Int J Clin Exp Med 2014;7(1):247-254
www.ijcem.com /ISSN:1940-5901/IJCEM1311018

Original Article

Association of LRP5 gene polymorphism with type 2 diabetes mellitus and osteoporosis in postmenopausal women

Miao Xuan*, Yonglan Wang*, Wenxing Wang, Jun Yang, Ying Li, Xiuzhen Zhang

*Department of Endocrinology, Tongji Hospital of Tongji University, Shanghai 200065, China. *Equal contributors.*

Received November 19, 2013; Accepted December 10, 2013; Epub January 15, 2014; Published January 30, 2014

Abstract: This study was to explore the association of low-density lipoprotein receptor related protein 5 (LRP5) gene polymorphism with bone mineral density (BMD), bone turnover markers and glycometabolism in postmenopausal women with type 2 diabetes mellitus (T2DM) and/or osteoporosis (OP) in Shanghai. 354 unrelated Han Chinese post-menopausal women were recruited from Shanghai and divided into 4 groups: OP group (n=90), T2DM group (n=96), T2DM + OP group (n=90) and control group (n=78). The LRP5 genotypes were determined by DNA sequencing. The BMD was measured by dual-energy X-ray absorptiometry. The bone transformation indicators and glycometabolism index (HbA1c and Fasting insulin) were also detected. The association of LRP5 polymorphism with BMD, bone turnover markers and glycometabolism was evaluated. Result showed that, In OP group, the BMD of L_{2-4} was higher in patients with rs3736228 CC genotype than those with CT/TT genotypes ($P<0.05$). After adjustment for age, body mass index (BMI) and years of menopause, A1330V polymorphism was still associated with BMD of L_{2-4} ($P<0.01$). In the control group, HbA1c was significantly higher in patients with A1330V CC genotype than those with CT/TT genotypes ($P<0.05$), but no significant difference was found after adjustment for BMI, age and years of menopause ($P>0.05$). Thus, LRP5 gene is an impressionable gene in postmenopausal women with OP in Shanghai. T2DM patients have a high BMD when compared with controls, which may be related to BMI and FINS. LRP5 genotype is not an impressionable gene in postmenopausal women with T2DM in Shanghai.

Keywords: Low density lipoprotein receptor related protein 5 gene, gene polymorphism, type 2 diabetes mellitus, osteoporosis, bone mineral density

Introduction

Both osteoporosis (OP) and diabetes mellitus (DM) manifest as chronic metabolic syndrome involving the whole body and can be affected by the environment, gene and other factors. Therefore, searching for osteoporosis and diabetes mellitus susceptibility genes has been an important task for human genome research.

Low-density lipoprotein receptor-related protein 5 (LRP5) gene is a member of LDL receptor family [1] and locates on chromosome 11q13.4 [2, 3]. It was found initially during searching for type I diabetes candidate gene by Hey et al [3] and seeking the new osteoblast-related gene by Dong et al [4]. As the receptor of Wnt ligand, LRP5 plays an important role in Wnt signaling pathway [5] and has an important effect for blood glucose and blood lipid metabolism [6]. LRP5 can express in the insulin-secreting pancreatic B cells [7], and sugar tolerance is impaired significantly due to decrease of glucose-induced insulin secretion in LRP5 knockout mice [6]. Guo et al found that the single rs498830 (SNP5-2) for LRP5 was associated with BMI in Caucasian with diabetes [8]. Various observations indicate that LRP5 is a potential T2DM susceptibility gene.

Meanwhile, a decrease of BMD in human and mice due to LRP passivation and mutation can cause autosome recessive genetic disease-osteoporosis-pseudoglioma syndrome (OPS) [9]. In addition, LRP5 activation and mutation can cause autosomal dominant high-bone-mass trait [10]. These data suggest that LRP5 can regulate bone metabolism.

