IL-1R2 Polymorphisms and its Interaction Associates With Osteoporosis Susceptibility in Chinese Han Population

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Research article

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Abstract

Background: IL-1R2, serves as a negative regulator of IL-1 signaling, is involved in the pathogenesis of osteoporosis. This study aimed to determine the correlation between *IL-1R2* polymorphism and osteoporosis susceptibility among the Chinese Han population.

Methods: We recruited 594 osteoporosis patients and 599 healthy controls. Six single nucleotide polymorphisms (SNPs) in *IL-1R2* were selected for genotyping using Agena MassARRAY platform. Odds ratio (OR) and 95% confidence interval (CI) was calculated through logistic regression analysis with adjustment for age and sex. Linkage disequilibrium analysis was plotted by Haploview v4.2. Multifactor dimension reduction (MDR) was performed to estimate the SNP-SNP interaction of *IL-1R2* variants.

Results: Our result revealed that rs11674595 (OR = 1.86, $p = 0.020$), rs2072472 (OR = 1.26, $p = 0.019$) and rs4851527 (OR = 0.78, $p = 0.007$) were related to the risk of osteoporosis. Moreover, the contribution of *IL-1R2* polymorphisms to osteoporosis risk presented age, sex and BMI difference. We found the relationship of $T_{rs11674595}A_{rs4851527}$ (OR = 0.80, $p = 0.015$), $C_{rs11674595}G_{rs4851527}$ (OR = 1.22, $p = 0.043$) and $A_{rs3218977}G_{rs2072472}$ (OR = 1.25, $p = 0.022$) haplotypes to osteoporosis occurrence, and a potential accumulated effect of *IL-1R2* SNPs (testing accuracy = 0.5783 and CVC = 10/10) on osteoporosis susceptibility.

Conclusion: *IL-1R2* polymorphisms (rs11674595, rs4851527, rs2072472 and rs3218977) might contribute to osteoporosis risk among the Chinese Han population. Our finding may increase our understanding of the effects of *IL-1R2* polymorphisms on the predisposition of osteoporosis.

Introduction

Osteoporosis is a common metabolic bone disease, which characterized by the progressive bone mass loss and the degeneration of bone microarchitecture [1]. Osteoporosis is caused by the reduction of osteoblastic proliferation and the abnormal activation of osteoclasts, consequently decreasing bone strength and increasing fracture risk [2]. Worldwide, about 200 million people suffer from osteoporosis [3]. The prevalence of osteoporosis in Asian and European populations is higher than in African populations. Among Chinese populations aged above 50 years old, the standardized prevalence of osteoporosis ranged from 5.04–7.46% in males and from 26.28–39.19% in females from 1990 to 2050 [4]. The occurrence of osteoporosis increases with the aging of a population annually, which poses a serious global health burden and reduces quality of life. Osteoporosis is a complex multifactorial disease influenced by genetic and environmental factors. To date, multiple risk factors affecting osteoporosis have been identified, including gender, age, ethnicity, lack of physical exercise, and low body mass [5]. Some studies estimated that genetic factors can account for 60–85% of the risk factors for osteoporosis occurrence [6]. Although various genetic variants have been reported to be related to the risk of osteoporosis [7, 8], a number of variants remain to be uncovered.

The immune system has an important role in bone pathology, such as osteoporosis. Osteoporosis is induced by abnormal osteoclast formation and activity, and cytokines are potentially implicated in its pathogenesis [9, 10]. Interleukins (ILs), a group of cytokines, are reported to be related to the development of osteoporosis, which is characterized by an imbalance between bone formation and bone resorption [11, 12]. IL-1 can upregulate the differentiation and activation of osteoclasts and motivate the release of degradative products, thereby increasing bone resorption [13]. Moreover, IL-1 induces the occurrence and development of osteoporosis by interacting synergistically with other cytokines, including, IL-6, IL-31, and TNF [14]. IL-1 inhibitory receptor type 2 (IL-1R2), belongs to IL-1 family members, can inhibit the activity of the ligands by binding IL-1 α and IL-1 β . The expression level of IL-1R2 is suppressed in pathologically activated osteoclasts, performing severe bone destruction [15]. Several studies have tested the relationship between IL-1 family gene polymorphisms (such as *IL-1A*, and *IL-1B*) and the risk of osteoporosis occurrence [16, 17]. However, the correlation of *IL-1R2* variants with osteoporosis susceptibility was not reported.

