The relationship between *LRP5* rs556442 and rs638051 polymorphisms and mutations and their influence on bone metabolism in postmenopausal Xinjiang women with type 2 diabetes

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Abstract

Background. The Wnt/β-catenin signaling pathway plays a crucial role in bone development and metabolism. The low-density lipoprotein receptor-related protein 5 (LRP5), an important receptor in the Wnt signaling pathway, promotes the osteogenesis of osteoblasts and curbs bone resorption by osteoclasts.

Objectives. To determine the expression of *LRP5* polymorphisms (rs556442 and rs638051) and their relationship with bone mineral density (BMD) and bone metabolism markers in postmenopausal patients with type 2 diabetes mellitus (T2DM) in Xinjiang, China.

Materials and methods. According to dual-energy X-ray (DEXA) and oral glucose tolerance test (OGTT) results, 226 postmenopausal women from Xinjiang were divided into the following groups: normal glucose tolerance (NGT) + normal bone mass group (group A), NGT + abnormal bone mass group (group B), T2DM + normal bone mass group (group C), and T2DM + abnormal bone mass group (group D).

Results. Femoral neck BMD was lower in group B women with the AG/GG genotype (mutant type) compared to women with the AA genotype (wild-type) at rs556442. Alkaline phosphatase (ALP) levels were lower in group D women with the AG/GG genotype (mutant type) compared to women with the AA genotype (wild-type) at rs556442 and rs638051. The factors influencing BMD (lumbar spine vertebrae 1–4 (L1–L4)) were triglyceride (TG) levels, body mass index (BMI), menopausal transition age, and age for rs556442 patients, and TG levels and menopausal transition age for rs638051 patients in group D. The factors affecting BMD (hip) were TG levels, BMI and age for rs556442 patients, and TG levels and age for rs638051 patients.

Conclusions. The *LRP5* gene mutations are linked to bone metabolism disorders in postmenopausal women with T2DM and abnormal bone mass. High BMI and TG were positively associated with BMD, while increased age and menopausal transition age were negatively associated with BMD.

Key words: type 2 diabetes, bone metabolism, postmenopausal women, *LRP5* polymorphism

Background

Increased blood glucose levels caused by insufficient insulin secretion or islet cell dysfunction in type 2 diabetes mellitus (T2DM) affect a number of metabolic processes in the human body. Genetic susceptibility is of great importance in T2DM.[1] This condition not only affects the quality of life and survival status of patients but also causes considerable economic and psychological burden.[2,3]

Osteoporosis (OP) is a common chronic complication in T2DM patients that is related to a decrease in estrogen levels in postmenopausal women. Goldshtein et al. showed that postmenopausal women with T2DM have an increased risk of osteoporotic fractures.[4]

The low-density lipoprotein receptor-associated protein 5 (LRP5) is a transmembrane receptor protein belonging to the low-density lipoprotein (LDL) receptor family. The *LRP5* gene is located on chromosome 11q12–13 and encodes an accessory receptor for the Wnt ligand 5.[5] The *LRP5* promotes the production of insulin, which is conducive to islet signal transduction and bone formation of osteoblasts.[6] It has been found that bone mineral density (BMD) is increased in mice with elevated *LRP5* expression.[7] In addition, *LRP5* is involved in lipid metabolism.[8] Recently, the relationship between the *LRP5* rs41494349 gene polymorphism and OP in postmenopausal women with T2DM was reported.[9] However, the relationship between *LRP5* rs556442 and rs638051 gene polymorphisms and bone metabolism in postmenopausal women with T2DM in Xinjiang remains unknown. Therefore, we aim to fill this research gap and lay the foundation for the prevention and treatment of OP in postmenopausal women with T2DM in Xinjiang.

Objective

The study aimed to explore the expression of *LRP5* polymorphisms rs556442 and rs638051, and their relationship with BMD and bone metabolism markers in postmenopausal patients with T2DM in Xinjiang.