Table 1. Clinical indicators at baseline in different groups (X ± S)

Groups	OP	T2DM	OP + T2DM	Control
Number (N)	90	96	90	78
Age (yr)	60.0 ± 3.0	60.4 ± 3.2	60.2 ± 3.3	60.5 ± 2.7
menopausal period (years)	7.4 ± 1.7	7.3 ± 1.6	7.3 ± 1.5	7.5 ± 1.4
BMI (kg/m^2)	20.5 ± 1.7	24.2 ± 1.7	21.4 ± 1.8	21.8 ± 1.5
TRACP-5b (U/L)	3.5 ± 1.0	2.9 ± 0.4	3.2 ± 0.7	3.0 ± 0.4
BALP (μg/l)	21.4 ± 7.2	17.3 ± 5.6	17.3 ± 5.5	15.8 ± 4.4
HbA1c (%)	5.0 ± 0.5	7.6 ± 1.0	7.7 ± 1.0	5.0 ± 0.5
FINS (mmol/L)	6.6 ± 1.6	15.2 ± 5.4	9.2 ± 1.2	6.5 ± 1.7
L_{2-4} (g/m^2)	0.78 ± 0.10	1.03 ± 0.13	0.80 ± 0.10	0.95 ± 0.14
femoral neck (g/m^2)	0.61 ± 0.08	0.76 ± 0.10	0.64 ± 0.11	0.73 ± 0.11

In order to investigate what is the association of LRP5 gene with DM, OP and whether LRP5 is related with T2DM with osteoporosis, our study intended to use gene sequencing for exploration the association of LRP5 gene polymorphism with bone mineral density (BMD), bone turnover markers and glycometabolism in patients with OP, T2DM patients with normal bone mass and T2DM patients accompanied by OP among the postmenopausal women in Shanghai, thus having a in-depth understanding of the pathogenesis of T2DM and OP, providing scientific and theoretical guidance for the prevention and treatment of T2DM and OP.

Subjects and methods

Subjects

The T2DM was diagnosed according to the criteria developed by WHO in 1999 [11] and OP was diagnosed according to Guidelines for Primary Osteoporosis from Osteoporosis and Bone Mineral Disease Branch of Chinese Medical Association [12]. A total of 354 unrelated Han Chinese post-menopausal women were recruited from the Department of Internal Medicine of Tongji Hospital from February 2009 to December 2011 from Shanghai. The patients had a menopausal period of 7.40 ± 1.52 years and were 60.26 ± 3.07 year-old. The mean BMI was 22.04 ± 2.22 kg/m^2.

They were divided into 4 groups: OP group (n=90), T2DM group (n=96), T2DM + OP group (n=90) and G control group (n=78). Informed consent was obtained from all patients, and the whole protocol was approved by the ethics committee of Tongji Hospital of Tongji University. Exclusion criteria: patients using the drugs affecting bone metabolism (such as sex hormones, parathyroid hormone, bisphosphonates, calcitonin, glucocorticoids hormones); patients with a history of hyperparathyroidism, multiple myeloma, rheumatoid arthritis and other bone diseases; patients suffering from T1DM, cancer or hematological diseases; patients with a history of severe heart, lung, liver, renal diseases, long-term heavy drinking and smoking. The age, menopausal period (years), height and body weight were recorded, and the BMI was calculated. Fasting blood was collected for the detection of indicators of glucose, bone metabolism and genotyping.

Genomic DNA extraction

The genomic DNA isolation kit (Jin You, Anhui, China) was used to perform the genomic DNA extraction from venous blood (300 ul).

PCR for LRP5

The primers for LRP5 were designed and synthesized in the Shanghai Biological Engineering Technology Co., Ltd. The forward primer with rs3736228 (p.Ala1330Val/c.3989C>T) was 5'-GCTGGGCTGTTGATGTTTAGA-3' and the reverse primer was 5'-AGAGGCAAGGTTTTC-CCATAA-3'. PCR conditions were as follows: predenaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 45 s and extension at 72°C for 1 min, and a final extension at 72°C for 10 min.

Gene sequencing

The sequencing of PCR products was done in the Shanghai Biological Engineering Co., Ltd. The PCR products were subjected to an auto-

Table 2. LRP5 A1330V loci genotype distribution and allele frequency

Groups	N	CC	CT	TT	χ^2	P
OP	90	64 (71.1)	23 (25.6)	3 (3.3)	0.95	0.622
T2DM	96	60 (62.5)	34 (35.4)	2 (2.08)	0.63	0.731
OP + T2DM	90	57 (63.3)	31 (34.4)	2 (2.2)	0.45	0.800
control	78	50 (64.10)	25 (32.05)	3 (3.85)		
Total	354	231 (65.25)	113 (31.92)	10 (2.82)		

matic ABI3730 sequencer, and the Chromas software was used to analyze the results.

