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Association of insulin-like growth factor I gene and vitamin D receptor gene polymorphisms with the risk of osteoporosis in a Chinese population.

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Abstract

Objective: Osteoporosis is a systemic metabolic disease. The etiology of osteoporosis is involved in many environmental and genetic factors. We investigated the association of IGF-I (rs35767, rs2288377 and rs5742612) and VDR (rs7975232, rs2228570, rs1544410 and rs11568820) polymorphisms with the risk of osteoporosis. We also assessed the effect of gene-environment interactions in the development of this disease.

Methods: A total of 320 patients with osteoporosis and 320 controls were recruited. IGF-I (rs35767, rs2288377 and rs5742612) and VDR (rs7975232, rs2228570, rs1544410 and rs11568820) were amplified and genotyped by the polymerase chain reaction-restriction fragment length polymorphism method.

Results: The AA genotype of rs2288377 was associated with an elevated risk of osteoporosis compared to the TT genotype (OR=1.90, 95% CI=1.08-3.39). In dominant model, the TA+AA genotype of rs2288377 had an increased risk of developing osteoporosis in comparison to the TT genotype (OR=1.50, 95% CI=1.08-2.08). In recessive model, the AA genotype of rs2288377 revealed a higher risk of developing osteoporosis than the TA+TT genotype (OR=2.19, 95% CI=1.28-3.79). Moreover, IGF-I rs35767 and rs5742612, and VDR rs7975232, rs2228570, rs1544410 and rs11568820 were not significantly associated with the risk of osteoporosis. Conclusions: Our study suggests a significant association between the IGF-I rs2288377 polymorphism and the risk of osteoporosis in the Chinese population.

Keywords: Osteoporosis, IGF-I, VDR, Polymorphism, Gene-environmental interaction.

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Introduction

Osteoporosis is a systemic metabolic disease, and it is a public health problem worldwide. Osteoporosis is an important risk factor for the occurrence of fracture [1]. The etiology of osteoporosis is involved in many environmental factors, such as lack of vitamins, microelement and minerals [2,3].

Moreover, recent studies have reported that genetic factors play an important role in the pathogenesis of osteoporosis, such as collagen 1A1, endothelial nitric oxide synthases gene, estrogen metabolism-related genes and human leukocyte antigen-A [3-7].

Insulin-like growth factors (IGFs) are important regulators for bone cell function [8,9]. Many experimental studies have shown that IGF-I expression plays an important role in bone formation and bone loss [10,11].

The function of vitamin D is mediated by vitamin D receptor (VDR), and VDR plays an important role in binding 1,25-dihydroxyvitamin D3 to regulate the development of skeleton, and maintains bone structure [12]. The genes encoding for VDR are regarded to be a candidate gene for regulating bone strength and metabolism [13].

Up to now, several studies investigated the association between IGF-I and VDR genetic polymorphisms and development of osteoporosis, but the results are inconsistent [14-18]. Moreover, few studies investigated the association between IGF-I and VDR genetic polymorphisms and environmental factors in the risk of this disease [19].

Therefore, we evaluated the association of IGF-I (rs35767, rs2288377 and rs5742612) and VDR (rs7975232, rs2228570, rs1544410 and rs11568820) genetic polymorphisms with the risk of osteoporosis. We also assessed the effect of gene-environment interactions in the development of this disease.

Subjects and Methods

Subjects

A total of 320 patients with osteoporosis were recruited, between January 2015 and June 2016, the Second Clinical Medical College (Shenzhen People's Hospital) of Jinan University. The diagnosis of osteoporosis was according to the criteria from the World Health Organization [20]. Patients with intake of drugs disturbing the balance of bone metabolism, and with a history of any serious kidney or liver diseases were excluded from this study.

At the same time, 320 healthy subjects without osteoporosis, designated as controls, were recruited from the health examination center or outpatient clinics from the Second Clinical Medical College (Shenzhen People's Hospital). These controls were confirmed to be free of osteoporosis, have no history of serious kidney or liver diseases. The protocol of our study was approved by the ethics committee of the Second Clinical Medical College (Shenzhen People's Hospital) of Jinan University. The BMD was determined by dual-energy X-ray absorptiometry (Hologic®, Waltham, MA, USA). The L1-L4 vertebrae, femoral neck hip, total hip and trochanter were evaluated.

