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Original Article

Association of insulin-like growth factor-I receptor and -II receptor gene polymorphisms with osteoporosis in postmenopausal women of Han Chinese

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Abstract: Objective: The purpose of the current study was to identify the association between polymorphisms +3174G/A of insulin-like growth factor-I receptor (IGF-IR) gene and c5002G/A of insulin-like growth factor-II receptor (IGF-IIR) gene and osteoporosis in postmenopausal women of Han Chinese. Materials and methods: This case-control study involved 218 patients with postmenopausal osteoporosis and 270 age-matched controls to genotype polymorphisms +3174G/A of IGF-IR and c5002G/A of IGF-IIR using restriction fragment length polymorphisms (RFLP)-polymerase chain reaction (PCR) assay. BMD values at lumbar spine, femoral neck and forearm were measured using dual-energy X-ray absorptiometry. Serum IGF-I was investigated by enzyme-linked immunosorbent assay. Results: Our data showed the distributions of AA genotype (29% vs. 17%) and A allele (51% vs. 40%) of IGF-IR gene +3174G/A polymorphism were significantly more frequent in the osteoporotic women than controls. Conditional logistic regression revealed that AA genotype of IGF-IR was statistically associated with osteoporosis (OR=2.40, 95% CI=1.46-3.95, P=0.001). After adjustment for age, menopause duration and BMI, AA genotype was still significantly associated with an increased risk of osteoporosis (OR=2.10, 95% CI=1.26-3.51, P=0.004). And osteoporotic women with AA genotype of IGF-IR were found to have a lower values of serum IGF-I and BMD at forearm and femoral neck compared with other genotype. Conclusions: Our results suggested that the IGF-IR gene +3174G/A polymorphism may affect the susceptibility to osteoporosis in postmenopausal women of Han Chinese and may change serum IGF-I level.

Keywords: Osteoporosis, polymorphism, insulin-like growth factor-I receptor, insulin-like growth factor-II receptor

Introduction

Osteoporosis (OP), one of the most prevalent metabolic diseases in the postmenopausal women, is a systemic skeletal disorder characterized by decreased bone mass, low bone mineral density (BMD) and micro-architecture deterioration of bone tissue, resulting in bone fragility and fracture [1, 2]. Osteoporotic fracture closely associated with chronic pain, disability and mortality has become an important and complex health concern in the world [3, 4]. According to previous studies, approximately 40 percent of women over 50 will suffer a fracture related to post-menopausal osteoporosis during their lifetime [5] and the fastigium of bone mass loss occurring at this moment [6]. In addition to age-related bone loss and estrogen deficiency [7], the process of postmenopausal women osteoporosis is caused by the combined effects of genetic and environmental factors [8-10]. As an important risk factor for osteoporosis, at least 50-80% of the variation of BMD is account for genetic factor, as revealed by twins and family studies [11-14].

The association between genetics and osteoporosis has become a focus of investigation in recent years. Numerous genetic variants, which contained in genes encoding component of bone matrix, growth factor, cytokines and their receptors related to bone metabolism, have been proved to associated with BMD, osteoporosis and osteoporotic fracture in lots of GWAS [15], candidate-gene association study [16, 17], and meta-analysis [18]. The List of

these genetic variants also includes single nucleotide polymorphism (SNP) in Insulin-like growth factor (IGF) system genes [19-21].