Materials and methods

Research subjects

A total of 226 naturally postmenopausal women treated in the First Affiliated Hospital of Xinjiang Medicine School in Xinjiang, China, were enrolled into the study. Type 2 diabetes mellitus was diagnosed based on the 1999 World Health Organization (WHO) recommendations, and BMD was measured based on the 1994 WHO recommendations. Subjects were divided based on the oral glucose tolerance test (OGTT) and dual-energy X-ray (DEXA) results for BMD. Group A patients had a normal glucose tolerance and normal bone mass (50 patients), and group B consisted of patients with NGT and abnormal bone mass (49 patients). The T2DM patients with normal bone mass were constituted group C (47 patients), and T2DM patients with abnormal bone mass group D (80 patients).

This research complied with the Declaration of Helsinki. Approval from the Ethics Committee of the First Affiliated Hospital of Shihezi University School of Medicine, China, was obtained (approval No. 2015-125-01). We explained the risks, benefits and goals of the study to each participant. Those who agreed to participate signed a written informed consent form.

Data acquisition

General descriptive data of the subjects were collected, and body mass index (BMI) and waist-to-hip ratio (WHR) were calculated (Table 1). The subjects fasted for 8–10 h and antecubital blood was collected the next morning. Triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose (FPG) calcium (Ca), and alkaline phosphatase (ALP) levels, as well as other indices were measured using an automatic biochemical analyzer (bs-280; Mindray, Shenzen, China). Using high-performance liquid chromatography (Bio-Rad D10; Bio-Rad, Hercules, USA), the level of glycosylated hemoglobin (HbA1c) was determined. Bone mineral density was analyzed in the lumbar spine and femur using the DEXA method. The detection of the rs556442 and rs638051 polymorphisms of the *LRP5* gene was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Table 1. Comparison of baseline data among groups using Welch analysis of variance (ANOVA)

Variable	Group A (n = 50)	Group B (n = 49)	Group C (n = 47)	Group D (n = 80)	Welch F	p-value
Age [years]	59.54 ±9.50	65.61 ±12.00[a]	60.30 ±8.49[b]	69.28 ±8.10[abc]	17.503	<0.001
Menopausal transition age [years]	15.14 ±10.00	19.00 ±9.55[a]	13.49 ±8.68[b]	22.24 ±7.75[abc]	13.157	<0.001
BMI [kg/m^2]	27.70 ±3.56	24.80 ±4.38[a]	26.19 ±3.77	24.84 ±3.55[a]	7.634	<0.001
WHR	0.84 ±0.14	0.89 ±0.01[a]	0.91 ±0.06[a]	0.91 ±0.07[a]	5.461	0.002

Data are presented as mean ± standard deviation (M ±SD). BMI – body mass index; WHR – waist-to-hip ratio. [a] age, menopausal transition age, BMI and WHR of groups B and D, and WHR of group C compared with group A ($p < 0.001$); [b] age and menopausal transition age of groups C and D compared with group B ($p < 0.001$); [c] age and menopausal transition age of group D compared with group C ($p < 0.001$).

Determination of single nucleotide polymorphism sites

According to the general principles for selecting single nucleotide polymorphisms (SNPs), we consulted the relevant literature and selected the SNPs related to the *LRP5* gene. Then, we selected functionally related and important sites, such as missense mutations, which change the amino acid sequence and affect protein function. The best locus for the functional region of the *LRP5* gene according to the National Center for Biotechnology Information (NCBI) website was chosen (https://www.ncbi.nlm.nih.gov/gene/?term=LRP5+and+human).[10,11] In this study, two SNPs of the *LRP5* gene were selected – rs556442 and rs638051.

DNA extraction

Five milliliters of antecubital blood were collected from each subject in ethylenediaminetetraacetic acid (EDTA)-coated tubes. The DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and stored at –80°C. The concentration and purity of DNA were assessed using a NanoDrop 2000 UV spectrophotometer (Thermo Fisher Scientific, Waltham, USA), and the absorption of nucleic acids at 260 nm was quantified.