Information collection

The demographic and clinical information of participants was collected from medical records or face-to-face investigations, such as body mass index (BMI), tobacco smoking and alcohol drinking habits. The BMD levels in L1-L4 vertebrae, femoral neck, total hip and trochanter were collected from medical records.

Genotyping

Peripheral venous blood samples of 5 mL, collected from each participant after study enrollment, were stored in tubes with EDTA anticoagulant. Genomic DNA was extracted by the TIANamp DNA Blood Mini Kit (QIAGEN GmbH, Germany) following with the instruction. Three SNPs in IGF-I (rs35767, rs2288377 and rs5742612) and four SNPs in VDR (rs7975232, rs2228570, rs1544410 and rs11568820) were amplified and genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Primers of IGF-I (rs35767, rs2288377 and rs5742612) and VDR (rs7975232, rs2228570, rs1544410 and rs11568820) were designed using the Primer Premier 5.0 (PREMIER Biosoft Ltd., Palo Alto CA, USA). PCR reaction was performed with an initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 sec, 58°C for 30 sec, and 72°C for 30 sec; and then an extension of 72°C for 7 min. 8 µl PCR products were analyzed in 3% agarose gel electrophoresis and observed under ethidium bromide staining.

Statistical analysis

The results were analyzed by SPSS Statistics for Windows, Version 18.0. (SPSS Inc., Chicago, USA). Student's t-tests, Pearson's chi-square (χ^2) or Fisher's exact tests were taken to assess differences between groups. Whether the IGF-I and VDR genotype frequencies conformed to Hardy-Weinberg equilibrium (HWE) in controls was analyzed by the goodness-of-fit chi-square test. The association of IGF-I and VDR polymorphisms with the risk of osteoporosis was evaluated using the multiple logistic regression analysis, and the results were shown by the odds ratios (ORs) and 95% confidence intervals (CI). The association between IGF-I and VDR genetic polymorphisms and the risk of osteoporosis was assessed by dominant, co-dominant and recessive genetic models. The gene-environment interaction was performed by Spearman correlation analysis.

Results

In comparison with the controls, patients with osteoporosis were more likely to have a habit of tobacco smoking and alcohol drinking, and have a lower BMD values of L1-L4 vertebrae, femoral neck, total hip and trochanter ($P<0.001$; Table 1). The Chi-square test indicated significant differences in the genotype distributions of rs2288377 and rs5742612 between the patients with osteoporosis and controls ($P<0.001$, Table 2). However, no significant differences were found between the two groups in terms of the genotype distributions of rs35767, rs7975232, rs2228570, rs1544410 and rs11568820.

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

Variables	Patients N=320	%	Controls N=320	%	χ2 or t values	P values
Sex						
Females	229	71.56	229	71.56		
Males	91	28.44	91	28.44	0	1
Age, years		69.5 ± 9.5		70.1 ± 9.7	0.79	0.21
BMI, kg/m^2						
<24	153	47.81	165	51.56		
≥ 24	167	52.19	155	48.44	0.9	0.34
Tobacco smoking						
No	196	61.25	232	72.5		
Yes	124	38.75	88	27.5	9.14	0.002
Alcohol drinking						
No	187	58.44	226	70.63		
Yes	133	41.56	94	29.38	10.38	0.001
BMD, g/cm^2						
L1-L4 vertebrae	0.92 ± 0.092		0.97 ± 0.113		6.14	<0.001
Femoral neck	0.58 ± 0.029		0.66 ± 0.027		36.12	<0.001

Total hip	0.60 ± 0.036	0.65 ± 0.037	17.33	<0.001	Trochanter	0.53 ± 0.041	0.61 ± 0.042	24.38	<0.001

Table 2. *Genotype distributions of IGF-I and VDR genetic polymorphisms between the two investigated groups.*

