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Original Article

Influence of polymorphisms in insulin-like growth factor-1 on the risk of osteoporosis in a Chinese postmenopausal female population

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Abstract: We conducted a case-control study in a Chinese postmenopausal population, and explore the potential role of the promoter region variation of the IGF-1 gene in bone mineral density and osteoporosis risk. 485 postmenopausal women with a primary diagnosis of osteoporosis and 485 age-matched controls were selected between 2012 and 2014. The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used for rs35767, rs2288377 and rs5742612 of IGF-1 genotyping. By conditional regression analysis, individuals carrying TT genotype and CT+TT genotype of rs35767 were found to be correlated with an elevated risk of osteoporosis, with adjusted ORs (95% CI) of 1.90 (1.23-2.93) and 1.35 (1.04-1.76), respectively. Our study found that CT+TT genotype of rs35767 was significantly associated with moderate increased risk of osteoporosis in smokers and drinkers, and the ORs (95% CI) were 2.11 (1.06-4.20) and 2.36 (1.29-4.32), respectively. We found that those carrying CT+TT genotype of rs35767 had a significant lower BMD levels at L1-L4 vertebrae, femoral neck, total hip and trochanter compared to those with CC genotype. Our study suggests that TT genotype and CT+TT genotype of IGF-I rs35767 were associated with risk of osteoporosis and BMD levels.

Keywords: Insulin-like growth factors, polymorphism, osteoporosis, women, bone mineral density

Introduction

Osteoporosis, characterized by decreased bone strength, is prevalent among postmenopausal women but also occurs in men and women with underlying conditions or major risk factors associated with bone demineralization [1]. It is reported that 55% population aged above 50 years old are suffered from osteoporosis [2]. Epidemiologic studies show that about 8 million women and 2 million men suffer from osteoporosis and over 18 million have such a low bone mineral density that are prone to osteoporosis. This trend increases with age because bone structure deteriorates progressively [1].

It is well known that bone remodeling is influenced by systemic hormones and locally produced factors [3, 4]. Insulin-like growth factors (IGFs) are ones of the important regulators for bone cell function, and they have important role in synthesis of the skeleton [5, 6]. The main role of the IGF system in the local regulation of bone formation is determined by blocking basal bone cell proliferation [6]. IGF-1 is one of the main factors in the IGF-1 signaling pathway. Previous experimental studies reported that expression of IGF-1 could influence the size, shape and composition of bones, and regulate osteoblast differentiation, mineralization and proliferation in vitro [7]. But some studies reported inconsistent results. Two previous studies reported no significant association between IGF-1 expression and risk of osteoporosis [8, 9]. In humans, several epidemiological studies suggested that genetic variations of IGF-1 signaling pathway may play an important role in influence the bone mineral density and osteoporosis, but the results are inconsistent [10-13]. Therefore, we conducted a case-control study in a Chinese postmenopausal population, and explored the

potential role of the promoter region variation of the IGF-1 gene in bone mineral density and osteoporosis risk.

Materials and methods

Subjects

In this cases-control study, 508 postmenopausal women with a primary diagnosis of osteoporosis were selected from the Second Affiliated Hospital of Inner Mongolia Medical University between 2012 and 2014. Postmenopausal women who had more than 3 months of amenorrhea, a history of hysterectomy or ovariectomy or the possibility of pregnancy, or taking drugs to affect the skeletal homeostasis or interfere with bone metabolism were excluded from our study. Finally, 485 postmenopausal women were included into our study, with a participation rate of 95.47%. The osteoporosis was defined according to T score based on WHO criteria. The osteoporosis was defined as women who had a decrease of bone mineral density (BMD) T-score less than 2.5 at the femoral neck without an evidence of vertebral fractures. Otherwise those who had a decrease of BMD T-score less than 1.5 with two or more vertebral fractures. A total of 485 subjects without a diagnosis of osteoporosis were selected from the health check-up examination in our hospital. The control subjects were matched with postmenopausal women by age.

In order to gather information, the demographic and clinical characteristics were collected from medical records and demographic data questionnaires. The demographic and clinical characteristics included age, height, weight, smoking, drinking and BMD in L1-L4 vertebrae, femoral neck, total hip and trochanter. The protocol of our study was approved by the Ethics Committee of the Second Affiliated Hospital of Inner Mongolia Medical University, and our study was conducted according to the Declaration of Helsinki. A total of 428 age-matched healthy controls were selected from the health check-up center. All subjects who were suffering disease or taking drugs to influence the skeletal homeostasis or interfere with bone metabolism were excluded from this study. All the subjects signed an informed consent form for blood sample collection before participating into our study. The protocol of our study was approved by ethics committee of the General Hospital of Chinese PLA.

