Association of the *IGF-1* rs35767 and rs972936 polymorphisms with the risk of osteoporosis in a Chinese postmenopausal female population

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ABSTRACT. The aim of our study was to conduct a case-control study in a Chinese postmenopausal population to evaluate the roles of the *IGF-1* rs35767 and rs972936 polymorphisms on bone mineral density (BMD) levels and osteoporosis risk. A total of 272 consecutive postmenopausal women with a primary diagnosis of osteoporosis and 272 controls were enrolled in the study between 2012 and 2014. The polymerase chain reaction-restriction fragment length polymorphism method was used to genotype the rs35767 and rs972936 *IGF-1* polymorphisms. By comparing the demographic characteristics between patients and controls, patients with osteoporosis were found to be more likely to have a habit of alcohol drinking (P = 0.023). Furthermore, the BMD levels of the L_1-L_4 vertebrae, femoral necks, total hips, and trochanters in patients with osteoporosis were significantly lower than those in controls. By conditional regression analysis, we found that the *IGF-1* rs2288377 and rs972936 gene polymorphisms

were not associated with the risk of osteoporosis (P < 0.05). However, the CT+TT genotype of rs35767 and the AG+GG genotype of rs972936 were significantly associated with lower BMD levels in the femoral neck. Overall, our study suggests that *IGF-1* rs2288377 and rs972936 gene polymorphisms do not influence the risk osteoporosis.

Key words: Insulin-like growth factors; Polymorphism; Osteoporosis; Postmenopausal female

INTRODUCTION

Osteoporosis is prevalent in females, especially in postmenopausal women (Kasper et al., 2005). It is estimated that about 60% of all osteoporotic fractures occur in females. Previous studies have reported that approximately 55% of females above 50 years old suffer from osteoporosis (Watts et al, 2008). The process of osteoporosis is caused by multiple factors including both environmental and genetic factors, and the disease features low bone mineral density (BMD) and loss of bone tissue. BMD is influenced by physical exercise, dietary factors, and sex hormones (Canalis, 1983; Mohan and Baylink, 1991).

Insulin-like growth factors (IGFs) play an important role in the regulation of bone metabolism and bone cell function, and play a critical role in the synthesis of many tissues including osteoblasts. The skeleton is an important reservoir of circulating IGF-1 (McCarthy et al., 1989; Mohan, 1993). Previous studies have reported that IGF-1 could increase matrix apposition and reduce collagen degradation (Mohan, 1993). It has also been demonstrated that single nucleotide polymorphisms (SNPs) might influence the expression of the *IGF-1* gene, and thus affect BMD levels and the risk of osteoporosis (Kim et al., 2002; Mezquita-Raya et al., 2004; Jiang et al., 2005; Yun-Kai et al., 2014). There are two common SNPs in *IGF-1*: rs35767 and rs972936, however, few studies have reported their association with osteoporosis.

Therefore, the aim of our study was to conduct a case-control study in a Chinese postmenopausal population, and evaluate the role of the *IGF-1* rs35767 and rs972936 polymorphisms on BMD levels and osteoporosis risk.

MATERIAL AND METHODS

Subjects

A total of 272 consecutive postmenopausal women with a primary diagnosis of osteoporosis and 272 controls were enrolled in the study between 2012 and 2014. The diagnostic criteria were postmenopausal women who had no more than 3 months of amenorrhea, no history of hysterectomy or ovariectomy, and no possibility of pregnancy. Osteoporosis was diagnosed according to the T-score from World Health Organization criteria. Individuals with a BMD T-score less than 2.5 at the femoral neck without any evidence of vertebral fractures, or with a BMD T-score less than 1.5 with two or more vertebral fractures were diagnosed as having osteoporosis. For the control group, a total of 272 subjects without a diagnosis of osteoporosis were recruited from among individuals getting a routine check-up in the health examination center of our hospital. The control subjects were matched with women with osteoporosis by age (± 5 years).

The demographic and clinical characteristics of patients with osteoporosis and controls

were collected from the medical records. The demographic characteristics included age, height, weight, and tobacco smoking and alcohol drinking status. The clinical characteristics included BMD in the L_1-L_4 vertebrae, femoral neck, total hip, and trochanter. The BMD levels evaluated using dual-energy X-ray absorptiometry (Hologic®, Waltham, MA, USA). The ages of the patients and controls ranged from 53 to 79 years old, and the mean ages of the patients and controls were 65.70 ± 8.10 and 66.40 ± 7.90 years, respectively. Informed consent was obtained from all patients and control subjects or their relatives before enrollment in this study. The study protocol was approved by the Ethics Committee of the 3201 Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, China.

DNA isolation

Each patient and control subject was asked to provide 5 mL blood for DNA sequencing after agreeing to participate in our study. The collected blood samples were kept at -20°C until use; 0.5 mg/mL ethylenediaminetetraacetic acid was used as the anticoagulant. The *IGF-1* rs35767 and rs972936 polymorphisms were analyzed using genomic DNA purified from the peripheral blood samples using the TIANamp Blood DNA kit (Tiangen, Beijing, China) according to the manufacturer instructions, and the genomic DNA was stored at -20°C until use.