Genotypes		Patients	%	Controls	%	χ^2 values	P values	HWE values	P values
IGF-I									
rs35767	CC	152	47.50	156	48.75	0.16	0.92	0.49	0.48
	TC	136	42.50	131	40.94				
	TT	32	10.00	33	10.31				
rs2288377	TT	125	39.06	157	49.06	12.14	0.002	0.50	0.48
	TA	145	45.31	138	43.13				
	AA	50	15.63	25	7.81				
rs5742612	TT	264	82.50	245	76.56	23.38	<0.001	0.20	0.66
	TG	33	10.31	69	21.56				
	GG	23	7.19	6	1.88				
VDR									
rs7975232	CC	139	43.44	143	44.69	0.43	0.81	0.51	0.47
	AC	145	45.31	146	45.63				
	AA	36	11.25	31	9.69				
rs2228570	CC	113	35.31	124	38.75	1.58	0.45	0.29	0.59
	CT	155	48.44	154	48.13				
	TT	52	16.25	42	13.13				
rs1544410	GG	279	87.19	282	88.13	0.69	0.71	0.51	0.47
	GA	37	11.56	36	11.25				
	AA	4	1.25	2	0.63				
rs11568820	GG	114	35.63	126	39.38	1.27	0.53	0.96	0.33
	GA	161	50.31	156	48.75				
	AA	45	14.06	38	11.88				

The multiple logistic regression analysis revealed that the AA genotype of rs2288377 was associated with an elevated risk of osteoporosis compared to the TT genotype, with an OR (95% CI) of 1.90 (1.08-3.39). In dominant model, the TA+AA genotype of rs2288377 had an increased risk of developing osteoporosis in comparison to the TT genotype (OR=1.50, 95% CI=1.08-2.08) (Table 3). In recessive model, the AA genotype of rs2288377 showed a higher risk of osteoporosis than the TA+TT genotype (OR=2.19, 95% CI=1.28-3.79). Moreover, we did not find a significantly association of the rs35767 and rs5742612, rs7975232, rs2228570, rs1544410 and rs11568820 polymorphisms with the risk of osteoporosis in co-dominant, dominant and recessive models. A gene-environment interaction analysis was carried out between the IGF-I rs2288377 polymorphism and smoking, drinking and BMD values in the risk of osteoporosis (Table 4). However, we did not observe any interaction between them (All P value>0.05).

Table 3. *Association between IGF-I and VDR genetic polymorphisms and risk of osteoporosis by multiple logistic regression analysis.*

Genotypes		Patients	%	Controls	%	OR (95% CI)*	P values
IGF-I							
rs35767	CC	152	47.5	156	48.75	1.0	-

Co-dominant	TC	136	42.5	131	40.94	1.07 (0.76-1.50)	0.70
	TT	32	10	33	10.31	1.00 (0.56-1.76)	0.99
Dominant	CC	152	47.5	156	48.75	1.0	-
	TC+TT	168	52.5	164	51.25	1.05 (0.76-1.45)	0.75
Recessive	TC+CC	288	90	287	89.69	1.0	-
	TT	32	10	33	10.31	0.97 (0.56-1.67)	0.89
rs2288377	TT	125	39.06	157	49.06	1.0	-
Co-dominant	TA	145	45.31	138	43.13	1.32 (0.94-1.86)	0.10
	AA	50	15.63	25	7.81	1.90 (1.08-3.39)	0.02
Dominant	TT	125	39.06	157	49.06	1.0	-
	TA+AA	195	60.94	163	50.94	1.50 (1.08-2.08)	0.01
Recessive	TA+TT	270	84.37	295	92.19	1.0	-
	AA	50	15.63	25	7.81	2.19 (1.28-3.79)	0.002
rs5742612	TT	264	82.5	275	85.94	1.0	-
Co-dominant	TG	33	10.31	29	9.06	1.19 (0.68-2.08)	0.53
	GG	23	7.19	16	5.00	1.50 (0.74-3.10)	0.23
Dominant	TT	264	82.5	275	85.94	1.0	-
	TG+GG	56	17.5	45	14.06	1.30 (0.83-2.04)	0.23
Recessive	TC+TT	297	92.81	304	95.00	1.0	-
	GG	23	7.19	16	5.00	1.47 (0.73-3.04)	0.25
VDR							
rs7975232	AA	139	43.44	143	44.69	1.0	-
Co-dominant	AC	145	45.31	146	45.63	1.02 (0.73-1.44)	0.89
	CC	36	11.25	31	9.69	1.19 (0.68-2.12)	0.51
Dominant	AA	139	43.44	143	44.69	1.0	-
	AC+CC	181	56.56	177	55.32	1.05 (0.76-1.46)	0.75
Recessive	AC+AA	284	88.75	289	90.32	1.0	-
	CC	36	11.25	31	9.69	1.18 (0.69-2.03)	0.52
rs2228570	CC	113	35.31	124	38.75	1.0	-
Co-dominant	CT	155	48.44	154	48.13	1.10 (0.78-1.57)	0.56
	TT	52	16.25	42	13.13	1.23 (0.75-2.01)	0.38
Dominant	CC	113	35.31	124	38.75	1.0	-
	CT+TT	207	64.69	196	61.26	1.16 (0.83-1.62)	0.37
Recessive	CT+CC	268	83.75	278	86.88	1.0	-
	TT	52	16.25	42	13.13	1.28 (0.81-2.05)	0.26
rs1544410	AA	279	87.19	282	88.13	1.0	-
Co-dominant	AG	37	11.56	36	11.25	1.04 (0.62-1.74)	0.88
	GG	4	1.25	2	0.63	2.02 (0.29-22.49)	0.41

