at the lumbar spine and hips was measured using dual-energy X-ray absorptiometry (DXA; PRODIGY, GE Healthcare, Chicago, IL). The regions of interest for lumbar and hip BMD were the L2–4 spinal and bilateral total hip regions, respectively. Subjects with BMD values of $\leq 70\%$ of the young adult mean (YAM) for either the lumbar region or total hips were diagnosed as having osteoporosis.

Determination of genetic variants

Cell-free DNA (cfDNA) was extracted from plasma samples of study subjects using a QIAamp Circulating Nucleic Acid Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's instructions. Genotyping assays were performed by a droplet digital polymerase chain reaction (ddPCR) QX200 system (Bio-Rad, Hercules, CA). Reaction mixture aliquots of 20 μ L containing 10 μ L 2 \times ddPCR Supermix, 5 μ L cfDNA sample, and 0.5 μ L 40 \times TaqMan SNP Genotyping Assay for each variant (Applied Biosystems, Waltham, MA) were prepared. The droplets were generated with a QX200 droplet generator and carefully transferred to 96-well PCR plates. After PCR cycling (40 cycles of 94°C for 30 s and 60°C for 1 min), the fluorescence of each droplet was determined using a QX200 droplet reader followed by analysis with QuantaSoft version 1.7.4 software (Bio-Rad). The present study examined the following genetic variants: *LRP5* rs312009 and rs3736228, *growth differentiation factor 5 (GDF5)* rs143383, *SMAD family member 3 (SMAD3)* rs12901499, and *MTHFR* rs1801133.

Statistical analysis

The background characteristic data of each study group (healthy control, OA, osteoporosis, and comorbid with OA and osteoporosis) are presented as the mean \pm standard deviation (SD) together with the median value. Fisher's exact test was performed to calculate the odds ratio (OR) and 95% confidence interval (CI) of variant genotypes and alleles for the prevalences of OA and osteoporosis versus healthy controls using R version 3.4 software⁷. A two-tailed *P*-value of < 0.05 was considered statistically significant in this study.

Results

Background characteristics of study subjects

The average \pm SD age of the 206 female subjects at enrollment was 69.7 \pm 11.0 years. The number of patients diagnosed as having OA and osteoporosis was 51 (24.8%; knee: 40, hip: 3, knee and hip: 8) and 22 (10.7%), respectively. Eight patients (3.9%) suffered from both osteoporosis and OA (knee: 6, hip: 1, knee and hip: 1) and were classified into the comorbid group. One hundred and twenty-five subjects having neither OA nor osteoporosis were defined as healthy controls in this study. The background characteristics of the study groups are summarized in Table 1.

Associations of genotype and allele frequencies with OA and osteoporosis

In the present cohort, we observed no remarkable associations for *LRP5* rs312009, *GDF5* rs143383, or *SMAD3* rs12901499 with OA or osteoporosis prevalence (Tables 2, 3 and Figures 1, 2). In contrast, the minor T allele of *LRP5* rs3736228 and its homozygotic genotype showed significant relationships with the prevalence rate of knee/hip OA. The ORs of the TT genotype and T allele for OA compared with healthy controls were 7.28 (95% CI 2.22–28.08; *P* < 0.001) and 1.80 (95% CI 1.07–3.00; *P* < 0.05), respectively (Table 2 and Figure 1). Although not significantly, the common C allele of *MTHFR* rs1801133 tended to protect against knee/hip OA prevalence. The respective ORs of the CC genotype and C allele for OA were 0.55 (95% CI 0.23–1.22; *P* = 0.15) and 0.70 (95% CI 0.43–1.14; *P* = 0.13) versus the healthy

control group (Table 2 and Figure 1). The prevalence rate of osteoporosis was significantly correlated with the TT genotype of *LRP5* rs3736228 (OR 5.24, 95% CI 0.95–26.98; $P < 0.05$) (Table 3 and Figure 2).

