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Correlation between LRP5 Protein Expression and rs41494349, rs3736228 Polymorphism and Bone Metabolism in Postmenopausal Women with Type 2 Diabetes Mellitus

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Abstract

Objective: To analyze the relationship between Low density lipoprotein receptor related protein 5 (LRP5) expression, rs41494349 and rs3736228 polymorphisms and bone mineral density (BMD) and bone metabolism indexes in postmenopausal women with type 2 diabetes, and to provide the basis for the prevention and treatment of OP in this population.

Methods: 136 postmenopausal women admitted to our hospital were divided into normal control group (n=26), T2DM group (n=28), OP group (n=27) and T2DM combined with OP group (n=55) according to 75g glucose tolerance test (OGTT) and dual energy X-ray bone density determination (DEXA). Fasting blood glucose (FBG), triacylglycerin (TG), calcium and other clinical indicators were measured. DEXA measures bone mineral density in the lumbar spine (L1-4), hip joint, and femur neck; LRP5 protein expression was determined by enzyme-linked immunosorbent assay (ELISA). rs41494349 and rs3736228 polymorphisms of LRP5 gene were detected by matrix-assisted laser desorption/ionisation, time-of-flight mass spectrometry (MALDI-TOF-MS). Correlation analysis of LRP5 protein and clinical indicators; Multiple stepwise linear regression analysis of BMD influencing factors.

Results: 1. Compared with Control group, HbA1c and FPG were increased in T2DM group and T2DM+OP group (P <0.01); TG, BMD(L1-4) and BMD (femur neck) in OP group and T2DM+OP group were decreased (P<0.05 or P<0.01). 2. Compared with the Control group, the protein concentration of LRP5 in OP group, T2DM group and T2DM+OP group was decreased (P<0.01). 3. Correlation analysis showed that LRP5 protein concentration was negatively correlated with age, menopausal years and FPG (r<0, P<0.05). It was positively correlated with BMI, BMD (L1-4) and BMD (femur neck) (r>0, P<0.05). 4. BMD of AA type (L1-4) in T2DM+OP group at rs41494349 of LRP5 gene was higher than that of AG/GG type. CC TG of LRP5 gene rs3736228 locus in Control group was higher than TT/TC TG, WHILE CC P and HDL of LRP5 gene rs3736228 locus in T2DM group were lower than TT/TC TG. CC type BMD (L1-4) was higher than TT/TC type in T2DM+OP group. (P<0.05). 5. Stepwise multiple linear regression analysis showed that BMI was a positive influence factor

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on BMD (femur neck) and BMD (L1-4) levels, while years of menopause was a negative influence factor. rs41494349 polymorphism was a negative factor of BMD (femur neck). TG was a positive influence factor of BMD (L1-4) level.
Conclusion: LRP5 protein expression and rs41494349 and rs3736228 polymorphism may be related to bone metabolism in postmenopausal women with type 2 diabetes mellitus, rs3736228 mutation may also be related to lipid metabolism in this population.

Keywords: ***Type 2 diabetes mellitus; Osteoporosis; LRP5; Bone mineral density; Gene polymorphism***

1. Introduction

Type 2 Diabetes mellitus (T2DM) is a metabolic disease mainly characterized by increased blood glucose levels caused by insufficient insulin secretion or islet cell dysfunction, which affects multiple systems in the human body. Genetic susceptibility plays an important role in T2DM [1]. T2DM and its complications seriously affect patients' quality of life and bring economic and psychological burden [2,3]. Osteoporosis (OP) is one of the common chronic complications in T2DM patients. OP patients have a significantly increased risk of fracture. Studies have found [4] that decreased estrogen levels in postmenopausal women are connected with an increased risk of osteoporosis and fracture. Low density lipoprotein receptor related protein 5 (LRP5) is a transmembrane receptor protein belonging to the low-density lipoprotein receptor family, whose encoding gene is located on chromosome 11q12-13 and is the receptor of Wnt signaling pathway [5]. LRP5 can promote insulin production, islet signal transduction and bone formation of osteoblasts. Studies have found that mice with elevated LRP5 expression had increased Bone mineral density (BMD) [6]. In addition, other studies have confirmed that LRP5 also plays a crucial part in lipid metabolism [7]. The relationship between LRP5 gene and OP has been reported in the past [8]. However, there are few reports on the expression of LRP5 protein concentration in T2DM combined with OP at home and abroad, and the study of rs41494349 and rs3736228 gene polymorphism of LRP5 gene and bone metabolism in postmenopausal women with T2DM has not been reported. This study aims to explore this problem and provide evidence for the prevention and treatment of postmenopausal T2DM women with OP.