The insulin-like growth factor (IGF) system is a complex system composed of two ligands (IGF-I and IGF-II), two receptors (IGF-IR and IGF-IIR), 6 binding proteins (IGFBP1-IGFBP6) and multiple IGFBP proteases. Previous studies have demonstrated the important effects of IGF system on bone metabolism. In vitro, both IGF-I and IGF-II were reported to stimulate human osteoblast proliferation, differentiation and mineralization [22-24]. Similarly, in vivo, administration of them to human subjects and rats increases biochemical markers of osteoblast function [25]. What is more, studies have shown that serum IGF-I and IGF-II were significantly correlated with BMD in postmenopausal women [26, 27]. The effects on bone metabolism of IGF-I and IGF-II are mediated through binding to the IGF-IR. Interestingly, the IGF-IIR has evolved a binding site for IGF-II to negatively regulate IGF-II level [28]. In previous study, the IGF-IR and IGF-IIR gene polymorphisms have been found to be associated with obesity [29], autoimmune diseases [30] and cancer [31] in different populations. But so far, the associations between genetic variants of IGF-IR and IGF-IIR gene and osteoporosis have not been investigated in Chinese population. So we performed this case-control study to identify the potential association between polymorphisms +3174G/A of IGF-IR gene (rs2229765) and c5002G/A of IGF-IIR gene (rs629849) and osteoporosis in postmenopausal women of Han Chinese.

Material and methods

Study subjects

We totally enrolled 218 patients with diagnosis of postmenopausal osteoporosis (aged 45-73) and 270 age-matched healthy controls with normal BMD (aged 45-73) in this case-control study. All subjects who had no menstruation at least 1 year were selected from Gansu Provincial Hospital between 2014 and 2016. All participants were unrelated Han ethnicity from Gansu province without migration history in the last three generations. Subjects with primary postmenopausal osteoporosis and normal BMD were diagnosed according to World Health organization criteria [32]: patients with a T-score value less than -2.5 at femoral neck or lumbar spine were considered to be osteoporotic, and subjects with T-score values higher than -1.0 were considered to have normal BMD. Questionnaires were used to collect the information of basic demographic characteristics including age, ethnicity, age of menopause and clinical characteristics including height, weight, blood pressure (BP) by physical examination. Body mass index (BMI) was calculated as body weight (kg)/height (m^2).

All women who were suffered skeletal disorders, chronic diseases involving the vital organs, serious metabolic disorders such as diabetes, hyperthyroidism and any systemic diseases known to affect BMD were excluded from this study. In addition, women taking drugs which could influence bone metabolism prior were also ruled out. Informed consent was obtained from all participants. The protocol of this study was approved by the Medicine Ethical Committee of Gansu Province People's Hospital.

BMD measurements

The BMD of lumbar spine (LS L2-4), femoral neck (FN) was assessed using the by dual-energy X-ray absorptiometry (DXA; Lunar DPX-L, Madison, Lunar, WI, USA). Forearm BMD was measured by dual-energy X-ray absorptiometry (DXA: Osteometer MediTech, USA). The value of BMD was automatically calculated from bone mineral content (g) and bone area (cm^2) and expressed as g/cm^2 adjusted by age, height, and weight. Trained technicians carried out all examinations and performed daily calibrations of the densitometers with equipment-specific phantoms.

Measurement of bone turnover markers and serum Ca, P, 25-$(OH)_2D_3$, IGF-I

Blood samples of all subjects were collected for biochemical analyses after an overnight fast of at least 8 h and centrifuged within 30 min. Fasting blood samples were collected and stored at -20°C. All serum samples remained frozen until analysis. Serum osteocalcin (OC), the C-terminal cross-linked telopeptides of type I collagen (β-CTX) were measured by Lectin affinity method (Zhong Sheng Jin Yu, Beijing, China). Serum calcium was measured by Arsenazo 2I Method used Calcium Assay Kit (Beijing Strong Biotechnologies, Beijing, China).

Serum phosphorus was measured by phosphomolybdate reduction method (Beijing Strong Biotechnologies, Beijing, China). Serum 25-$(OH)_2D_3$ was measured by chemiluminescence particles immunoassays (Abbott Laboratories, Barcelona, Spain). Serum IGF-I was determined using commercially available Quantikine Human IGF-I ELISA kits (R&D Systems, Minneapolis, MN, USA).