Subgroup analysis for knee OA prevalence

In a subgroup analysis, we focused on the prevalence of knee OA, which was the most common disorder witnessed in this study. In knee OA only or knee OA + comorbid osteoporosis patients, both the TT genotype ($P < 0.001$) and T allele ($P < 0.05$) of *LRP5* rs3736228 associated significantly with knee OA prevalence as compared with healthy controls (Table 4). Moreover, the C allele of *MTHFR* rs1801133 demonstrated a statistically significant protective association with the prevalence rate of knee OA (OR 0.58, 95% CI 0.35–0.97; $P < 0.05$) in the knee OA + comorbid osteoporosis subgroup (Table 4).

Discussion

This study demonstrated a significant relationship between *LRP5* rs3736228 and the skeletal disorders of OA and osteoporosis in elderly community-dwelling female residents randomly sampled from a Japanese town resident registry. A statistically significant protective association of the common allele of *MTHFR* rs1801133 with knee OA prevalence was also observed. As the population sampling of our cohort minimized selection bias, our results might be considered reflective of the Japanese general population.

LRP5 and 6 (*LRP5/6*) are required as co-receptors for canonical Wnt signaling^{8,9} and play important roles in skeletal development and metabolism. A number of *LRP5* gene variants have been reported. Of those, associations of the missense variants *LRP5* rs3736228 (Ala1330Val) and rs4988321 (Val667Met) with decreased BMD and the risk of osteoporotic fracture are well described^{10,11}. In particular, a relationship between *LRP5* A1330V and diminished BMD has been identified in the Japanese population^{12,13}. A loss of function in *LRP5* increased cartilage degradation in a mouse model¹⁴ and was also suggested to be associated with OA. However, little is known on the precise connection between OA and *LRP5* gene variants. Although associations of *LRP5* rs41494349 (Gln89Arg) with spinal OA¹⁵ and *LRP5* rs3736228 with knee OA¹⁶ have been reported, no information has been recorded in the GWAS catalog to date (<https://www.ebi.ac.uk/gwas/>)³. Therefore, the findings of this study demonstrating a relationship between the T allele of *LRP5* rs3736228 and knee/hip OA prevalence in a randomly sampled population cohort will be of value for further understanding the relationship between OA development and the pathophysiological role of *LRP5* dysfunction.

In the subgroup analysis for knee OA, there was a protective association for the common C allele of *MTHFR* rs1801133 (Ala222Val) rather than a risk association of the minor T allele with the prevalence rate of knee OA. *MTHFR* is known to act within the methionine cycle and play an essential role in homocysteine clearance. A functional deficiency of the *MTHFR* enzyme leads to mild elevation of circulating homocysteine levels¹⁷. The A222V missense variant is a common mutation in the *MTHFR* gene that causes dysfunctional enzymatic activity. Notably, the T allele of *MTHFR* rs1801133 has been implicated in decreased BMD and the occurrence of osteoporotic fractures^{18,19}, and we very recently

uncovered a relationship among homocysteine, *MTHFR* rs1801133, and spinal OA in Japanese postmenopausal women [under review]. The results of the present study imply a correlation between diminished homocysteine levels and a lower risk of knee OA prevalence. Since circulating homocysteine levels can be decreased by vitamin B group supplementation²⁰, the significance of B-vitamins intervention in individuals bearing the T allele of *MTHFR* rs1801133 for preventing OA development may warrant further investigation.

An intron variant of *LRP5* gene rs312009 as well as *GDF5* rs143383 and *SMAD3* rs12901499 showed no remarkable correlations with OA or osteoporosis prevalence in this study. The rs143383 is located in the 5'-untranslated region core promotor of *GDF5*, which encodes a chondrogenic protein. A relationship of rs143383 with OA has been demonstrated in various racial groups, including a Japanese cohort^{21,22}. On the other hand, *SMAD3* is a member of the SMAD family of proteins and plays an essential role in mediating the transforming growth factor-beta signaling pathway. A genetic variant, rs12901499, within the intron 1 of *SMAD3* is reportedly associated with OA in Caucasian and Asian populations^{23,24}. However, other studies have shown no relationship for either *GDF5* rs143383 or *SMAD3* rs12901499 with OA prevalence^{25,26}. Relatively small number of samples limited to female subjects is a limitation of the current study. Besides, although the subjects were randomly sampled from a resident registry, there was a potential for selection bias due to the low participation rate (32.0%) as a result of the noncompulsory survey design. Furthermore, since it sampled from a single town in Japan, this study might contain local features that should be considered when interpreting the data. Future studies with larger sample sizes and male subjects that include multiple regions in Japan and/or other Asian countries will overcome the controversial issues.