2. Objects of Research

136 cases of natural postmenopausal women admitted to our hospital from 2020 to 2021 were included. According to OGTT and dual-energy X-ray detection results, they were divided into Control group(n=26), T2DM group(n=28) and OP group (n=27), T2DM+ OP group (n=55). This study was approved by the Ethics Committee and all subjects signed informed consent.

3. Data Acquisition

General data of subjects were collected, and body mass index (BMI) and waist-to-hip ratio (WHR) were calculated. Triacylglycerol (TG), low density lipoprotein cholesterol (LDL-C), fasting blood glucose (FPG), calcium (Ca), alkaline phosphatase (ALP) and other indicators were detected by automatic biochemical analyzer. HbA1c was detected by HPLC affinity chromatography. BMD (lumbar L4-1, g/cm2) and BMD (femur neck, g/cm^2) were measured by dual-energy X-ray bone density measuring instrument. BMD measurements of all patients were performed by the same technician, machine calibration was performed once a day, and the results were stored in the database. The coefficient of variation of

anteroposterial-lateral lumbar examination was 0.49% and 0.66%, and the 3-year QC was <1%. The expression of LRP5 protein was determined by enzyme-linked immunosorbent assay (ELISA). rs41494349 and rs3736228 polymorphisms of LRP5 gene were detected by Sequenom time-of-flight mass spectrometry. A spectrophotometer measures the concentration and purity of DNA.

4. Statistical Analysis

Statistical software SPSS 22.0 analyzed the data. In accordance with the normal distribution of measurement data. Analysis of variance (ANOVA) was used for uniform baseline data, and covariance analysis was used for inconsistent baseline data. The hardy-Weinberg genetic balance test was performed by $\chi 2$ goodness of fit test. The correlation between LRP5 protein and clinical indicators was analyzed by correlation analysis. The influence factors of bone mineral density were analyzed by multiple stepwise linear regression. P<0.05 was considered statistically significant.

5. Results

5.1 Comparison of general data between groups

The results of ANOVA showed that there was no significant difference in BMI and WHR between groups (P>0.05). Age and duration of menopause in OP group and T2DM+OP group were higher than those in control group (P<0.05 or P<0.01). Baseline data were of different quality between groups, and covariance was used for other indicators. The results of covariance analysis showed that there were no significant differences in LDL-C, HDL-C, Ca, P and ALP between groups (P>0.05). Compared with Control group, HbA1c and FPG were increased in T2DM group and T2DM+OP group (P<0.01). TG, BMD(L1-4) and BMD (femur neck) in OP group and T2DM+OP group were decreased (P<0.05 or P<0.01)(TABLE 1).

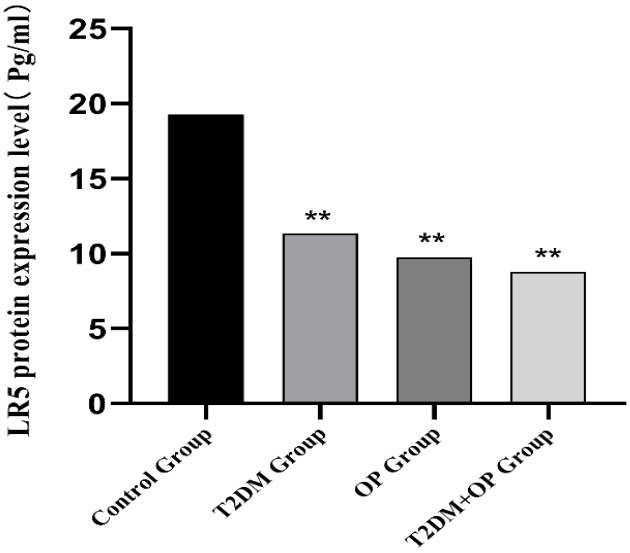
TABLE 1. **Comparison of general data between groups [$\bar{x} \pm s$].**