Measurement of BMD

The BMD was assessed using dual-energy X-ray absorptiometry (Hologic®, Waltham, MA, USA) at L_1-L_4 vertebrae, femoral neck hip and total hip as well as trochanter. The BMD was assessed using dividing bone mineral content (g) by bone area (cm^2), and the BMD was expressed as g/cm^2.

Genotype analyses

Venous blood samples from forearm were collected from each participant. All study participants were asked to provide 5 ml peripheral venous blood, and their blood samples were kept at -20°C placed in EDTA anticoagulant and centrifuged at 2700rpm for ten min at room temperatureuntil analyzed. The blood was kept at -20°C in a refrigerator maintained until use. The genomic DNA was extracted by DNA Extraction Kit Genomic DNA was extracted using the TIANamp DNA Blood Mini Kit (QIAGEN GmbH, Germany) according to the supplier's instructions. The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used for rs35767, rs2288377 and rs5742612 of IGF-1 genotyping. The primer sequences for rs35767, rs2288377 and rs5742612 of IGF-1 were shown in **Table 1**. The PCR reactions were conducted in a 50 μl volume, including 6 μl genomic DNA, 0.4 μl Taq enzyme, 5 μl 10×PCR mix, 2.5 μl 5 mol/L dNTP Mixture, 80 μmol/L forward primer and 80 μmol/L reverse primer. The PCR reaction was carried out at 94°C for 5 min for initial denaturation and then at 94°C for 30 s, 58°C 30 s and 72°C for 30 s. After 35-cycles of amplification, additional extensions were done at 72°C for 7 min. Finally, each 8 μl PCR product was analyzed by 3% agarose gel electrophoresis to identify the purity and integrity, observed under ultraviolet lamp and recorded by photography. For quality control, 5% of the samples were randomly selected to repeat genotyping, and the genotyping results showed 100% concordant.

Statistical analysis

All the statistical analyses were conducted with the SPSS 16.0 statistical software (SPSS, Chicago, IL). Frequencies and percentages (%)

Table 1. Demographic and clinical characteristics of included osteoporosis cases and controls

Parameters	Cases (%) N=485	Controls (%) N=485	χ^2 or t value	*P*-value
Age, years	66.7±7.2	67.2±6.8	1.11	0.13
Height (cm)	155.7±7.4	156.2±7.1	1.07	0.14
Weight (kg)	57.2±6.6	57.6±6.8	0.93	0.18
Age of menarche, years	13.8±1.5	13.6±1.6	2.01	<0.05
Number of pregnancies	1.7±1.3	1.9±1.5	2.22	<0.05
Duration of menopause, years	17.4±3.8	17.2±4.1	0.79	0.22
Smoking				
No	358 (86.27)	415 (85.57)		
Current or former	127 (13.73)	70 (14.43)	20.70	<0.05
Drinking				
No	356 (73.40)	399 (82.27)		
Current or former	129 (26.60)	86 (17.73)	11.05	<0.05
BMD (g/cm^2)				
L_1–L_4 vertebrae	0.93±0.082	0.95±0.120	3.03	<0.05
Femoral neck	0.61±0.035	0.65±0.031	18.84	<0.05
Total hip	0.62±0.044	0.66±0.042	14.48	<0.05
Trochanter	0.56±0.043	0.60±0.048	13.67	<0.05

were used to describe the distribution of categorical variables, and mean ± standard deviation (SD) was used for continuous variables. Deviations from Hardy-Weinberg equilibrium of the genotyped IGF-1 genetic polymorphisms were evaluated by χ^2-test. Logistic regression was used to assess the influence of IGF-1 genetic polymorphisms for the osteoporosis risk, and the results were determined as odds ratios (ORs) and their 95% confidence intervals (CIs). The OR (95% CI) was adjusted for potential confounding factors, such as age, BMI, smoking and drinking. *P* values <0.05 with two-sided were considered statistical differences.

Results

Patients' demographic and clinical characteristics

The demographic and clinical characteristics of included osteoporosis cases and controls were shown in **Table 1**. The mean ages of included osteoporosis cases and controls were 66.7±7.2 and 67.2±6.8 years at diagnosis, respectively.