Determination of BMD

BMD was measured according to the Guideline of International Society of Clinical Densitometry [13]. All patients lied in supine position, and the BMD of L2~4 and left femoral neck was measured. BMD was measured using dual-energy X-ray absorptiometry (model Lunar DPX-IQ) by the same technician. Machine calibration was done once daily, and data were stored in the database. The coefficient of variation (CV) of the lateral L2~4 test was 0.49% and 0.66%, respectively, and the three-year QC was lower than 1%.

Detection of indicators for bone and glucose metabolism

Serum tartrate-resistant acid phosphatase 5b (TRACP-5b): The enzyme-linked immunosorbent assay (ELISA) was performed to detect the serum TRACP-5b. In normal postmenopausal women (41~81 years), the reference range was 2.34~4.04 U/L. The intra-group CV was 6.0~13.9%, and inter-group CV was 5.8~9.2%; the recovery rate was 89.6~112.2%.

Serum bone alkaline phosphatase (BALP): ELISA was done to detect serum BALP. In normal postmenopausal women (41~81 years), the reference range was 12.5~22.4 μg/L. The intra-group CV was 2.6~6.5%, and the inter-group CV was 3.7~6.4%; the recovery rate was 90.1~100.9% (n=100); the range of standard curve was 0.28~92.08 μg/L.

Glycated hemoglobin (HbA1c): High pressure liquid chromatography was done to measure serum HbA1c on an automated glycosylated hemoglobin analyzer. The normal reference range was 4.5~6.2%.

Fasting insulin (FINS): Radioimmunoassay was done to measure serum FINS on the rationale of competitive binding reaction. The normal range was 1.22~25.5 uU/ml. The automatic biochemical analyzer was applied for test. The intra-group CV was <7%, and inter-group CV was <8%.

Statistical analysis

The A1330V polymorphism and allele frequency of LRP5 gene were determined. The genotype distribution of genetic equilibrium was compared with chi square test. Statistical analysis was done with SPSS version 17.0 for Windows, and a difference with $P<0.05$ was considered statistically significant. Clinical variables were expressed as mean ± standard deviation (SD, $\bar{x} \pm s$). Data between groups were compared with the independent *t*-test (homogeneity of variance: *t*-test, and heterogeneity of variance: t'-test). The genotype, BMD, bone metabolism and glucose metabolism were compared with ANOVA after adjustment for age, BMI and menopause period (years).

The clinical variables were expressed as mean ± standard deviation ($X \pm s$); analysis of variance was employed for the inter-group comparison of measurement data, and non-parametric test was used for non-normal distribution; ANOVA was adopted for comparison of the difference between genotypes in the four groups after adjustment of age, BMI and menopause period and BMD, bone metabolism with glucose metabolism.

Results

Clinical indicators at baseline in different groups

The age, menopausal period (years), BMI, TRACP-5b, BALP, HbA1c, FINS, BMD of the lumbar spine and femoral neck in different groups are shown in **Table 1**.

The age and menopausal period (years) were comparable among 4 groups ($P>0.05$). The BMI in OP and T2DM group was significantly different from that in the control group ($P<0.01$), but it was comparable between T2DM + OP group

Table 3. Relationship between LRP5 gene polymorphisms and lumbar BMD (X ± S, g/cm^2)

Groups	CC	CT/TT	t	*P*	*P'*
OP	0.8 ± 0.1	0.7 ± 0.1	2.49	0.016	0.005
T2DM	1.02 ± 0.14	1.04 ± 0.13	-0.53	0.595	0.678
OP + T2DM	0.81 ± 0.09	0.80 ± 0.11	0.79	0.430	0.752
control	0.96 ± 0.13	0.94 ± 0.15	0.80	0.428	0.496

Table 4. Relationship between LRP5 gene polymorphisms and FNBMD (X ± S, g/cm^2)

Group	CC	CT/TT	t	*P*	*P'*
OP	0.62 ± 0.77	0.61 ± 0.10	0.364	0.718	0.901
T2DM	0.76 ± 0.11	0.76 ± 0.10	-0.060	0.953	0.700
OP + T2DM	0.643 ± 0.11	0.638 ± 0.11	0.177	0.860	0.935
control	0.74 ± 0.10	0.72 ± 0.12	0.553	0.582	0.361

and control group (P>0.05). The TRACP-5b and BALP in OP group were significantly higher than those in the control group (P<0.01), but both in T2DM group and T2DM + OP group were similar to those in the control group (P>0.05). The HbA1c and FINS in T2DM group and T2DM + OP group were markedly higher than those in the control group (P<0.01), while there were no remarkable differences between OP group and the control group (P>0.05). The lumbar BMD in OP group and T2DM + OP group was dramatically lower than that in the control group; the lumbar BMD in OP, T2DM, and T2DM + OP group was significantly different from that in the control group (P<0.01). When compared with the control group, the BMD of femoral neck in OP and T2DM + OP group was significantly reduced (P<0.01), while there was no significant difference between T2DM and T2DM + OP group (P>0.05).