Parameter	Control (n=26)	T2DM (n=27)	OP (n=28)	T2DM+OP (n=55)
Age(years)	65.60 ± 8.20	66.50 ± 7.50	69.70 ± 7.30*	70.50 ± 6.40 **
Menopausal period	15.70 ± 8.00	16.70 ± 7.10	19.70 ± 7.10*	20.40 ± 6.00 **
WHR	0.87 ± 0.18	0.90 ± 0.05	0.88 ± 0.10	0.91 ± 0.06
BMI(Kg/m^2)	25.70 ± 3.00	27.10 ± 4.00	25.70 ± 4.70	25.68 ± 3.64
HbA1c(%)	5.92 ± 1.06	7.20 ± 1.10 **	6.00 ± 1.06	7.50 ± 1.05 **
FPG(mmol/l)	5.20 ± 2.10	7.60 ± 2.10 **	5.15 ± 2.11	7.50 ± 2.16 **
LDL-C (mmol/l)	3.06 ± 1.00	3.18 ± 1.01	3.17 ± 1.00	3.50 ± 1.00
TG(mmol/l)	2.49 ± 1.49	2.26 ± 1.40	1.49 ± 1.00*	1.70 ± 1.00 **
HDL-C (mmol/l)	1.36 ± 0.30	1.18 ± 0.33	1.31 ± 0.32	1.21 ± 0.33
Ca (mmol/l)	2.26 ± 0.13	2.25 ± 0.14	2.29 ± 0.14	2.28 ± 0.15
P (mmol/l)	1.10 ± 0.80	1.10 ± 0.79	1.07 ± 0.78	1.24 ± 0.79
ALP (U/l)	80.30 ± 21.00	72.40 ± 21.20	82.10 ± 21.00	73.80 ± 21.21
BMD(L_{1-4}) (g/cm^2)	1.20 ± 0.14	1.22 ± 0.15	0.85 ± 0.14 **	0.91 ± 0.15 **
BMD(Femur neck) (g/cm^2)	0.88 ± 0.18	0.85 ± 0.19	0.72 ± 0.19 **	0.75 ± 0.19 **

Compared with control group, *P<0.05, **P<0.01(BMI: body mass index, WHR: waist-hip ratio)

5.2 Comparison of LR5 protein expression levels between groups

Covariance analysis showed that compared with the Control group, the protein concentration of LRP5 in OP group, T2DM group and T2DM+OP group was decreased (P<0.01). (FIG. 1).



Compared with control group, **P<0.01

FIG. 1. **Comparison of LR5 protein expression levels between groups.**

5.3 Correlation analysis between LRP5 protein and clinical indicators

Correlation analysis showed that LRP5 protein concentration was negatively correlated with age, menopausal years and FPG (r<0, P<0.05). It was positively correlated with BMI, BMD (L1-4) and BMD (Femur neck) (r>0, P<0.05) (TABLE 2).

TABLE 2. **Correlation analysis between LRP5 protein and clinical indicators.**

	LRP5 protein	
	r	P value
Age	-0.322	0.001
Menopausal years	-0.267	0.008
BMI	0.226	0.024
WHR	-0.076	0.454
FPG	-0.225	0.025
HbA1c %	-0.195	0.053
TG	0.169	0.094
HDL	-0.099	0.931
LDL	-0.021	0.838
Ga	0.099	0.330
P	-0.021	0.838
ALP	-0.164	0.105
BMD(L1-4)	0.284	0.004
BMD (Femur neck)	0.297	0.003

5.4 Genotype and allele frequency distribution of rs41494349 and rs3736228 in LRP5 gene

Genotypes of rs41494349 and rs3736228 of LRP5 gene in 4 groups were examined Hardy-weinberg equilibrium (P>0.05) and genotype and allele frequency between groups. The difference was not statistically significant (P>0.05). (FIG. 2-3).