Genotype analyses

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to genotype the rs35767 and rs972936 *IGF-1* polymorphisms. The primer sequences for rs35767 and rs972936 in *IGF-1* were as follows: for rs35767, the forward and reverse primers were 5'-GGA TTT CGC AGA ACT TCG TTT TCA-3' and 5'-GGT GGA ATA CTG GAT TCC TGA AT-3', respectively. For rs972936, the forward and reverse primers were 5'-GTG GTA TGT GTA GTT ATT CTG ACA TCC AG-3' and 5'-GTG TCT GGC TGT GGC TCT TAG-3', respectively. The restriction enzymes for rs35767 and rs972936 were *Ssi*I and *Mva*I, respectively. PCR was performed using the following conditions: initial denaturation at 95°C for 5 min, followed by 20 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 40 s, and final extension at 72°C for 6 min. The PCR products were visualized by 1.0% agarose gel electrophoresis with ethidium bromide staining and UV light.

Statistical analysis

Continuous variables are reported as means ± SD, and categorical variables are reported as N (%) of study participants. The Student *t*-test was used to compare continuous variables between patients and control subjects, and the χ^2-test was used to compare categorical variables between patients and control subjects. Deviations from Hardy-Weinberg equilibrium of the genetic distributions of *IGF-1* rs35767 and rs972936 variants in controls were evaluated by the χ^2-test. Conditional logistic regression was conducted to assess the effects of *IGF-1* rs35767 and rs972936 genetic polymorphisms on the risk of osteoporosis after adjusted for potential confounding factors, with results expressed as ORs and corresponding 95%CIs. Homozygotes of the most frequent genotype were regarded as the reference group. All P values were two-sided, and a P value less than 0.05 was considered as statistically significant. All statistical analyses were conducted using the SPSS 19.0 statistical software (SPSS, Chicago, IL, USA).

RESULTS

The demographic and clinical characteristics of all patients with osteoporosis and controls are shown in Table 1. As expected, no significant differences were observed between patients with osteoporosis and controls in terms of age. The mean ages of patients and controls were 65.2 ± 8.1 and 66.4 ± 7.9 years at the time of enrollment into our study, respectively. By comparing the demographic characteristics between patients and controls, patients with osteoporosis were more likely to have a habit of alcohol drinking (P = 0.023). In addition, the BMD levels of the L_1-L_4 vertebrae, femoral necks, total hips, and trochanters in patients with osteoporosis were significantly lower than those in controls.

Table 1. Demographic and clinical characteristics of patients included with osteoporosis and controls.

Parameter	Patients (N = 272)	%	Controls (N = 272)	%	χ^2 or *t* value	P value
Age (years)	65.70 ± 8.10		66.40 ± 7.90		1.02	0.154
Height (cm)	155.30 ± 8.20		156.10 ± 7.50		1.19	0.119
Weight (kg)	59.70 ± 7.20		58.80 ± 7.50		1.43	0.077
Smoking						
No	223	81.99	238	87.50	3.20	0.074
Current or former	49	18.01	34	12.50		
Drinking						
No	181	66.54	205	75.37	5.13	0.023
Current or former	91	33.46	67	24.63		
BMD (g/cm^2)						
L_1-L_4 vertebrae	0.922 ± 0.123		0.956 ± 0.154		2.58	0.005
Femoral neck	0.604 ± 0.052		0.645 ± 0.062		8.15	<0.001
Total hip	0.625 ± 0.044		0.662 ± 0.042		10.85	<0.001
Trochanter	0.642 ± 0.120		0.664 ± 0.105		2.276	0.012

BMD = bone mineral density.

The genotype frequencies of rs35767 and rs972936 are shown in Table 2. By χ^2-test, we found that *IGF-1* rs2288377 and rs972936 were in line with Hardy-Weinberg equilibrium in the control group. Furthermore, the minor allele frequencies in controls for *IGF-1* rs2288377 and rs972936 were similar to the distributions listed in the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/). By conditional regression analysis, we found that the *IGF-1* rs2288377 and rs972936 gene polymorphisms were not associated with the risk of osteoporosis (P < 0.05).

Table 2. *IGF-1* genotype frequencies and their association with osteoporosis risk.

IGF-1		Patients (N = 272)	%	Controls (N = 272)	%	MAF		P value for HWE	OR (95%CI)[a]	P value
						In controls	In database			
rs35767	CC	124	45.59	132	48.4				1.0 (Ref.)	-
	CT	118	43.38	116	42.7				1.08 (0.75-1.57)	0.66
	TT	30	11.03	24	8.9	0.3015	0.3037	0.834	1.33 (0.71-2.52)	0.342
	CT+TT	148	54.41	140	51.6				1.13 (0.79-1.60)	0.492
rs972936	AA	104	38.24	119	43.6				1.0 (Ref.)	-
	AG	124	45.59	121	44.5				1.17 (0.80-1.71)	0.39
	GG	43	16.18	32	11.9	0.3415	0.3419	0.883	1.54 (0.88-2.70)	0.109
	AG+GG	167	61.76	153	56.4				1.25 (0.87-1.79)	0.203

[a]Adjusted for age, weight, height, smoking, and drinking. MAF = minor allele frequency; HWE = Hardy-Weinberg equilibrium.