In conclusion, we observed significant associations of *LRP5* rs3736228 and *MTHFR* rs1801133 with knee/hip OA and osteoporosis prevalences and knee OA prevalence, respectively, in Japanese elderly women from the randomly sampled Obuse study cohort. The results of the present study will help further the understanding of OA pathogenesis and related genetic risk factors, which will contribute to improved disease prevention and therapeutic management.

Data availability statements

The data analyzed and/or generated during the current study are available from the corresponding author on reasonable request.

Declarations

Acknowledgements

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Author contributions

Y.N. directed and designed the study. M.N., H.Y., S.K., R.T., and N.S. analyzed the data and performed the statistical analysis. N.K., N.E., H.H., H.S., T.S., H.K., J.T., and Y.N. interpreted the data. M.N., H.Y., S.K., and Y.N. drafted the manuscript. All authors approved the final version of the manuscript for publication.

Competing interests

The authors declare no competing interests related to this work.

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Tables

Table 1. Background characteristics of the study groups

	Healthy control (<i>n</i> = 125)	Osteoarthritis (<i>n</i> = 51)	Osteoporosis (<i>n</i> = 22)	Comorbid group [†] (<i>n</i> = 8)
	mean ± SD (median)	mean ± SD (median)	mean ± SD (median)	mean ± SD (median)
Age, years	64.0 ± 8.2 (64.0)	79.3 ± 7.8 (82.0)	75.1 ± 10.3 (77.5)	83.1 ± 6.5 (84.5)
Height, cm	154.0 ± 6.4 (154.5)	148.1 ± 6.5 (147.2)	148.8 ± 5.1 (147.9)	141.7 ± 4.2 (141.3)
Weight, kg	53.0 ± 8.7 (53.0)	52.4 ± 7.1 (52.6)	45.2 ± 7.0 (44.8)	44.5 ± 3.6 (45.2)
BMI, kg/m ²	22.3 ± 3.4 (22.0)	23.9 ± 2.9 (23.6)	20.3 ± 2.3 (20.0)	22.2 ± 1.8 (21.9)
Hip BMD, % YAM	91.1 ± 11.1 (90.0)	86.4 ± 12.3 (84.0)	69.6 ± 8.5 (67.5)	65.4 ± 9.8 (63.8)
Lumbar BMD, % YAM	93.6 ± 13.7 (93.0)	100.0 ± 20.3 (95.0)	71.8 ± 10.4 (69.5)	80.5 ± 10.5 (77.0)

SD, standard deviation; BMI, body mass index; BMD, bone mineral density; YAM, young adult mean

[†]Comorbid with osteoarthritis and osteoporosis

Table 2. Genotype and allele frequencies in patients with osteoarthritis

	Healthy control (<i>n</i> = 125)	Osteoarthritis (<i>n</i> = 51)	OR (95% CI)	<i>P</i> -value
<i>LRP5</i> rs312009				
CC genotype	69 (55.2%)	27 (52.9%)	0.91 (0.45–1.85)	0.87
C allele	190 (76.0%)	76 (74.5%)	0.92 (0.53–1.64)	0.79
<i>LRP5</i> rs3736228				
TT genotype	5 (4.0%)	12 (23.5%)	7.28 (2.22–28.08)	< 0.001
T allele	68 (27.2%)	41 (40.2%)	1.80 (1.07–3.00)	< 0.05
<i>GDF5</i> rs143383				
TT genotype	74 (59.2%)	26 (51.0%)	0.72 (0.35–1.46)	0.40
T allele	195 (78.0%)	73 (71.6%)	0.71 (0.41–1.25)	0.22
<i>SMAD3</i> rs12901499				
GG genotype	20 (16.0%)	13 (25.5%)	1.79 (0.74–4.22)	0.20
G allele	105 (42.0%)	46 (45.1%)	1.13 (0.69–1.85)	0.64
<i>MTHFR</i> rs1801133				
CC genotype	42 (33.6%)	11 (21.6%)	0.55 (0.23–1.22)	0.15
C allele	145 (58.0%)	50 (49.0%)	0.70 (0.43–1.14)	0.13