Gene sequencing

Primers for amplification and single base extensions were designed using Sequenom assay designer software v. 3.0 (Sequenom, San Diego, USA). The primer sequences were as follows: rs556442, forward: 5'-GGGCAGCCAAGATCGAAC-3' and reverse: 5'-CGTCCACCCAGAACAGCTT-3'; rs638051, forward: 5'-CTTTGGGCAGTGGGCTTAG-3' and reverse: 5'-CACCCTCTGGACATAGCTCTGA-3'. Polymerase chain reaction (PCR) conditions involved 1) preheating the samples to 94°C for 4 min (1 cycle); 2) 94°C for 20 s (45 cycles); 3) 56°C for 30 s (45 cycles); 4) 72°C for 1 min (45 cycles); and 5) final extension at 72°C for 3 min (1 cycle). The PCR products were obtained using a 384-pad spectral chip (JL-PZY96BT; Yibaiju, Shanghai, China). Each PCR product was treated with shrimp alkaline phosphatase (SAP; Sequenom). The Iplex (Sequenom) single base extension reaction was performed. Resin (Xingruikebo, Shihezi, China) purification procedure was also performed using the mass array nanodispenser RS1000 spotter (Sequenom), and the extension product was moved to the 384-pad spectral chip.[12–14] The MALDI-TOF MS (Sequenom) was performed to detect rs556442 and rs638051 genotypes under different conditions.[15] After the above reaction steps, the chip was put into the mass spectrometer (Sequenom). The flight time of these ionized products depended on the quality of each allele, measured using mass spectrometer. The smaller the ion mass, the faster it arrives. Finally, the molecular weight and base type were determined according to the position of the simplicity peak to succeed in typing.[16]

Statistical analyses

The IBM SPSS v. 22.0 software (IBM Corp., Armonk, USA) was used to analyze the data. The data conforming to a normal distribution are presented as the mean ± standard deviation (M ±SD). General data and biochemical parameters were compared between the groups using independent sample t-tests. When the baseline data was homogeneous (age [years], menopausal transition age [years], BMI [kg/m^2], and WHR), one-way analysis of variance (ANOVA) testing was used to conduct intergroup comparisons. Otherwise, analysis of covariance (ACNOVA) was used. The χ^2 goodness-of-fit was determined using the Hardy–Weinberg equilibrium (HWE) test. Multiple linear regression was adopted to analyze the factors influencing BMD, and independent variables were selected using the best subset regression, which was based on adjusted R^2 values. A p-value <0.05 was considered statistically significant.

The results of the statistical tests are available as Supplementary data.

Results

There were statistically significant differences in menopausal transition age, BMI, and WHR within groups A, B and D ($p < 0.05$, Table 1).

The ACNOVA demonstrated that FPG and HbA1c levels were statistically higher in groups C and D compared to group A ($p < 0.01$). Bone mineral density (femoral neck) and BMD (lumbar spine vertebrae 1–4 (L1–L4)) were statistically lower in groups B and D compared to group A ($p < 0.01$, Table 2).

The genotype frequencies of the 2 loci were consistent with the HWE ($p > 0.05$). There were no significant differences in genotype frequency and gene frequency between the rs556442 and rs638051 loci on the *LRP5* gene ($p > 0.05$, Table 3).

At the rs556442 locus in the group B, femoral neck BMD of the AG/GG genotype was statistically lower than that of the AA genotype ($p < 0.05$). In group D, ALP level was statistically lower for the AG/GG genotype compared to the AA genotype ($p < 0.05$, Table 4). At the rs638051 locus in group D, ALP level was statistically lower for the AG/GG genotype compared to the AA genotype ($p < 0.05$, Table 5).