LRP5 gene polymorphism and allele frequency distribution

In this study, genetic equilibrium test showed that the LRP5 rs3736228 genotype distribution in OP, T2DM, T2DM + OP and the control group were in line with the Hardy-Weinberg equilibrium (OP group: χ^2=0.268, P=0.604; T2DM group: χ^2=1.281, P=0.258; T2DM + OP group: χ^2=0.891, P=0.345; the control group: χ^2=0.03, P=0.954).

As shown in **Table 2**, Pearson Chi-Square distribution frequency test revealed the CC, CT and TT genotype distribution in the control group was comparable to that in Group A, B and D (χ^2=0.95, 0.63 and 0.45, respectively; P=0.63, 0.731 and 0.80 respectively). The C and T allele frequency distribution in the control group was also similar to that in OP, T2DM and T2DM + OP group (χ^2=0.69, 0.099 and 0.04 respectively; P=0.41, 0.75 and 0.84, respectively).

LRP5 gene polymorphisms and BMD

Due to the small number of patients with TT genotype in this study (N=10), the patients with CT or TT genotype were compared with those with CC genotype. In OP group, the lumbar BMD in patients with LRP5 rs3736228 CC genotype was significantly higher than that in those with CT/TT genotype (*P*=0.016). After adjustment for age, menopausal period (years) and BMI, statistical difference was still observed (*P*=0.005). In T2DM, T2DM + OP and the control group, the lumbar BMD in patients with LRP5 A1330V CC genotype was comparable to that in patients with CT/TT genotype (**Table 3**).

Note: P': *P* value after adjustment for age, menopausal period (years) and BMI.

In all groups, the femoral neck BMD in patients with LRP5 rs3736228 CC genotype was similar to that in patients with CT/TT femoral neck BMD, even after adjustment for age, menopausal period (years) and BMI (**Table 4**).

Note: *P' values represent the results after comparison between the two genotypes after adjustment of age, menopausal period (years) and BMI.*

LRP5 gene polymorphisms and bone metabolism

In all groups, the TRACP-5b and BALP were comparable between patients with LRP5 rs3736228 CC genotype and patients with CT/TT genotype.

LRP5 gene polymorphisms and glucose metabolism

In the control group, the HbA1c in patients with LRP5 gene rs3736228 CC genotype was significantly different from that in patients with CT/TT

(P<0.05), and after adjustment of age, BMI and menopausal period (years), no correlation was still found (P>0.05). In all groups, there was no marked correlation between FINS and LRP5 rs3736228 polymorphism.

Discussion

T2DM and OP are common geriatric diseases and their incidences increase over age. Their pathogeneses are complex and are usually influenced by genetic and environmental factors. Multiple genes have been found to be associated with T2DM and OP. Studies on T2DM and OP-related genes mainly focus on the regulation of calcium and phosphorus metabolism by hormones and their receptors, such as sex hormone receptor genes [14, 15] and cytokine genes. In recent years, LRP5 gene has attracted increasing attention. A large number of studies [16-19] have reported the association between LRP5 gene polymorphisms and OP, but the correlations of LRP5 gene polymorphisms with T2DM or T2DM combined with OP are less reported.

The present study found that the LRP5 rs3736228 genotype distribution frequencies were 65.3% in CC genotype, 31.9% in CT genotype, and 2.8% in TT genotype in post-menopausal women of Shanghai, which were similar to the findings in Greece [20] and the United States [21]. However, a study from sub-Saharan Africa indicated that the detection rate of LRP5 gene allele presented with racial differences. Our study confirmed that the LRP5 gene rs3736228 polymorphism was related to the lumbar BMD in post-menopausal OP women: lumbar BMD in patients with CC genotype was higher than that in those with CT/TT genotype, but there was no association between LRP5 gene rs3736228 polymorphism and femoral neck BMD [22-25]. In T2DM or T2DM with OP patients with normal bone mass, the BMD was comparable between patients with CC and CT/TT genotypes suggesting that LRP5 genotype may not be a gene affecting BMD in T2DM patients.