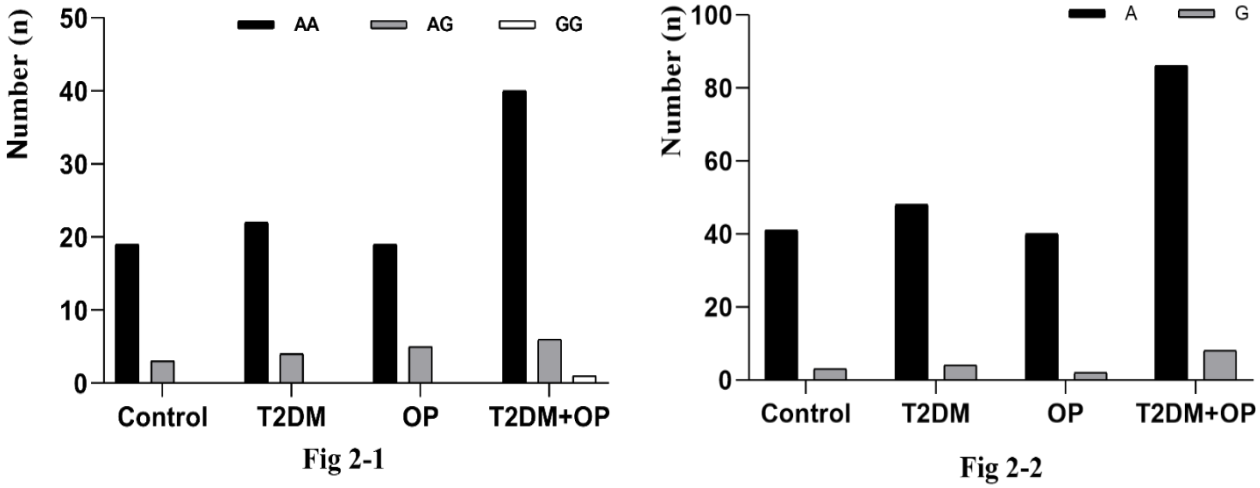


FIG. 2. **Genotype and allele distribution frequency of rs41494349 locus of LRP5 gene (n/%).**

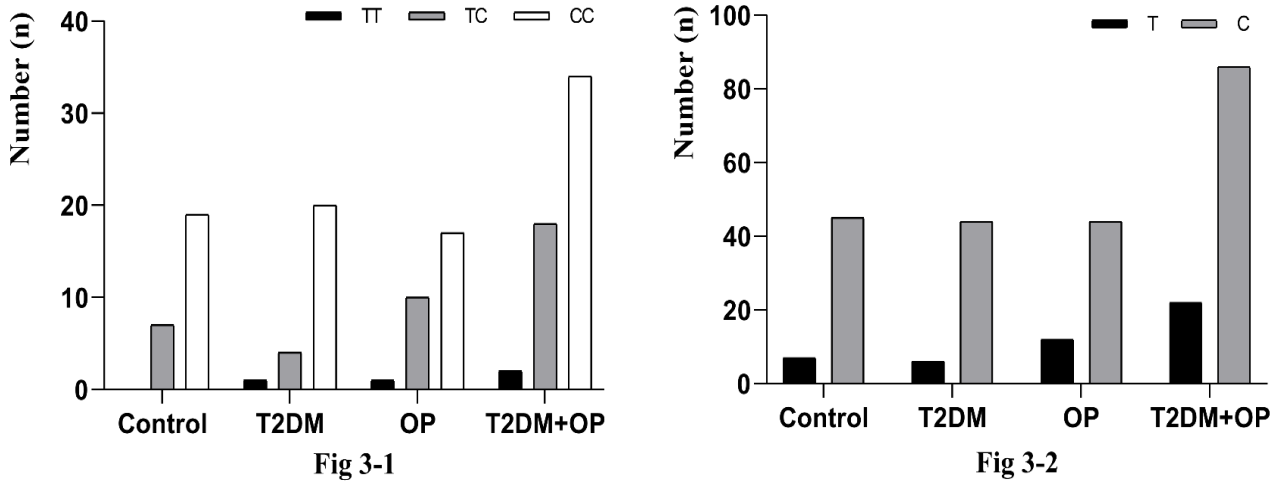


FIG. 3. **Genotype and allele distribution frequency of rs3736228 locus of LRP5 gene (n/%).**

5.5 Comparison of different genotypes at rs41494349 and rs3736228 of LRP5 gene

Due to the few research subjects of rs41494349 and rs3736228 mutated genotypes, the mutated genotypes (AG, GG) were compared with the wild type (AA), and the mutated genotypes (TT, TC) were compared with the wild type (CC). BMD(L1-4) of AA type in T2DM+OP group was higher than that in AG/GG type (P<0.05).