Here, six single nucleotide polymorphisms (SNPs) including rs11674595, rs4851527, rs719250, rs3218896, rs3218977, and rs2072472 in *IL-1R2* were selected as the candidate SNPs and were detected using Agena MassARRAY platform to explore the effect of *IL-1R2* genetic variants, haplotypes and SNP-SNP interactions on osteoporosis susceptibility. The study is designed to improve understanding of the contribution of *IL-1R2* variants to the risk of osteoporosis among the Chinese Han population.

Materials And Methods

Study participants

In the present study, we recruited 1193 subjects from the Xi'an 630 hospital, including 594 osteoporosis patients and 599 healthy controls. All participants were unrelated genetically Chinese Han population. Patients with osteoporosis was diagnosed and confirmed by bone mineral density (BMD) T score of the lumbar spine (L1–L4) and hip measuring by dual-energy X-ray absorptiometry, according to the WHO diagnosis criteria for osteoporosis (T score ≤ -2.5) [18]. The exclusion criteria were the following: 1) taken antiosteoporosis drugs; 2) suffered from osteoarthritis or other diseases influencing bone metabolism, and had determined history of hip fracture; and 3) with dysfunction of major organs, such as renal or hepatic disease, and hyper- or hypothyroidism. The controls were age-, gender- and frequency-matched healthy subjects from the physical examination center of same hospital, who were free from any history of hip fracture, no any acute or chronic inflammatory disease, and no other metabolic disorders that can affect bone metabolism. The basic characteristics of subjects were recorded by self-administered questionnaires or medical record. All subjects signed a written consent before participating in the study. The research protocol was in accordance with the Research and Ethical Committee of the Xi'an 630 hospital and with the 1964 Helsinki Declaration.

Candidate gene selection and genotyping

Six candidate SNPs (rs11674595, rs4851527, rs719250, rs3218896, rs3218977 and rs2072472) located in *IL-1R2* as candidate polymorphisms were selected by the 1000 Genomes Project data (<http://www.internationalgenome.org/>) and the NCBI dbSNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP>) based on minor allele frequency (MAF) > 5% and r^2 of ≥ 0.8 .

About 2-mL peripheral venous blood was obtained from each subject in the tube with disodium-EDTA anticoagulant. gDNA was obtained from a blood sample of each participant using the commercial DNA isolation kit (GoldMag Co. Ltd, Xi'an, China), and was stored at -20°C. Agena MassARRAY system (Agena, San Diego, CA, USA) was used to conduct the genotype analysis in double-blind fashion [19–21]. Primers for PCR amplification and single base extension were synthesized in Invitrogen, Shanghai, and the detailed sequences was shown in Table S1. The results were sequenced using Agena MassARRAY Analyzer 4.0 software. About 1/10 samples were randomly re-assayed to guarantee the validity of the genotyping results, and the consistent rate was 100%.

Statistical analysis

The variables comparison between osteoporosis patients and healthy controls was performed using chi-square for categorical variables described as mean $\pm$ standard deviation ($M \pm SD$), and student's t-test for the continuous variables as percentages (%). Hardy–Weinberg equilibrium for the genotype distribution of *IL-1R2* polymorphisms in the control group was examined by χ^2 test. The genotype and allele frequencies of polymorphisms in *IL-1R2* were compared χ^2 tests. Odds ratio (OR) and 95% confidence interval (CI) were used to estimate the relationship between the susceptibility of osteoporosis and *IL-1R2* variants through logistic regression analysis with adjustment for age and sex. Linkage disequilibrium analysis was plotted by Haploview v4.2 software, and the correlation of haplotypes with the occurrence of osteoporosis was conducted using logistic regression analysis. Multifactor dimension reduction (MDR) was performed to estimate the optimal interaction of *IL-1R2* variants on osteoporosis risk. SPSS v18.0 software (IBM, Chicago, IL, USA) and PLINK software were used for statistical analysis, and two-tailed p values < 0.05 was considered as statistical significance.