Measurement of IGF-IR and IGF-IIR gene polymorphism

Genomic DNA was extracted from peripheral blood leukocytes using QiaAmp blood kits (Qiagen, Santa Clarita, CA, USA) and stored at -20°C until analyzed. The IGF-IR and IGF-IIR gene polymorphisms were detected by PCR restriction fragment length polymorphism assay. Polymerase chain reaction (PCR) primers designed to amplify the polymorphism rs2229765 were identical to those used by Kimberley et al [33]. The sequence of the forward and reverse oligonucleotide primers was 5'-CAGGGGTCGTTTGGGATGGTC-3' (forward) and 5'-CCTGTGCTGCATTTTGGCTTTTC-3' (reverse). In Each PCR reaction (25 μl volume), 100 ng genomic DNA was amplified from these primers (TaKaRa, Dalian, China) in a buffer that contained 50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 250 μmol of dNTP mix, with 1.25 U of Taq DNA polymerase (TaKaRa, Dalian, China). All PCR reactions were performed using a Multicolor Real-time PCR Detection System (iCycler iQ, BIO-RAD, USA). PCR was performed with 4 min incubation at 94°C, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, dropping 0.5°C per cycle, and 72°C for 30 s. This was followed by 30 cycles of 94°C for 30 s, 45°C for 30 s and 72°C for 30 s and a final 7 min incubation at 72°C. PCR products, 207 bp fragment, were electrophoresed through a 2% agarose gel to confirm reaction and then digested with 1*enzyme buffer and 5 U of Mn1I (New England Biolabs, Hitchin, UK) at 37°C for 12 h. Different banding patterns of wild-type and polymorphic variants were identified by electrophoresis in a 4% agarose gel as follows: the wildtype G allele had two restriction sites resulting in three bands (103 bp, 84 bp and 20 bp), the polymorphic A allele had a single restriction sites resulting in two bands (123 bp and 84 bp). The polymorphism rs629849 was detected by PCR-RFLP analysis according to Petry et al [34]. The sequences of oligonucleotide primers were 5'--AACAATGGTTAAAGCCGGATTGGACACTTGAAGTT3' (forward) and 5'--GGCCCGGGTGCAGCCAGGCACTG-3' (reverse). Each PCR reaction (25 μl volume) contained 100 ng genomic DNA, 0.4 μM of each primer (TaKaRa, Dalian, China), 50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 250 μmol of dNTP mix, 1.25 U of Taq DNA polymerase (TaKaRa, Dalian, China), and 1.0*PCR buffer. PCR was performed with 5 min incubation at 94°C, followed by 20 cycles of 94°C for 30 s, 65°C for 30 s, dropping 0.5°C per cycle, and 72°C for 40 s. This was followed by 15 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 40 s and a final 10 min incubation at 72°C. PCR products, 456 bp fragment, were electrophoresed through a 2% agarose gel and then digested with 1*enzyme buffer and 20 U of NciI (New England Biolabs, Hitchin, UK) at 37°C for 12 h. After the restriction digest, separating the products by 3% agarose gel electrophoresis produced a 456 bp band for A allele, 149 and 307 bp bands for G allele. All assays were performed in duplicate by the same individual blinded to the subject case-control status.

Statistical analysis

Continuous variables were presented as mean ± standard deviation and tested with the independent samples t-test, one-way analysis of variance (ANOVA) and one-way analysis of covariance (ANCOVA). Categorical variables were expressed as number (n) and percentage (%) and compared using the χ^2 test. Hardy-Weinberg equilibrium in controls was estimated by the χ^2 test. Multivariate conditional regression analyses were performed to assess the independent role of the genotype and other risk factors, odds ratio (OR) and respective 95% confidence intervals (CI) are calculated to test the association between genotypes and OP. All statistical analyses already described were conducted using SPSS 21 (SPSS Inc., Chicago, IL, USA). *P* values <0.05 were considered to be statistically significant.