In Control group, CC type TG was higher than TT/TC type TG (P<0.05), and in T2DM group, CC type P and HDL were lower than TT/TC type TG (P<0.05). CC type BMD (L1-4) in T2DM+OP group was higher than TT/TC type (P<0.05). (TABLE 3).

TABLE 3. **Comparison of rs41494349 and rs3736228 genotypes of LRP5 gene [$\bar{x} \pm s$]**

Groups	Parameter	rs41494349		rs3736228	
		AA	AG/GG	CC	TT/TC
Control	FPG	5.49 ± 2.49	4.54 ± 0.35	5.62 ± 2.49	4.48 ± 0.36
	HbA1c	5.97 ± 0.98	5.63 ± 0.49	6.08 ± 0.97	5.59 ± 0.32
	TG	2.50 ± 1.72	1.98 ± 1.20	2.85 ± 1.91	1.60 ± 0.77*
	LDL-C	3.07 ± 1.11	3.31 ± 0.96	2.99 ± 1.10	3.41 ± 0.87
	HDL-C	1.38 ± 0.48	1.23 ± 0.41	1.31 ± 0.45	1.42 ± 0.38
	Ca	2.27 ± 0.06	2.25 ± 0.06	2.27 ± 0.07	2.29 ± 0.05
	P	1.09 ± 0.09	1.05 ± 0.12	1.07 ± 0.09	1.10 ± 0.07
	ALP	79.58 ± 16.04	81.67 ± 10.21	81.00 ± 16.33	79.86 ± 12.84
	BMD(L_{1-4})	1.20 ± 0.14	1.10 ± 0.05	1.32 ± 0.16	1.22 ± 0.14
	BMD(Femurneck)	0.91 ± 0.13	0.85 ± 0.08	0.97 ± 0.13	0.87 ± 0.11
T2DM	FPG	8.26 ± 3.55	6.07 ± 1.57	7.73 ± 3.48	6.99 ± 1.13
	HbA1c	7.50 ± 1.45	6.73 ± 0.81	7.24 ± 1.46	7.02 ± 1.20
	TG	2.66 ± 2.84	1.50 ± 0.93	1.98 ± 1.92	2.19 ± 1.70
	LDL-C	3.22 ± 1.08	2.69 ± 1.16	3.47 ± 0.94	2.67 ± 0.93
	HDL-C	1.21 ± 0.28	1.12 ± 0.08	1.11 ± 0.19	1.49 ± 0.29*
	Ca	2.25 ± 0.27	2.37 ± 0.36	2.26 ± 0.30	2.24 ± 0.06
	P	1.11 ± 0.12	1.15 ± 0.04	1.07 ± 0.10	1.19 ± 0.15*
	ALP	73.14 ± 21.18	61.25 ± 13.05	77.00 ± 22.39	59.80 ± 11.43
	BMD(L_{1-4})	1.24 ± 0.19	1.11 ± 0.10	1.24 ± 0.19	1.16 ± 0.11
	BMD(Femurneck)	0.88 ± 0.10	0.90 ± 0.02	0.89 ± 0.08	0.83 ± 0.08
OP	FPG	5.17 ± 0.75	4.66 ± 0.59	5.24 ± 0.69	4.89 ± 0.68
	HbA1c	5.96 ± 0.37	6.30 ± 1.13	5.98 ± 0.39	5.95 ± 0.49
	TG	1.63 ± 1.13	1.69 ± 0.22	1.47 ± 1.06	1.59 ± 0.87
	LDL-C	3.09 ± 0.58	2.60 ± 0.01	3.27 ± 0.72	2.97 ± 0.90
	HDL-C	1.27 ± 0.30	1.12 ± 0.23	1.29 ± 0.28	1.35 ± 0.35
	Ca	2.29 ± 0.10	2.22 ± 0.00	2.28 ± 0.09	2.28 ± 0.09
	P	1.10 ± 0.12	0.89 ± 0.04	1.11 ± 0.12	1.02 ± 0.12
	ALP	79.26 ± 16.53	81.50 ± 7.78	76.29 ± 19.60	90.45 ± 22.39
	BMD(L_{1-4})	0.87 ± 0.10	0.86 ± 0.11	0.82 ± 0.21	0.88 ± 0.09
	BMD(Femurneck)	0.71 ± 0.12	0.70 ± 0.02	0.72 ± 0.12	0.69 ± 0.05
T2DM+OP	FPG	7.47 ± 2.01	7.24 ± 1.67	7.54 ± 2.10	7.10 ± 1.33
	HbA1c	7.55 ± 1.23	7.07 ± 0.82	7.65 ± 1.29	7.18 ± 0.81
	TG	1.62 ± 0.72	1.62 ± 1.54	1.77 ± 0.95	1.65 ± 1.04
	LDL-C	3.52 ± 0.94	3.33 ± 1.37	3.49 ± 1.11	3.39 ± 0.97
	HDL-C	1.20 ± 0.28	1.15 ± 0.28	1.23 ± 0.34	1.18 ± 0.23
	Ca	2.27 ± 0.09	2.28 ± 0.08	2.27 ± 0.11	2.29 ± 0.09
	P	1.09 ± 0.12	1.00 ± 0.14	1.37 ± 1.52	1.07 ± 0.12*
	ALP	75.33 ± 22.11	63.29 ± 22.81	76.56 ± 22.11	68.30 ± 23.54
	BMD(L_{1-4})	0.92 ± 0.11	0.81 ± 0.10*	1.00 ± 0.15	0.87 ± 0.23*
	BMD(Femur neck)	0.75 ± 0.12	0.70 ± 0.10	0.74 ± 0.10	0.75 ± 0.14