We also assessed the correlation between the *IGF-1* rs35767 and rs972936 polymorphisms and BMD levels in patients with osteoporosis. We found that individuals carrying the CT+TT genotype of rs35767 had a significantly lower BMD level at the femoral neck than did those with the CC genotype (Table 3). Furthermore, the BMD levels in the femoral necks of patients with osteoporosis carrying the AG+GG genotype of rs972936 were significantly lower than in those patients with the AA genotype.

Table 3. Association between *IGF-1* rs35767 and rs972936 polymorphisms and BMD levels in patients with osteoporosis.

Region	rs35767		*t* value	P value	rs972936		*t* value	P value
	CC	CT+TT			AA	AG+GG		
L_1-L_4 vertebrae	0.924 ± 0.112	0.917 ± 0.101	0.766	0.222	0.922 ± 0.108	0.918 ± 0.122	0.405	0.343
Femoral neck	0.602 ± 0.035	0.591 ± 0.041	3.365	<0.001	0.608 ± 0.052	0.597 ± 0.042	2.714	0.003
Total hip	0.624 ± 0.049	0.618 ± 0.053	1.371	0.086	0.625 ± 0.042	0.622 ± 0.037	0.884	0.189
Trochanter	0.646 ± 0.081	0.640 ± 0.079	0.875	0.191	0.644 ± 0.102	0.639 ± 0.106	0.561	0.288

BMD = bone mineral density.

DISCUSSION

It is well known that osteoporosis is caused by many environmental factors such as high intake of alcohol and tobacco smoking, low weight, low calcium intake, low vitamin D absorption due to lack of sunshine, and it leads to frequent falls as well as to a lack of physical activity (Leslie and Morin, 2014). Previous studies have reported that the expression of *IGF-1* polymorphisms might affect the development of osteoporosis; however, the results have been inconsistent (Kim et al., 2002; Rivadeneira et al., 2003; Jiang et al., 2005; Niu and Rosen, 2005; Yun-Kai et al., 2014). In our study, we suggested that the *IGF-1* rs2288377 and rs972936 gene polymorphisms were not associated with osteoporosis risk in Chinese postmenopausal women, and that the CT+TT genotype of rs35767 and the AG+GG genotype of rs972936 were significantly associated with lower BMD levels in the femoral necks of these patients.

Several studies have suggested that variation in *IGF-1* is associated with osteoporosis risk (Kim et al., 2002; Jiang et al., 2005; Lee et al., 2008; Yun-Kai et al., 2014). Yun-Kai et al. (2014) suggested that the *IGF-1* rs35767 polymorphism significantly influenced BMD levels and osteoporosis risk in a postmenopausal female population. Kim et al. (2002) conducted a study in a Korean population and suggested that *IGF-1* gene polymorphisms are significantly associated with BMDs of the lumbar spine and proximal femur. Another study in a postmenopausal Korean population reported that the *IGF-1* rs2229765 gene polymorphism was correlated with BMD level in the lumbar spine (Lee et al., 2008). However, Jiang et al. (2005) conducted a study in premenopausal Chinese women that showed that the *IGF-1* gene had no role in BMD variation at any skeletal site. In our study, we also did not find significant association of the *IGF-1* rs2288377 and rs972936 gene polymorphisms with the risk of osteoporosis. Our study also found that the *IGF-1* rs2288377 and rs972936 gene polymorphisms were associated with BMD level at the femoral neck, which is in accordance with the finding reported by Kim et al. (2002). The discrepancies of the reported results might be caused by differences in ethnicities, selection of patients and controls, study design, or sample size.

We identified three limitations in our study. First, patients were selected from a single hospital, which might not be representative of the general population. Second, other genetic

polymorphisms might have influenced the risk of osteoporosis in addition to *IGF-1* rs2288377 and rs972936. Third, the sample size of this study was relatively small, which could limit the statistical power to identify the differences between groups. Further studies with large sample sizes are greatly needed to clarify the association of *IGF-1* gene polymorphisms with the risk of osteoporosis.

In conclusion, our study suggests that the *IGF-1* rs2288377 and rs972936 gene polymorphisms do not influence the risk of osteoporosis, but that the CT+TT genotype of rs35767 and the AG+GG genotype of rs972936 might be related to the lower BMD levels in the femoral neck of patients with osteoporosis. Further well designed and large sample size studies on the *IGF-I* rs35767 polymorphism and the risk of osteoporosis are warranted.

Conflicts of interest

The authors declare no conflict of interest.

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