OR, odds ratio; CI, confidence interval; *LRP5*, LDL receptor related protein 5; *GDF5*, growth differentiation factor 5; *SMAD3*, SMAD family member 3; *MTHFR*, methylenetetrahydrofolate reductase

Table 3. Genotype and allele frequencies in patients with osteoporosis

	Healthy control (<i>n</i> = 125)	Osteoporosis (<i>n</i> = 22)	OR (95% CI)	<i>P</i> -value
<i>LRP5</i> rs312009				
CC genotype	69 (55.2%)	12 (54.5%)	0.97 (0.36–2.72)	1.00
C allele	190 (76.0%)	33 (75.0%)	0.95 (0.43–2.21)	0.85
<i>LRP5</i> rs3736228				
TT genotype	5 (4.0%)	4 (18.2%)	5.24 (0.95–26.98)	< 0.05
T allele	68 (27.2%)	16 (36.4%)	1.53 (0.72–3.14)	0.21
<i>GDF5</i> rs143383				
TT genotype	74 (59.2%)	11 (50.0%)	0.69 (0.25–1.91)	0.49
T allele	195 (78.0%)	32 (72.7%)	0.75 (0.35–1.72)	0.44
<i>SMAD3</i> rs12901499				
GG genotype	20 (16.0%)	4 (18.2%)	1.17 (0.26–4.08)	0.76
G allele	105 (42.0%)	18 (40.9%)	0.96 (0.47–1.92)	1.00
<i>MTHFR</i> rs1801133				
CC genotype	42 (33.6%)	4 (18.2%)	0.44 (0.10–1.46)	0.21
C allele	145 (58.0%)	22 (50.0%)	0.72 (0.36–1.45)	0.33

OR, odds ratio; CI, confidence interval; *LRP5*, *LDL receptor related protein 5*; *GDF5*, *growth differentiation factor 5*; *SMAD3*, *SMAD family member 3*; *MTHFR*, *methylenetetrahydrofolate reductase*

Table 4. Subgroup analysis of patients with knee osteoarthritis

	Knee osteoarthritis only (<i>n</i> = 40)	<i>P</i> - value	Knee osteoarthritis + comorbid osteoporosis (<i>n</i> = 46)	<i>P</i> - value
	OR (95% CI)		OR (95% CI)	
<i>LRP5</i> rs3736228				
TT genotype	10 (25.0%) 7.86 (2.25–31.62)	< 0.001	10 (21.7%) 6.57 (1.90–26.17)	< 0.001
T allele	33 (41.3%) 1.88 (1.07–3.27)	< 0.05	37 (40.2%) 1.80 (1.05–3.06)	< 0.05
<i>MTHFR</i> rs1801133				
CC genotype	7 (17.5%) 0.42 (0.14–1.08)	0.07	8 (17.4%) 0.42 (0.15–1.02)	0.06
C allele	36 (45.0%) 0.59 (0.35–1.01)	0.05	41 (44.6%) 0.58 (0.35–0.97)	< 0.05

OR, odds ratio; CI, confidence interval; *LRP5*, LDL receptor related protein 5; *MTHFR*, methylenetetrahydrofolate reductase