Results

Baseline characteristics of the studied groups

The general characteristics of the 488 postmenopausal women in the study were presented in **Table 1**. There were no significant differences in age (P=0.56), menopause duration

Table 1. Baseline characteristics of postmenopausal women with osteoporosis and control groups

Parameters	*OP (N=218) Mean ± SD*	*Control (N=270) Mean ± SD*	*P[a]*
Age (years)	60.82±5.95	60.51±5.65	0.56
Menopause duration (years)	11.27±5.54	10.89±5.28	0.44
Height (cm)	158.38±7.32	158.84±7.00	0.48
Weight (Kg)	57.77±8.40	58.79±8.33	0.18
BMI (Kg/m^2)	23.02±2.89	23.27±2.70	0.32
LSBMD (g/cm^2)	0.75±0.08	0.99±0.08	0.00
FNBMD (g/cm^2)	0.64±0.06	0.86±0.04	0.00
Forearm BMD (g/cm^2)	0.23±0.05	0.48±0.08	0.00
Serum Ca (mmol/L)	2.41±0.20	2.44±0.20	0.15
Serum P (mmol/L)	1.17±0.23	1.16±0.21	0.51
Serum 25-$(OH)_2D_3$ (ng/ml)	14.94±5.24	15.89±5.11	0.04
Serum OC (ng/ml)	25.00±5.84	20.78±6.44	0.00
Serum β-CTX (ng/ml)	0.44±0.15	0.39±0.16	0.00
Serum IGF-I (ng/ml)	168.16±65.37	181.24±56.37	0.02

[a]t test for baseline characteristics between patients and controls.

Table 2. Genotype and allele distributions of IGF-IR and IGF-IIR gene polymorphisms in the patients and controls groups

Gene	*Polymorphism*	*Type*	*OP n (%)*	*Control n (%)*	*X^2*	*P[a]*
IGF-IR	rs2229765					
	Genotype	G/G	58 (27)	100 (37)		
		G/A	96 (44)	124 (46)	1.828	0.176
		A/A	64 (29)	46 (17)	12.057	0.001
	Allele	G	212 (49)	324 (60)		
		A	224 (51)	216 (40)	12.61	0.000
IGF-IIR	rs629849					
	Genotype	G/G	167 (77)	216 (80)		
		G/A	49 (22)	52 (19)	0.780	0.377
		A/A	2 (1)	2 (1)	0.00	1.00
	Allele	G	383 (88)	484 (90)		
		A	53 (12)	56 (10)	0.775	0.379

[a]χ^2 test for genotype and allele distribution of IGF-IR and IGF-IIR gene polymorphisms between patients and controls.

(P=0.44) and BMI (P=0.32) between osteoporotic women and controls. Compared to controls, patients had statistically significant lower mean values of BMD (P<0.01) at forearm/lumbar spine and femoral neck and serum β-CTX, OST levels (P<0.01). Osteoporotic women had significant lower mean levels of serum 25-$(OH)_2D_3$ (P=0.04) and IGF-I (P=0.02) than controls, this result suggested that low level of 25-$(OH)_2D_3$ and IGF-I increased the risk of OP in postmenopausal women. But there was no statistical significance between two groups as regard serum calcium (P=0.15) and phosphorus levels (P=0.51).

Genotype and allele distributions of IGF-IR and IGF-IIR gene polymorphisms

Genotypic distributions of the IGF-IR and IGF-IIR gene polymorphism were consistent with the Hardy-Weinberg equilibrium in control subjects (rs2229765: P=0.53; rs629849: P=0.75). As shown in **Table 2**, the distribution of the genotypes frequencies of IGF-IR gene +3174G/A polymorphism were GG 27%, GA 44%, and AA 29% in osteoporotic women and GG 37%, GA 46%, AA 17% in controls. There was no significant difference in the distribution of GA genotype (P=0.176) of IGF-IR between osteoporotic women and controls compared with GG genotype. Instead the AA genotype (P=0.001) was significantly more frequent in patients than in controls compared with GG genotype. The distribution of the alleles frequencies of IGF-IR were G 49%, A 51% in patients and G 60%, A 40% in controls. Osteoporotic women group showed an increased frequency of the A allele (P=0.000) of IGF-IR compared to healthy controls. Concerning IGF-IIR gene c5002-G/A polymorphism, there was no significant difference in distributions of genotype (P>0.05) and allele (P>0.05) between osteoporotic women and controls.