In group D (T2DM + abnormal bone mass), the best subset regression analysis was performed with BMD (L1–L4) and BMD (femoral neck) as the response variables and age (X1), menopausal transition age (X2), BMI (X3), WHR (X4), FPG (X5), HbA1c (X6), TG (X7), high-density lipoprotein (HDL)

Table 2. Comparison of biochemical indexes among groups after analysis of covariance (ANCOVA)

Variable	Group A (n = 50)	Group B (n = 49)	Group C (n = 47)	Group D (n = 80)	F	p-value
FPG [mmol/L]	5.39 ±1.58	5.08 ±0.58	8.24 ±3.00[ab]	7.57 ±2.36[ab]	28.861	<0.001
HbA1c [%]	5.73 ±0.80	6.10 ±0.77	7.73 ±1.21[ab]	7.94 ±1.51[ab]	52.059	<0.001
TG [mmol/L]	2.12 ±1.29	1.31 ±0.59[a]	2.07 ±1.27[b]	1.50 ±0.99[ac]	7.676	<0.001
HDL-C [mmol/L]	1.33 ±0.39	1.35 ±0.42	1.42 ±0.68	1.29 ±0.39	0.840	0.473
LDL-C [mmol/L]	2.66 ±0.89	2.56 ±0.82	2.99 ±1.09[b]	3.43 ±1.19[abc]	9.370	<0.001
Ca [mmol/L]	2.28 ±0.07	2.28 ±0.11	2.44 ±0.43[ab]	2.32 ±0.24[c]	4.511	0.004
P [mmol/L]	1.11 ±0.15	1.12 ±0.19	1.13 ±0.14	1.20 ±0.47	1.259	0.289
ALP [U/L]	78.72 ±17.54	82.77 ±27.30	69.33 ±18.85[ab]	75.99 ±19.54	3.495	0.016
BMD (L1–L4) [g/cm^2]	1.21 ±0.15	0.89 ±0.17[a]	1.17 ±0.17[b]	0.92 ±0.11[ac]	68.544	<0.001
BMD (femoral neck) [g/cm^2]	0.93 ±0.13	0.72 ±0.10[a]	0.96 ±0.12[b]	0.75 ±0.11[ac]	63.717	<0.001

Data are presented as mean ± standard deviation (M ±SD). [a] FPG of groups C and D, HbA1c ($p < 0.001$), TG of groups B and D ($p < 0.001$), LDL-C of group D ($p < 0.001$), Ca of group C ($p = 0.004$), ALP of group C ($p = 0.016$), and BMD (L1-L4) and BMD (femoral neck) of groups B and D ($p < 0.001$) compared with group A; [b] FPG, HbA1c and LDL-C of groups C and D ($p < 0.001$), TG of group C ($p < 0.001$), Ca of group C ($p = 0.004$), ALP of group C ($p = 0.016$), and BMD (L1-L4) and BMD (femoral neck) of group C ($p < 0.001$) compared with group B; [c] TG, LDL-C, BMD (L1-L4), and BMD (femoral neck) of group D ($p < 0.001$), and Ca of group D ($p = 0.004$) compared with group C. FPG – fasting plasma glucose; HbA1c – glycosylated hemoglobin; TG – triglyceride; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; Ca – calcium; P – phosphorus; ALP – alkaline phosphatase; BMD – bone mineral density; L1–L4 – lumbar spine vertebrae 1–4.

Table 3. Genotype and allele distribution frequencies of the *LRP5* gene rs556442 and rs638051 locus calculated using the χ^2 test (n (%))