Compared with control group, *P<0.05

5.6 Stepwise multiple linear regression analysis affecting BMD

BMD (femur neck) and BMD (L1-4) were used as dependent variables, and age, menopausal years, BMI, WHR, FPG, HbA1c, ALP, TG, HDL-C, LDL-C, Ca, P and genotype were used as independent variables. The results showed that BMI was a positive influence factor on BMD (femur neck) and BMD (L1-4) levels, while the length of menopause was a negative influence factor. rs41494349 polymorphism was a negative factor of BMD (femur neck). TG was a positive influence factor of BMD (L1-4) level (TABLE 4).

TABLE 4. **Stepwise multiple linear regression analysis affecting BMD.**

BMD	Independent variable	β	t value	P value
Femur neck	BMI	0.018	3.406	0.008
	rs41494349	-0.113	-2.557	0.014
	Menopausal years	-0.008	-2.856	0.019
L_{1-4}	BMI	0 .109	2.969	0.004
	TG	0.311	3.379	0.001
	Menopausal years	-0.063	-3.315	0.001

6. Discussion

Both T2DM and OP are common diseases of the elderly, with complex pathogenesis and influenced by multiple factors such as genetics and environment [9,10]. At present, studies have confirmed that multiple genes are related to the occurrence and development of T2DM and OP respectively [11,12]. Studies on T2DM with OP mainly focus on hormones and receptor genes concerned with calcium and phosphorus regulation, such as nuclear factor kappa-B ligand (RANKL). In recent years, LRP5, as a receptor of Wnt signaling pathway, has attracted more and more attention for its relationship between gene polymorphism and bone metabolism. However, there are few reports on the expression of LRP5 protein concentration in T2DM with OP at home and abroad, and there are also few reports on the correlation between LRP5 gene polymorphism and T2DM and T2DM with OP. This study aims to investigate the relationship between LRP5 protein expression, rs41494349 and rs3736228 polymorphisms and bone metabolism in postmenopausal women with type 2 diabetes mellitus.