Dominant	AA	279	87.19	282	88.13	1.0	-
	AG+GG	41	12.81	38	11.88	1.09 (0.66-1.79)	0.72
Recessive	AG+AA	316	98.75	318	99.38	1.0	-
	GG	4	1.25	2	0.63	2.01 (0.29-22.37)	0.41
rs11568820	AA	114	35.63	126	39.38	1.0	-
Co-dominant	AG	161	50.31	156	48.75	1.14 (0.80-1.62)	0.44
	GG	45	14.06	38	11.88	1.15 (0.69-1.92)	0.58
Dominant	AA	114	35.63	126	39.38	1.0	-
	AG+GG	206	64.37	194	60.63	1.17 (0.84-1.64)	0.33
Recessive	AG+AA	275	85.94	282	88.13	1.0	-
	GG	45	14.06	38	11.88	1.21 (0.75-1.99)	0.41

*Adjusted for tobacco smoking, alcohol drinking and BMD values.

Discussion

The candidate gene approach is increasingly adopted to pinpoint genes related to disease susceptibility that may trigger the onset and progression of various conditions. We reported that the IGF-I rs2288377 polymorphism was significantly associated with an increased risk of osteoporosis in co-dominant, dominant and recessive genetic models. *IGF-I* gene is located at chromosome 12q22-q24.1 with a length of 83kb, including six introns [21]. IGFs play an important role in human's physiological activities, and they contribute to the metabolism of carbohydrates, fats and proteins, and regulation of cell growth, differentiation and apoptosis [21]. IGF-I contribute to the proliferation and differentiation of osteoclasts, and promote the synthesis and mineralization of bone matrix. Finally, IGF-I could regulate the bone cell function and influence bone metabolism in the form of autocrine and paracrine [22,23].

Table 4. *Interaction between the rs2288377 and smoking, drinking and BMD values in the risk of osteoporosis.*

Variables	rs2288377	
	r	P values
Tobacco smoking	0.045	0.19
Alcohol drinking	0.061	0.10
BMD for L1-L4 vertebrae	0.042	0.24
BMD for femoral neck	0.039	0.39
BMD for total hip	0.037	0.45
BMD for trochanter	0.040	0.31

Polymorphisms in IGF-I could affect the expression of IGF-I, and influence the risk of bone mineral density. We also observed an association between IGF-I rs2288377 polymorphism and risk of osteoporosis in the Chinese population. Previous studies have reported the correlation between IGF-I polymorphisms and risk of osteoporosis, but the results of these studies are inconsistent [18,24-28]. Zhang et al. performed a study in a Chinese population, and they found that individuals carrying the TT genotype of IGF-I rs35767 had an increased risk of osteoporosis [18]. Yun-Kai al. suggested that T allele of rs35767 was significantly associated with BMD and osteoporosis in postmenopausal female population [28]. However, Jiang et al. performed a study with 1263 subjects in a Chinese population, but they did not find significant evidence of association of the *IGF-I* gene and BMD variation at any skeletal site in a Chinese population [25]. Inconsistent results of these studies may be attributed to the differences in population, study design, sample size and random by chance.

One limitation of the present study should be considered. First, the included patients with osteoporosis were recruited from only one hospital, and this sample may therefore not adequately represent other populations. Second, the sample size is relatively small in this study, which can cause low statistical power to find differences between groups.

Conclusions

Our study suggests a significant association between the IGF-I rs2288377 polymorphism and the risk of osteoporosis in this Chinese population. However, owing to the limitations of the present study, further studies with larger sample size and more populations need to be conducted.

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