The present study revealed that TRACP-5b and BALP (bone metabolic markers) were not associated with LRP5 gene rs3736228 polymorphism, which was in accordance with findings from the studies of Kruk et al and Anastasia et al [26]. In addition, our findings also indicated that, in the control group, the HbA1c level in patients with CC genotype was higher than that in patients with CT/TT genotype, even after adjustment for BMI, age and menopausal period (years). The FINS level was not associated with LRP5 rs3736228 polymorphism. The study of Zenibayashi et al [27] found that the LRP5 rs3736228 polymorphism was not related to HbA1c and insulin in Japanese patients with T2DM. Jiang et al [28] found LRP5 gene was not related to the fasting glucose in Chinese Han women. These findings suggest that LRP5 gene polymorphism may not predict the change in glucose metabolism.

Our study indicated that the BMI in OP patients was significantly lower than that in healthy control. Numerous studies have confirmed that the body weight is an important protective factor for OP within the appropriate range [29]. The effect of weight on BMD may be attributed to the bone under mechanical loading because appropriate increase in weight may enhance the bone mass under the mechanical load accordingly, which is beneficial for the improvement of bone strength and bone mineralization; the increase in fatty tissues can increase the amount of aromatase activity leading to an increase in circulating estrogen.

Numerous studies have been conducted to investigate the association between T2DM and BMD, but findings are conflicting. In the load-bearing parts such as the lumbar spine and femoral neck, some studies have revealed that the lumbar and femoral neck BMD of T2DM patients was similar to that in healthy controls [30, 31]. However, other studies revealed that the lumbar and femoral neck BMD of T2DM patients was higher than that in the control group [32], and some showed contrary findings [33]. Our study indicated that the lumbar BMD of T2DM patients increased as compared to controls.

Outlook

The relationship of LRP5 gene single nucleotide mutations with OP and T2DM may affect its binding to ligands and receptor signal transduction system, which then influence the occurrence and development of disease, but the specific pathophysiological mechanisms are still unclear and the related studies are still needed. T2DM and OP are both multifactorial dis-

eases related to many candidate genes. In future genetic studies on the basis of genetic screening, the linkage disequilibrium should be full considered between genes and gene interactions. The molecular biology may help to understand the role of candidate genes in disease and to choose a reasonable solution in clinical practice to improve the therapeutic effect on T2DM and OP, and reduce the morbidity in T2DM and/or OP patients.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xiuzhen Zhang or Miao Xuan, Department of Endocrinology, Tongji Hospital of Tongji University, Shanghai 200065, China. E-mail: xzzhang005@163.com; zhangxiuzhen@tongji.edu.cn (XZZ); mxuan321@126.com (MX)

References

[1] Brown SD, Twells RC, Hey PJ, Cox RD, Levy ER, Soderman AR, Metzker ML, Caskey CT, Todd JA and Hess JF. Isolation and characterization of LRP6, a novel member of the low density lipoprotein receptor gene family. Biochem Biophys Res Commun 1998; 248: 879-888.

[2] Twells RC, Metzker ML, Brown SD, Cox R, Garey C, Hammond H, Hey PJ, Levy E, Nakagawa Y, Philips MS, Todd JA and Hess JF. The sequence and gene characterization of a 400-kb candidate region for IDDM4 on chromosome 11q13. Genomics 2001; 72: 231-242.

[3] Hey PJ, Twells RC, Phillips MS, Yusuke N, Brown SD, Kawaguchi Y, Cox R, Guochun X, Dugan V, Hammond H, Metzker ML, Todd JA and Hess JF. Cloning of a novel member of the low-density lipoprotein receptor family. Gene 1998; 216: 103-111.

[4] Dong Y, Lathrop W, Weaver D, Qiu Q, Cini J, Bertolini D and Chen D. Molecular cloning and characterization of LR3, a novel LDL receptor family protein with mitogenic activity. Biochem Biophys Res Commun 1998; 251: 784-790.

[5] Pinson KI, Brennan J, Monkley S, Avery BJ and Skarnes WC. An LDL-receptor-related protein mediates Wnt signalling in mice. Nature 2000; 407: 535-538.