The results of this study showed that the age and duration of menopause in OP group and T2DM+OP group were higher than those in the control group, suggesting that with the increase of age and duration of menopause, the possibility of abnormal BMD is greater. John et al [13] found that postmenopausal women are more prone to BMD reduction and OP, and the results of this study are similar. Therefore, postmenopausal women should pay attention to screening, early intervention, alert to the occurrence of OP. In this study, it was found that LRP5 protein concentration in postmenopausal T2DM group and abnormal bone mass group was lower than that in normal population. BMD (L1-4) and BMD (femur neck) in abnormal bone mass group were both lower than that in normal bone mass group, and LRP5 protein concentration was positively correlated with BMD (L1-4) and BMD (femur neck). This is consistent with the research views of Deniza et al [14,15]. These results suggest that the decrease of LRP5 protein concentration is an influential factor for the decline of BMD in postmenopausal women in this region.

We found that LRP5 protein concentration was negatively correlated with FPG, and compared with normal glucose tolerance and normal bone mass, LRP5 protein concentration in T2DM patients was reduced. Previous studies have reported that LRP5 protein concentration decreased after knockdown of mouse LRP5 gene. The phosphorylation level of mediating insulin signaling protein and the cellular response to insulin stimulation are significantly reduced, and the blood glucose increases [16], which is consistent with our study. These results indicate that LRP5 protein concentration in human body is an influential factor of glucose metabolism index. However, it is not clear whether T2DM increases the risk of decreased BMD in postmenopausal women.

Previous studies have suggested that the occurrence of OP in postmenopausal women with T2DM may be related to gene polymorphism [17]. The results showed that the genotype of rs41494349 and rs3736228 of LRP5 gene was in line with Hardy-Weinberg equilibrium, and there was no significant difference in genotype and allele frequency between groups (P>0.05). However, BMD of the two mutated genotypes (L1-4) was lower than that of the wild genotypes, suggesting that the occurrence of T2DM with OP may be related to the polymorphism of rs41494349 and rs3736228 of LRP5 gene. Regression analysis further suggested that the polymorphism of rs41494349 was the influence factor of BMD reduction. Kitjaroentham A [18] found that LRP5 gene rs41494349 gene polymorphism was associated with decreased BMD in Thai menopausal women. Xu et al [19] also confirmed that T gene mutation in rs3736228 gene polymorphism was more likely to lead to osteoporosis and fracture. The results are similar to our study. At the same time, we also observed that P and HDL-C of rs3736228 mutant genotype were lower in T2DM+OP group than wild type, but higher in T2DM group than wild type, suggesting that rs3736228 gene polymorphism and mutation are not only involved in bone metabolism of postmenopausal women with T2DM in Xinjiang. It may also be associated with lipid metabolism in this population, which is similar to the results of previous studies [20].

Our study found that LRP5 protein concentration was negatively correlated with age and duration of menopause, which may be related to the decrease of Wnt signaling pathway activity with the increase of age. It was also found that the duration of menopause in the abnormal bone mass group was higher than that in the normal bone mass group. Regression analysis showed that the duration of menopause was a negative factor influencing BMD, and BMD decreased with the increase of the duration of menopause, suggesting that the increase of the duration of menopause may increase the risk of T2DM patients with OP, which was consistent with the research results of Karlamangla AS [21]. Some scholars believe [22] that BMI is a positive influence factor of BMD (femur neck) and BMD (L 1-4), and BMD increases with the increase of BMI. We found similar results, suggesting that increased BMI within a range can delay bone loss. In addition, interestingly, we also found that TG levels in people with abnormal bone mass were lower than those in the normal population, and TG was a positive factor influencing BMD level (L1-4), suggesting that the increase of TG may play a protective role in the increase of BMD. This phenomenon is similar to the research results of Xu et al [23]. The specific molecular mechanism and signal pathway of TG affecting BMD need further study in the future.

In conclusion, this study confirmed that polymorphism and mutation of rs41494349 and rs3736228 of LRP5 gene may be related to bone metabolism and decrease LRP5 protein expression in postmenopausal women with T2DM. In addition, mutation of rs3736228 may also be related to lipid metabolism in this population. In the future, related mechanisms should be further explored to find new targets for the prevention and treatment of OP in postmenopausal women with T2DM.

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