Figures

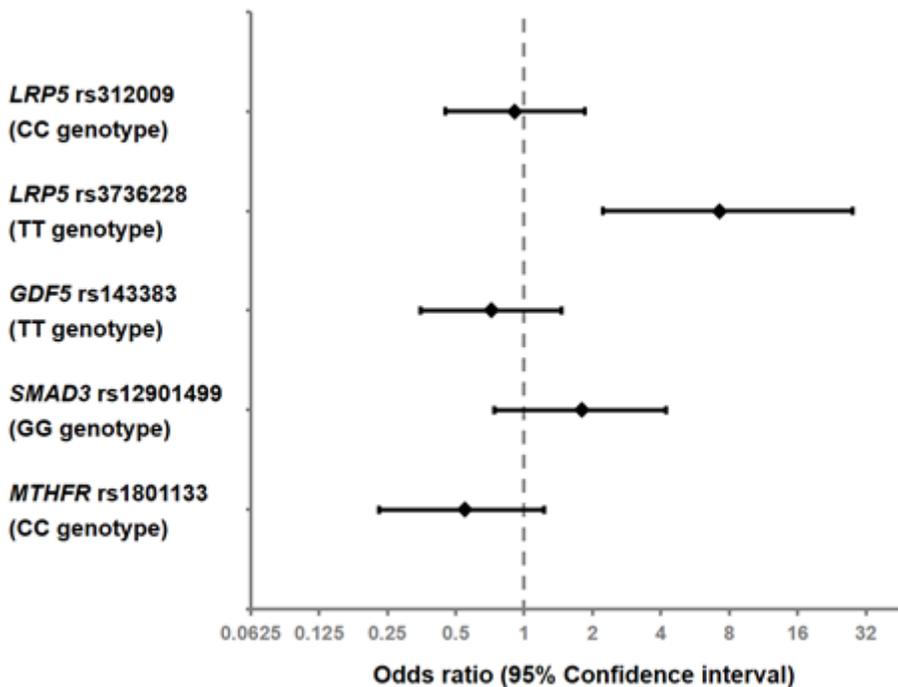


Figure 1

Odds ratios for osteoarthritis by each variant genotype. Fisher's exact test was employed to calculate the odds ratio and 95% confidence interval of variant genotypes for the prevalence of osteoarthritis versus the healthy control group. LRP5, LDL receptor related protein 5; GDF5, growth differentiation factor 5; SMAD3, SMAD family member 3; MTHFR, methylenetetrahydrofolate reductase

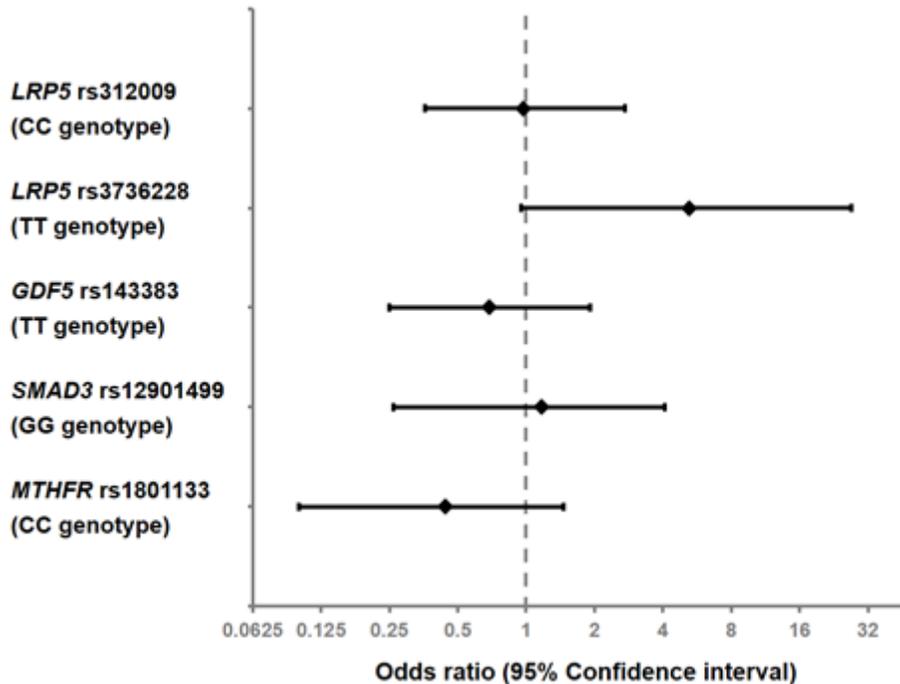


Figure 2

Odds ratios for osteoporosis by each variant genotype. Fisher's exact test was employed to calculate the odds ratio and 95% confidence interval of variant genotypes for the prevalence of osteoporosis versus the healthy control group. LRP5, LDL receptor related protein 5; GDF5, growth differentiation factor 5; SMAD3, SMAD family member 3; MTHFR, methylenetetrahydrofolate reductase