SNP	Genotype/allele	Group A	Group B	Group C	Group D	χ^2 value	p-value
rs556442	AA	31 (62.0)	30 (61.2)	33 (70.2)	52 (65.0)	3.433	0.763
	AG	17 (34.0)	17 (34.7)	10 (21.3)	23 (28.8)		
	GG	2 (4.0)	2 (4.1)	4 (8.5)	5 (6.2)		
	A	79 (79.0)	77 (78.6)	76 (80.9)	127 (79.4)	0.172	0.982
	G	21 (21.0)	21 (21.4)	18 (19.1)	33 (20.6)		
rs638051	AA	17 (65.4)	15 (55.6)	18 (58.0)	30 (53.6)	3.054	0.812
	AG	6 (23.1)	11 (40.7)	10 (32.3)	20 (35.7)		
	GG	3 (11.5)	1 (3.7)	3 (9.7)	6 (10.7)		
	A	40 (76.9)	41 (75.9)	46 (74.2)	80 (71.4)	0.719	0.869
	G	12 (23.1)	13 (24.1)	16 (25.8)	32 (28.6)		

SNP – single nucleotide polymorphism. The AA (wild-type), AG/GG (mutant-type) and A/G (allele gene) of groups B, C and D were compared with those of group A.

Table 4. Comparison of biochemical indices and bone mineral density (BMD) between genotypes at the rs556442 locus in groups using t-test

Variable	Group B		t-value	p-value	Group D		t-value	p-value
	AA	AG/GG			AA	AG/GG		
FPG [mmol/L]	5.23 ±0.65	4.93 ±0.58	1.639	0.108	8.22 ±2.69	7.31 ±1.36	2.009	0.050
HbA1c [%]	6.08 ±0.79	6.03 ±0.43	0.286	0.776	7.76 ±1.67	7.68 ±1.36	0.217	0.828
TG [mmol/L]	1.29 ±0.95	1.53 ±0.72	–0.942	0.351	1.85 ±1.25	1.51 ±0.95	1.256	0.213
HDL-C [mmol/L]	1.36 ±0.37	1.33 ±0.35	0.282	0.779	1.31 ±0.43	1.24 ±0.28	0.878	0.383
LDL-C [mmol/L]	2.51 ±0.93	3.03 ±0.80	–2.010	0.050	3.13 ±1.20	3.51 ±0.92	–1.459	0.149
Ca [mmol/L]	2.28 ±0.12	2.28 ±0.07	0.147	0.884	2.29 ±0.17	2.27 ±0.11	0.636	0.527
P [mmol/L]	1.15 ±0.19	1.09 ±0.16	1.143	0.259	1.26 ±1.24	1.06 ±0.13	1.151	0.255
ALP [U/L]	82.20 ±29.99	85.00 ±19.46	–0.396	0.694	77.46 ±20.66	66.36 ±21.02	2.278	0.025*
BMD (L1–L4) [g/cm^2]	0.91 ±0.19	0.86 ±0.10	1.202	0.235	0.93 ±0.10	0.90 ±0.12	1.192	0.237
BMD (femoral neck) [g/cm^2]	0.75 ±0.10	0.68 ±0.08	2.571	0.013*	0.76 ±0.10	0.74 ±0.13	0.767	0.446

Data are presented as mean ± standard deviation (M ±SD). *$p < 0.05$, **$p < 0.01$. FPG – fasting plasma glucose; HbA1c – glycosylated hemoglobin; TG – triglyceride; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; Ca – calcium; P – phosphorus; ALP – alkaline phosphatase; L1–L4 – lumbar spine vertebrae 1–4.

Table 5. Comparison of biochemical indices and bone mineral density (BMD) between genotypes at the rs638051 locus in the group D using t-test

Variable	AA	AG/GG	t-value	p-value
FPG [mmol/L]	7.61 ±2.21	7.21 ±1.34	0.831	0.410
HbA1c [%]	7.59 ±1.12	7.33 ±1.13	0.863	0.392
TG [mmol/L]	1.53 ±0.66	1.76 ±1.16	–0.893	0.377
HDL-C [mmol/L]	1.28 ±0.33	1.14 ±0.25	1.767	0.083
LDL-C [mmol/L]	3.39 ±1.01	3.62 ±1.14	–0.801	0.427
Ca [mmol/L]	2.27 ±0.09	2.29 ±0.12	–0.711	0.480
P [mmol/L]	1.12 ±0.12	1.16 ±0.18	–0.963	0.341
ALP [U/L]	80.00 ±16.83	66.54 ±25.38	2.301	0.026*
BMD (L1–L4) [g/cm^2]	0.92 ±0.12	0.89 ±0.11	0.970	0.337
BMD (femoral neck) [g/cm^2]	0.75 ±0.11	0.74 ±0.11	0.339	0.736