[6] Fujino T, Asaba H, Kang MJ, Ikeda Y, Sone H, Takada S, Kim DH, Ioka RX, Ono M, Tomoyori H, Okubo M, Murase T, Kamataki A, Yamamoto J, Magoori K, Takahashi S, Miyamoto Y, Oishi H, Nose M, Okazaki M, Usui S, Imaizumi K, Yanagisawa M, Sakai J and Yamamoto TT. Low-density lipoprotein receptor-related protein 5 (LRP5) is essential for normal cholesterol metabolism and glucose-induced insulin secretion. Proc Natl Acad Sci U S A 2003; 100: 229-234.

[7] Figueroa DJ, Hess JF, Ky B, Brown SD, Sandig V, Hermanowski-Vosatka A, Twells RC, Todd JA and Austin CP. Expression of the type I diabetes-associated gene LRP5 in macrophages, vitamin A system cells, and the Islets of Langerhans suggests multiple potential roles in diabetes. J Histochem Cytochem 2000; 48: 1357-1368.

[8] Guo YF, Xiong DH, Shen H, Zhao LJ, Xiao P, Guo Y, Wang W, Yang TL, Recker RR and Deng HW. Polymorphisms of the low-density lipoprotein receptor-related protein 5 (LRP5) gene are associated with obesity phenotypes in a large family-based association study. J Med Genet 2006; 43: 798-803.

[9] Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR and Warman ML. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 2001; 107: 513-523.

[10] Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao SC, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, FitzGerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogues X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recker SM, Van Eerdewegh P, Recker RR and Johnson ML. A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet 2002; 70: 11-19.

[11] Shaw JE, Zimmet PZ, McCarty D and de Courten M. Type 2 diabetes worldwide according to the new classification and criteria. Diabetes Care 2000; 23 Suppl 2: B5-10.

[12] Association OaBMDBoCM. Guidelines for Primary Osteoporosis (discussion paper). Chin J Gen Pract 2006; 8: 455-457.

[13] Lewiecki EM, Gordon CM, Baim S, Leonard MB, Bishop NJ, Bianchi ML, Kalkwarf HJ, Langman

CB, Plotkin H, Rauch F, Zemel BS, Binkley N, Bilezikian JP, Kendler DL, Hans DB and Silverman S. International Society for Clinical Densitometry 2007 Adult and Pediatric Official Positions. Bone 2008; 43: 1115-1121.

[14] Guan MP, Xue YM, Zhang Q, Liu SQ and Shen J. Relationship between estrogen receptor gene polymorphism and bone mineral density in type 2 diabetic patients. J Chin Clin Rehabt 2005; 7: 2140-2141.

[15] Jia R, Miao YD and Ji LN. Association between T950C and A163G polymorphism of the osteoprotegerin gene and bone mineral density in elderly type 2 diabetic patients. J Clin Rehab Tissue Engineer Res 2011; 15: 5290-5294.

[16] Falcon-Ramirez E, Casas-Avila L, Cerda-Flores RM, Castro-Hernandez C, Rubio-Lightbourn J, Velazquez-Cruz R, Diez GP, Penaloza-Espinosa R and Valdes-Flores M. Association of LRP5 haplotypes with osteoporosis in Mexican women. Mol Biol Rep 2013; 40: 2705-2710.

[17] Agueda L, Velazquez-Cruz R, Urreizti R, Yoskovitz G, Sarrion P, Jurado S, Guerri R, Garcia-Giralt N, Nogues X, Mellibovsky L, Diez-Perez A, Marie PJ, Balcells S and Grinberg D. Functional relevance of the BMD-associated polymorphism rs312009: novel involvement of RUNX2 in LRP5 transcriptional regulation. J Bone Miner Res 2011; 26: 1133-1144.

[18] Kim H, Choe SA, Ku SY, Kim SH and Kim JG. Association between Wnt signaling pathway gene polymorphisms and bone response to hormone therapy in postmenopausal Korean women. Menopause 2011; 18: 808-813.

[19] Koh JM, Jung MH, Hong JS, Park HJ, Chang JS, Shin HD, Kim SY and Kim GS. Association between bone mineral density and LDL receptor-related protein 5 gene polymorphisms in young Korean men. J Korean Med Sci 2004; 19: 407-412.

[20] Stathopoulou MG, Dedoussis GV, Trovas G, Katsalira A, Hammond N, Deloukas P and Lyritis GP. Low-density lipoprotein receptor-related protein 5 polymorphisms are associated with bone mineral density in Greek postmenopausal women: an interaction with calcium intake. J Am Diet Assoc 2010; 110: 1078-1083.