Association of the genotypes of IGF-IR and IGF-IIR gene polymorphisms with the risk of OP

The relationships between IGF-IR and IGF-IIR gene polymorphisms and osteoporosis were further assessed by multivariate logistic regression analysis. As shown in **Table 3**, conditional logistic regression revealed that compared with the GG genotype of IGF-IR, the AA genotype (GG vs. AA: OR=2.40, 95% CI=1.46-3.95; P=0.001) and GG/AA genotype (GG vs. GA/AA: OR=1.62, 95% CI=1.10-2.39; P=0.015) was

Table 3. Association of the genotypes of IGF-IR and IGF-IIR gene polymorphisms with the risk of OP

SNP	*Genotypes*	*Crude OR (95% CI)*	*P*	*Adjusted OR (95% CI)*	*P*[a]
Rs2229765	GG	1.00[b]		1.00[b]	
	GA	1.33 (0.88-2.03)	0.177	1.24 (0.81-1.91)	0.317
	AA	2.40 (1.46-3.95)	0.001	2.10 (1.26-3.51)	0.004
	GA+AA	1.62 (1.10-2.39)	0.015	1.48 (0.99-2.20)	0.056
Rs629849	GG	1.00[b]		1.00[b]	
	GA	1.22 (0.79-1.89)	0.377	1.30 (0.83-2.03)	0.260
	AA	1.29 (0.18-9.28)	0.798	1.27 (0.17-9.36)	0.818
	GA+AA	1.22 (0.79-1.88)	0.365	1.29 (0.83-2.02)	0.254

[a]Adjusted for age, BMI (0, BMI<25; 1, BMI≥25), menopause duration, serum IGF-I, Ca, P, 25-$(OH)_2D_3$ in logistic regression model. [b]Reference group.

associated with osteoporosis without adjustment for confounding factors. After adjustment for age, menopause duration, BMI (0, BMI<25; 1, BMI≥25), β-CTX, OST, calcium, phosphorus, IGF-I, AA genotype of rs2229765 was still statistically associated with an increased risk of osteoporosis compared with GG genotype (GG vs. AA OR=2.10, 95% CI: 1.26-3.51, P=0.004), but the association between GG/AA genotype and osteoporosis was no longer significant (GG vs. GA/AA: OR=1.48, 95% CI=0.99-2.20; P=0.056). However, the association between rs629849 polymorphism of IGF-IR gene and the risk of osteoporosis was not identified.

The association of +G3174A polymorphism of IGF-IR gene with characteristics of osteoporotic women

Further, we analyzed the relations between IGF-IR gene +3174G/A polymorphism and the baseline characteristics in osteoporotic women. As shown in **Table 4**, there was no significant difference in age, menopause duration, height, weight, BMI, serum Ca and P level and bone turnover markers such as OC, β-CTX for the three genotypes. But IGF-IR gene +3174G/A polymorphisms was significantly associated with adjusted BMD at forearm and femoral neck and serum IGF-I level. Osteoporotic women with the AA genotype were found to have a significant lower mean values of forearm BMD (P=0.04) compared to women with GG genotype. The mean values of femoral neck BMD were significantly lower in women with AA genotype in comparison with GG and GA genotype (GG vs. AA: P=0.01; GA vs. AA: P=0.03). Women with the AA genotype were found to have a relatively lower Lumbar spine BMD than women with other genotypes, but these differences were not statistically significant (P> 0.05). Serum IGF-I analysis shown that women with AA (P<0.01) and GA (P=0.02) genotype had a lower mean serum IGF-I level when compared with GG genotype.

Discussion

So far, osteoporosis in the postmenopausal women remains a universal problem. Studies have indicated that this complex health problem was caused by the interaction of genetic and multiple environmental factors [8-10]. Growing evidence supports that the IGF system genes are a series of important candidate genes for influencing BMD and the process of osteoporosis [19]. In this case-control study, we evaluated the association between IGF-IR gene +G3174A and IGF-IIR gene c500-2G/A polymorphisms and osteoporosis in postmenopausal women of Han Chinese.