Of the included cases and controls, osteoporosis cases were more likely to have higher age of menarche, more numbers of pregnancies, more smokers and more drinkers. Moreover, osteoporosis cases had lower BMD in L2-L4 vertebrae, femoral neck and total hip as well as trochanter when compared with them in controls.

Genotype distributions of IGF-1 gene polymorphisms and its association with risk of osteoporosis

The genotype frequencies of genotyping of rs35767were found to be in line with Hardy-Weinberg equilibrium in the control group, while rs2288377 and rs5742612 in IGF-1 in controls was not (**Table 2**). The minor allele frequencies (MAF) in controls of the six SNPs were similar to those in database (http://www.ncbi.nlm.nih.gov/snp). By conditional regression analysis, individuals carrying TT genotype and CT+TT genotype of rs35767 were found to be correlated with an elevated risk of osteoporosis, with adjusted ORs (95% CI) of 1.90 (1.23-2.93) and 1.35 (1.04-1.76), respectively. However, rs2288377 and rs5742612 polymorphisms were found to be no significant association with risk of osteoporosis.

Interaction analysis between the rs35767 polymorphism and demographics on the risk of osteoporosis

We conducted interaction analysis between the rs35767 polymorphism and demographics and BMD on the risk of osteoporosis (**Table 3**). Our study found that CT+TT genotype of rs35767 was significantly associated with moderate increased risk of osteoporosis in smokers and drinkers, and the ORs (95% CI) were 2.11 (1.06-4.20) and 2.36 (1.29-4.32), respectively. However, our study did not find any significant interaction of rs35767 polymorphism with age, age of menarche and number of pregnancies.

Interaction between IGF-1 rs35767 polymorphism and BMD levels in osteoporosis cases

We also assessed the correlation between IGF-1 rs35767 polymorphism and BMD value in

Table 2. The genotype frequencies of IGF-1 gene and their association with risk of osteoporosis

IGF-I		Cases % N=485		Controls % N=485		MAF		HWE	OR (95% CI)[a]	*P*-value	*P*-value
						In controls	In database				
rs35767	CC	202	41.6	238	49.1				1.0 (Ref.)		
	CT	210	43.2	201	41.5				1.23 (0.93-1.63)	0.13	
	TT	74	15.2	46	9.4	0.3015	0.3037	0.71	1.90 (1.23-2.93)	<0.05	30.15
	CT+TT	284	58.4	247	50.9				1.35 (1.04-1.76)	0.02	
rs2288377	AA	396	81.7	413	85.2				1.0 (Ref.)		
	AT	52	10.8	45	9.3				1.21 (0.77-1.88)	0.39	
	TT	36	7.5	27	5.5	0.1015	0.098	<0.05	1.39 (0.80-2.43)	0.21	10.15
	AT+TT	88	18.3	72	14.8				1.27 (0.89-1.82)	0.16	
rs5742612	CC	389	80.2	400	82.5				1.0 (Ref.)		
	CT	55	11.3	50	10.3				1.13 (0.74-1.74)	0.55	
	TT	41	8.5	35	7.2	0.1235	0.1146	<0.05	1.20 (0.73-1.99)	0.44	12.35
	CT+TT	96	19.8	85	17.5				1.16 (0.83-1.63)	0.36	

[a]Adjusted for age, weight, height, smoking and drinking.

Table 3. Association between IGF-1 rs35767 polymorphism and demographic characteristics in the risk of osteoporosis

Parameters	IGF rs35767				OR (95% CI)	*P*-value
	CC		CT+TT			
	Cases	Controls	Cases	Controls		
Age, years					1.11	0.13
<65	93	112	123	103	1.44 (0.97-2.14)	0.06
≥65	109	126	90	74	1.41 (0.92-2.14)	0.1
Age of menarche, years						
<13	77	81	114	85	1.41 (0.91-2.19)	0.11
≥13	125	157	99	92	1.35 (0.92-1.99)	0.11
Number of pregnancies						
≤1	61	87	85	88	1.37 (0.86-2.20)	0.16
>1	141	151	128	159	0.86 (0.61-1.21)	0.38
Smoking						
No	162	209	196	221	1.14 (0.86-1.53)	0.35
Current or former	40	29	87	26	2.11 (1.06-4.20)	<0.05
Drinking						
No	145	182	211	217	1.22 (0.90-1.65)	0.18
Current or former	57	56	72	30	2.36 (1.29-4.32)	<0.05

osteoporosis cases. We found that those carrying CT+TT genotype of rs35767 had a significant lower BMD value at L1-L4 vertebrae, femoral neck, total hip and trochanter compared to those with CC genotype (**Figure 1**). Moreover, the BMD value in L2-L4 vertebrae, femoral neck and total hip as well as trochanter in cases carrying CT+TT genotype of rs35767 were significantly lower by 4.81%, 7.20%, 3.13% and 2.41% when compared with those in cases with CC genotype, respectively.