Data are presented as mean ± standard deviation (M ±SD). * $p < 0.05$, ** $p < 0.01$. FPG – fasting plasma glucose; HbA1c – glycosylated hemoglobin; TG – triglyceride; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; Ca – calcium; P – phosphorus; ALP – alkaline phosphatase; L1–L4 – lumbar spine vertebrae 1–4.

(X8), LDL (X9), Ca (X10), phosphorus (P) (X11), ALP (X12), and genotype (X13) as independent variables.

The best subset regression analysis demonstrated that lower TG level and BMI, older age and higher menopausal transition age at the rs556442 locus were risk factors for a decreased BMD (L1–L4). Lower TG level and higher menopausal transition age at the rs638051 locus were risk factors for decreased BMD (L1–L4). Except for higher menopausal transition age, all variables were also risk factors for a decreased BMD (femoral neck, Table 6).

Discussion

In an aging population, the incidence rate of OP is increasing with each calendar year, and high disability rate among OP patients places a heavy financial burden on the society.[17] As confirmed in other studies, postmenopausal women with T2DM have a decreased bone mass due to a rapid decline in estrogen levels, putting them at high risk for OP.[18,19] Therefore, at the gene level, further study of the pathogenesis of OP can provide a theoretical basis for explaining the occurrence of T2DM with OP. Currently, it is unclear whether T2DM can lead to the reduction of BMD. However, an increasing number of studies have shown that T2DM can increase bone fragility,[20,21] and such fragility leads to an increase in T2DM with OP.[22,23] The pathogenesis of T2DM complicated by OP is multifactorial and influenced by genetic and environmental factors. The Wnt signaling pathway is crucial in the axis differentiation of multicellular organisms,[24] where LRP5 is a transmembrane receptor of the Wnt protein.[25] Regarding the Wnt signaling pathway, studies on *LRP5* gene polymorphisms and T2DM or OP have been published both in China and in other countries, but there are few reports on the relationship between *LRP5* gene polymorphisms and OP in postmenopausal women with T2DM.

This study found that the genotype and allele frequency distribution of the rs556442 and rs638051 loci of *LRP5* comply with the Hardy–Weinberg law of genetic balance. This indicates that the population selected for this study has relatively stable heritability and is a representative of the target population. The genotype distribution was dominated by the AA genotype, which was 64.6% and 57.1% for rs556442 and rs638051, respectively (Table 3). The wild-type homozygous genotype was the most common, the mutant heterozygous genotype was the 2nd most common, and the mutant homozygous genotype was the least common. Astiazar et al.[26] and Koay et al.[27] found that the *LRP5* gene mutation can reduce BMD and increase the incidence of OP. This study found that for rs556442 in group B, the BMD (femoral neck) of the AG/GG genotype (mutant) was lower compared to the AA genotype (wild-type, 0.68 ±0.08 compared to 0.75 ±0.10 g/cm^2, $p = 0.013$). At rs556442, ALP level for the AG/GG genotype (mutant-type) was lower in group D than in the AA genotype (wild-type, 66.36 ±21.02 compared to 77.46 ±20.66 U/L, $p = 0.025$; Table 4). In group D, at rs638051, ALP level for the AG/GG genotype (mutant-type) was lower compared to the AA genotype (wild-type, 66.54 ±25.38 compared to 80.00 ±16.83 U/L, $p = 0.026$). These findings suggest that mutations at rs556442 and rs638051 loci may be related to BMD and bone metabolism (Table 5). Wang et al. showed that *LRP5* gene polymorphisms are genetically linked to increases in blood lipid levels, BMI and obesity.[28] Through the best subset regression analysis, decreased BMI and TGs and higher menopausal transition age and age were found to be risk factors for decreased BMD, suggesting that higher BMI and TG levels are associated with a lower risk of abnormal BMD among diabetic patients, similar to the findings of Li et al.[29] Thus, postmenopausal women with dyslipidemia should receive screening to prevent the occurrence of OP.