Our results showed significant differences between osteoporotic women and controls regarding the distribution of the IGF-IR gene +3174G/A polymorphism (rs2229765). The distributions of AA genotype (P=0.001, **Table 2**) and A allele (P=0.000, **Table 2**) of IGF-IR were more frequent in osteoporosis patients than controls. AA genotype of rs2229765 was statistically associated with the increased risk of osteoporosis (P<0.05, **Table 3**) and decreased BMD at forearm and femoral neck in osteoporotic women (P<0.05, **Table 4**). These results differ from those previously reported in a cross-sectional study in Korean postmenopausal women [19]. Lee et al [19] found that the AA genotype and A allele of +G3174A polymorphism of IGF-IR gene were associated with a higher BMD at the lumbar spine. Women with the AA genotype were found to have about half the risk of a low BMD than women with other genotypes. The reasons for these conflicting results may lie in the differences in environment and population we selected. The participants in our study were older than subjects selected by Lee et al, especially osteoporotic postmenopausal women. According to the pre-

Table 4. Correlation between IGF-IR gene +3174G/A polymorphism and baseline characteristics in the osteoporotic women (n=218)

Parameters	*Genotypes OP (n=218)*			*P*
	GG (58)	GA (96)	AA (64)	
Age (years)	59.66±5.37	61.01±6.47	61.58±5.56	0.187[c]
Menopause duration (years)	10.14±5.21	11.56±5.64	11.84±5.62	0.181[c]
Height (cm)	157.97±7.65	158.39±7.53	158.73±6.76	0.845[c]
Weight (Kg)	56.47±7.77	57.90±8.69	58.75±8.47	0.310[c]
BMI (Kg/m^2)	22.62±2.62	23.06±2.97	23.31±3.02	0.410[c]
LS BMD (g/cm^2)	0.76±0.07	0.75±0.08	0.73±0.08	0.107[d]
FN BMD (g/cm^2)	0.65±0.06[a1]	0.64±0.06[a2]	0.62±0.07[a1,a2]	0.029[d]
Forearm BMD (g/cm^2)	0.24±0.04[a]	0.23±0.05	0.21±0.05[a]	0.035[d]
Serum Ca (mmol/L)	2.40±0.20	2.42±0.21	2.41±0.18	0.897[d]
Serum P (mmol/L)	1.17±0.25	1.17±0.24	1.17±0.20	0.988[d]
Serum 25-$(OH)_2D_3$ (ng/ml)	15.79±4.72	14.43±5.58	14.93±5.12	0.341[d]
Serum OC (ng/ml)	23.56±5.74	25.36±5.66	25.76±6.04	0.093[d]
Serum β-CTX (ng/ml)	0.45±0.15	0.43±0.16	0.46±0.15	0.386[d]
Serum IGF-I (ng/ml)	189.21±64.82[a,b]	163.46±64.12[a]	156.13±64.27[b]	0.015[d]

Data are expressed as mean ± SE. [a]P<0.05. [a1]P<0.05 (GG vs. AA). [a2]P<0.05 (GA vs. AA). [b]P<0.01. [c]ANOVA (analysis of variance). [d]ANCOVA (analysis of covariance), Values adjusted for age, duration of menopause, and BMI.