Discussion

Osteoporosis is the most common metabolic disease of bones which is defined as low BMD. This disease is caused by many environmental factors, such as smoking, low weight, low calcium intake, vitamin D absorption decrease due to lack of sunshine, alcoholism, frequent falls, lack of physical activity and poor health [14]. Genetic factors also play an important role in the development of osteoporosis, such as TGF-β1, LRP5, IL-17, Fok1 and Bsm1 [15-17]. In our study, we found that individuals carrying TT genotype and CT+TT genotype of rs35767 were found to be correlated with an elevated risk of osteoporosis, and CT+TT genotype of rs35767 was significantly associated with moderate increased risk of osteoporosis in smokers and drinkers.

It is reported that genetic modulation plays an important role of modifying the bone phenotype parameters, including bone mineral density and size as well as turnover [18], and genetic factors could contribute to 70% of the vari-

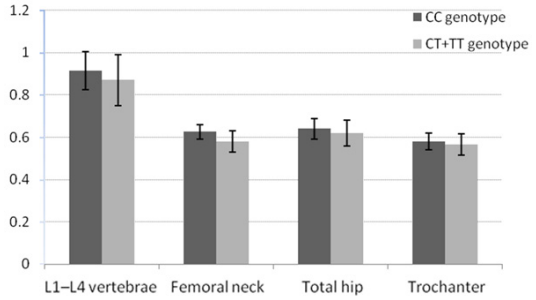

Figure 1. Association between IGF-1 rs35767 polymorphism and BMD levels in osteoporosis cases.

ance in bone phenotype [18]. Previous studies reported that IGF-1 expression contributes to influence the bone formation and pathogenesis of osteoporosis [13, 19]. Previous studies reported the IGF-1 polymorphisms are associated with BMD and osteoporosis [11, 12, 20-24]. Rivadeneira et al. reported that promoter polymorphisms in IGF-1 could influence the BMD levels and rate of bone loss in postmenopausal women [20]. Rivadeneira et al. conducted a prospective population-based cohort study, and found that polymorphism in the IGF-1 gene was associated with the risk for fragility fracture at old age in women and with bone structure [21]. Another study in Spain reported that IGF-1 serum levels were independently associated with BMD in postmenopausal Spanish women [11]. Lee et al. reported that IGF-I receptor gene G3174A polymorphism was correlated with lumbar spine BMD in postmenopausal women [23]. However, one study reported that IGF-1 gene polymorphisms did not influence the BMD levels in premenopausal women [12]. The inconsistent results might be caused by differences in populations, source of patients, sample size and also by chance.

Our study found that IGF-1 rs35767 polymorphism was associated with increased risk of osteoporosis, and correlated with BMD levels in osteoporosis cases. Only one previous study reported the association between IGF-1 rs35767 polymorphism and osteoporosis [13]. Yun-Kai et al. conducted a case-control study in a postmenopausal population, and suggested that polymorphism in IGF-I rs35767 was significantly associated with BMD and osteoporosis in postmenopausal female population [13]. The results of above-mentioned study were associated with our study. Further studies with more ethnicities are greatly needed to clarify the association between IGF gene polymorphisms and risk of osteoporosis.

Moreover, our study reported that CT+TT genotype of rs35767 was significantly associated with moderate increased risk of osteoporosis in smokers and drinkers. Previous studies reported that age, sex and smoking could influence the expression of IGF-1, and affect the pathogenesis of disease statues associated with IGF-1 [23]. Gapstur et al., suggested that IGF-1 levels could be influenced by alcohol intake and cigarette smoking [26].

In conclusion, our study suggests that TT genotype and CT+TT genotype of IGF-I rs35767 are associated with risk of osteoporosis and BMD levels, and IGF-I rs35767 polymorphism has interaction with alcohol intake and cigarette smoking. Moreover, further investigations on the IGF-I rs35767 polymorphism and risk of osteoporosis are warranted.

Disclosure of conflict of interest

None.

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