Table 6. Best subset regression analysis of the influencing factors of bone mineral density (BMD) in type 2 diabetes mellitus (T2DM) patients

SNP	BMD	Variable	Adjusted R^2	β	t-value	p-value
rs556442	L1–L4	TG	0.247	0.043	3.773	<0.001
		BMI	–	0.008	2.479	0.014
		menopausal transition age	–	–0.004	–3.162	0.002
		age	–	–0.003	–2.002	0.047
		WHR	–	–0.146	–1.010	0.314
		Ca	–	0.091	1.862	0.064
		HbA1c	–	–0.011	–1.331	0.184
	femoral neck	TG	0.210	0.025	2.845	0.005
		BMI	–	0.005	2.142	0.033
		age	–	–0.003	–2.797	0.006
		menopausal transition age	–	–0.001	–1.333	0.184
		FPG	–	0.006	1.437	0.152
		HbA1c	–	–0.008	–1.093	0.275
		HDL-C	–	0.022	1.068	0.287
		LDL-C	–	–0.013	–1.399	0.163
		ALP	–	–0.001	–1.130	0.260
rs638051	L1–L4	TG	0.272	0.035	3.580	<0.001
		menopausal transition age	–	–0.005	–3.217	0.002
		FPG	–	0.009	1.683	0.094
		BMI	–	0.004	1.065	0.289
		age	–	–0.001	–1.013	0.313
	femoral neck	TG	0.196	0.213	2.077	0.039
		age		–0.002	–2.262	0.025
		WHR	–	0.279	1.526	0.129
		LDL-C	–	–0.018	–1.886	0.061
		BMI	–	0.004	1.496	0.137
		menopausal transition age	–	–0.002	–1.861	0.064
		ALP	–	–0.001	–1.627	0.106

SNP – single nucleotide polymorphism; WHR – waist-to-hip ratio; BMI – body mass index; TG – triglyceride; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; Ca – calcium; ALP – alkaline phosphatase; FPG – fasting plasma glucose; HbA1c – glycosylated hemoglobin; L1–L4 – lumbar spine vertebrae 1–4.

Limitations of the study

The adjusted R^2 in the best subset regression analysis model established for rs556442 and rs638051 was relatively low, which was considered to be related to the quantity and quality of the included independent variables.

In the future, our research group will consider investigating more important factors that may affect the occurrence and development of diseases, such as FINS, FCP, ISI, PINP, CTX, NTX, etc., which include many invasive independent variables with high diagnostic value, so as to further improve the value of the model.

Conclusions

To summarize, mutations at rs556442 and rs638051 loci of the *LRP5* gene are related to bone metabolism in postmenopausal women in Xinjiang. Therefore, postmenopausal women with T2DM should undergo screening and early intervention strategies for the prevention of OP.

Supplementary data

The results of the statistical tests are available as Supplementary data at https://doi.org/10.5281/zenodo.7196566. The package contains the following files:

Supplementary Table 1. Normality test of the variables presented in Table 1,2.

Supplementary Table 2. Homogeneity of variances test of the variables presented in Table 1,2.

Supplementary Table 3. Welch ANOVA of the statistical values presented in Table 1.

Supplementary Table 4. ANCOVA of the statistical values presented in Table 2.

Supplementary Table 5. Post hoc tests of the statistical values presented in Table 1.

Supplementary Table 6. Post hoc tests of statistical values presented in Table 2.

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