[21] Koller DL, Ichikawa S, Johnson ML, Lai D, Xuei X, Edenberg HJ, Conneally PM, Hui SL, Johnston CC, Peacock M, Foroud T and Econs MJ. Contribution of the LRP5 gene to normal variation in peak BMD in women. J Bone Miner Res 2005; 20: 75-80.

[22] Bollerslev J, Wilson SG, Dick IM, Islam FM, Ueland T, Palmer L, Devine A and Prince RL. LRP5 gene polymorphisms predict bone mass and incident fractures in elderly Australian women. Bone 2005; 36: 599-606.

[23] Markatseli AE, Hatzi E, Bouba I, Georgiou I, Challa A, Tigas S and Tsatsoulis A. Association of the A1330V and V667M polymorphisms of LRP5 with bone mineral density in Greek peri- and postmenopausal women. Maturitas 2011; 70: 188-193.

[24] van Meurs JB, Trikalinos TA, Ralston SH, Balcells S, Brandi ML, Brixen K, Kiel DP, Langdahl BL, Lips P, Ljunggren O, Lorenc R, Obermayer-Pietsch B, Ohlsson C, Pettersson U, Reid DM, Rousseau F, Scollen S, Van Hul W, Agueda L, Akesson K, Benevolenskaya LI, Ferrari SL, Hallmans G, Hofman A, Husted LB, Kruk M, Kaptoge S, Karasik D, Karlsson MK, Lorentzon M, Masi L, McGuigan FE, Mellstrom D, Mosekilde L, Nogues X, Pols HA, Reeve J, Renner W, Rivadeneira F, van Schoor NM, Weber K, Ioannidis JP and Uitterlinden AG. Large-scale analysis of association between LRP5 and LRP6 variants and osteoporosis. JAMA 2008; 299: 1277-1290.

[25] Urano T, Shiraki M, Usui T, Sasaki N, Ouchi Y and Inoue S. A1330V variant of the low-density lipoprotein receptor-related protein 5 (LRP5) gene decreases Wnt signaling and affects the total body bone mineral density in Japanese women. Endocr J 2009; 56: 625-631.

[26] Kruk M, Ralston SH and Albagha OM. LRP5 Polymorphisms and response to risedronate treatment in osteoporotic men. Calcif Tissue Int 2009; 84: 171-179.

[27] Zenibayashi M, Miyake K, Horikawa Y, Hirota Y, Teranishi T, Kouyama K, Sakaguchi K, Takeda J and Kasuga M. Lack of association of LRP5 and LRP6 polymorphisms with type 2 diabetes mellitus in the Japanese population. Endocr J 2008; 55: 699-707.

[28] Jiang XY, Chen Y, Xu L, Li X, Cao FF, Li L, Lu M, Jin L and Wang XF. Association of LPR5 polymorphism with bone mass density and cholesterol level in population of Chinese Han. Exp Clin Endocrinol Diabetes 2010; 118: 388-391.

[29] Saarelainen J, Kiviniemi V, Kroger H, Tuppurainen M, Niskanen L, Jurvelin J and Honkanen R. Body mass index and bone loss among postmenopausal women: the 10-year follow-up of the OSTPRE cohort. J Bone Miner Metab 2012; 30: 208-216.

[30] Majima T, Komatsu Y, Yamada T, Koike Y, Shigemoto M, Takagi C, Hatanaka I and Nakao K. Decreased bone mineral density at the distal radius, but not at the lumbar spine or the femoral neck, in Japanese type 2 diabetic patients. Osteoporos Int 2005; 16: 907-913.

[31] Anaforoglu I, Nar-Demirer A, Bascil-Tutuncu N and Ertorer ME. Prevalence of osteoporosis and factors affecting bone mineral density among postmenopausal Turkish women with type 2 diabetes. J Diabetes Complications 2009; 23: 12-17.

[32] Schwartz AV, Sellmeyer DE, Vittinghoff E, Palermo L, Lecka-Czernik B, Feingold KR, Strot-

meyer ES, Resnick HE, Carbone L, Beamer BA, Park SW, Lane NE, Harris TB and Cummings SR. Thiazolidinedione use and bone loss in older diabetic adults. J Clin Endocrinol Metab 2006; 91: 3349-3354.

[33] Yaturu S, Humphrey S, Landry C and Jain SK. Decreased bone mineral density in men with metabolic syndrome alone and with type 2 diabetes. Med Sci Monit 2009; 15: CR5-9.