vious study [35, 36], distribution of AA genotype and A allele of IGF-IR +G3174A polymorphism were higher in older population. We also found some other differences between our study and others. Minor allele frequency (MAF) of rs2229765 in our study was 0.4 in the control group, which was consistent with MAF in Chinese population [37] and NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=2229765), but much lower than European [38] and Canadian populations [33]. These differences could be due to the diversity of populations and ancestries as well as the different scale of research. The +G3174A polymorphism in the exon 16 of IGF-IR gene is a synonymous mutation, which has been examined in several diseases and shown to affect the susceptibility to ischemic stroke in the Chinese population [37]. Possible roles of this genetic variant have been already investigated in numerous studies. But the molecular mechanism of the relationship between IGF-IR gene +G3174A polymorphism and osteoporosis has not yet been fully identified. One of the possible mechanisms may be that IGF-IR polymorphism changes plasma IGF-I levels by altering the function of binding site in IGF-IR. Furthermore, IGF-I involves in maintaining bone mass by enhancing bone matrix formation and inhibiting its degradation [39]. Another is that the change of allele from G to A in this polymorphism may lead to partial loss of the activity of IGF-IR protein. However, the synonymous amino acid substitution in IGF-IR protein resulted from rs2229765 suggests that this polymorphism does not have direct functional consequences on receptor function. Nevertheless, rs2229765 may be linked to another functional polymorphism that may alter receptor activity or influence gene transcription or mRNA stability in the process of the expression of genetic information. As recent studies have revealed that synonymous single nucleotide polymorphism (SNP), such as rs2229765, may represent genetic markers for functional molecular alterations and affect gene function and phenotype through various mechanisms. In recent years, more and more studies found the association between IGF-IR and bone metabolism. IGF-IR plays an important role in osteoblast differentiation and coordinates chondrocyte, osteoclast, and endothelial responses [40]. And IGF-I receptor insufficiency leads to age-dependent attenuation of osteoblast differentiation, which may phenocopy age-related decreases in bone formation [41]. Study in the T1D patients. It found that decreased IGF-IR expressions may contribute to low bone mineral density [42].

The association between c5002G/A polymorphism of IGF-IIR gene (rs629849) and the increased risk of osteoporosis was identified in

our study. The MAF of rs629849 was 0.1 in our controls group which was consistent with MAF in NCBI dbSNP. Rs629849 is in the exon 34 of IGF-IIR gene, and polymorphism in coding region may alter the ability of IGF-II receptor to bind and regulate IGF-II level [28]. Previous studies have shown that rs629849 polymorphism was associated obesity in Korean population, birth weight and birth length in Caucasian, Asian and African Americans infants [43], as well as circulating levels of IGF-II in women [44]. But in our study, there was no statistical difference between cases and controls in distributions of allele and genotype of rs629849. So IGF-IIR gene c5002G/A polymorphism may not influence susceptibility to osteoporosis.

We also investigated plasma IGF-I concentration between two groups and a possible correlation between IGF-IR gene +G3174A polymorphism and IGF-I levels. We found that serum IGF-I in osteoporosis patients was significantly lower than in age-matched control subjects. In this regard, our results were similar to the data reported by Kim et al [45]. Studies have also demonstrated that serum IGF-I, one of the most abundant growth factors in the bone microenvironment was found to be significantly and positively correlated with BMD at lumbar spine, radius, and total body [26]. Research in recent years shows that IGF-I can enhance osteogenic differentiation of human bone marrow mesenchymal stem cells and dental pulp stem cells via different pathways [46, 47]. Moreover, osteocyte-derived IGF-I has also been shown to be a key determinant in bone mechanic sensitivity, bone growth, remodeling and regeneration [48, 49]. In the entire osteoporosis sample we found that women with AA genotype of IGF-IR showed decreased level of IGF-I in contrast to AG and GG genotype. In this regard, our results were similar to data reported by Bonafe and Albani et al [35, 36]. Results of these investigations were that subjects carrying at least an A allele in rs2229765 had low levels of plasma IGF-I.

In conclusion, We provided the evidence that IGF-I was associated with increased risk of osteoporosis in postmenopausal women. Our results shown that IGF-IR gene +3174G/A polymorphism may be associated with increased risk of osteoporosis, decreased serum IGF-I, BMD at forearm and femoral neck in postmenopausal women of Han Chinese. So our study suggested that IGF-IR gene +3174G/A polymorphism may affect the susceptibility to osteoporosis in postmenopausal women. But there is no association between IGF-IIR gene c5002G/A polymorphism and osteoporosis in postmenopausal women was found in our study. To date, few studies have examined the association of IGF-IR gene +G3174A polymorphism, and IGF-IIR gene c5002G/A polymorphism with BMD or osteoporosis. So this hypothesis requires further investigation.

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Disclosure of conflict of interest

None.

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