Results

The characteristics of subjects

A total of 594 osteoporosis cases (58.09 ± 13.22 years) and 599 health controls (57.59 ± 13.11 years) were included. The characteristics of osteoporosis patients and controls were presented in Table 1. The distribution of age and sex between cases and controls was not significantly different ($p = 0.513$ and $p = 0.931$, respectively), but total protein, total bilirubin, serum uric acid, urea, fasting blood glucose, hemoglobin, total leukocyte count (TLC) and platelets were significantly different in both groups ($p < 0.05$).

Table 1
Characteristics of patients with osteoporosis and health controls

Characteristic	Cases (n = 594)	Controls (n = 599)	p
Age, year (mean ± SD)	58.09 ± 13.22	57.59 ± 13.11	0.513
Gender (Male/ Female)	298/296	299/300	0.931
BMI, kg/m ² (< 24/≥ 24)	404/190	187/230	
Total protein (g/L)	70.95 ± 5.97	72.04 ± 3.98	0.013
Total bilirubin (mmol/L)	12.02 ± 4.61	16.37 ± 7.43	< 0.001
Serum uric acid (mmol/L)	260.79 ± 82.36	321.06 ± 87.41	< 0.001
Urea (mmol/L)	5.67 ± 1.76	5.25 ± 1.23	0.002
Creatinine (mmol/L)	63.99 ± 15.26	65.1 ± 13.16	0.390
Fasting blood glucose (mmol/L)	4.88 ± 1.34	5.92 ± 1.07	< 0.001
Hemoglobin (g/L)	120.48 ± 17.17	143.36 ± 15.31	< 0.001
TLC (10 ⁹ /L)	7.27 ± 2.46	5.74 ± 1.42	< 0.001
Platelets (10 ⁹ /L)	258.2 ± 108.19	216.8 ± 62.23	< 0.001
ALP (U/L)	107.36 ± 35.98		
T score			
Total hip	-3.12 ± 0.51		
Lumbar spine L1	-3.05 ± 0.68		
Lumbar spine L2	-3.12 ± 0.67		
Lumbar spine L3	-3.04 ± 0.68		
Lumbar spine L4	-3.22 ± 0.76		
BMI, body mass index; TLC, total leukocyte count; ALP, alkaline phosphatase.			
p values were calculated by χ^2 test or the Student's t test.			
Bold indicate that $p < 0.05$ indicates statistical significance.			

Association analyses of IL-1R2 SNPs with osteoporosis susceptibility

The detail of candidate SNPs in *IL-1R2* was presented in Table S2. The genotype distribution of *IL-1R2* polymorphisms among controls was in Hardy-Weinberg equilibrium ($p > 0.05$). The MAF for each SNP was more than 0.05 in both groups, and the call rate of genotyping was > 99.0%.

The frequencies of rs11674595-C allele and rs2072472-G allele were higher in osteoporosis patients compared to controls, while rs4851527-A allele was lower (Table 2). In the analysis of the genetic model, rs11674595, rs4851527 and rs2072472 were related to osteoporosis risk. Rs11674595 and rs2072472 were risk factors for osteoporosis occurrence: for rs11674595 under the allele (OR = 1.23, 95% CI: 1.01–1.50, $p = 0.043$), homozygote (OR = 1.86, 95% CI: 1.10–3.15, $p = 0.020$), recessive (OR = 1.81, 95% CI: 1.08–3.03, $p = 0.025$) and additive (OR = 1.22, 95% CI: 1.00–1.48, $p = 0.048$) models; and for rs2072472 in the allele (OR = 1.26, 95% CI: 1.04–1.54, $p = 0.019$), dominant (OR = 1.27, 95% CI: 1.01–1.61, $p = 0.043$), and additive (OR = 1.26, 95% CI: 1.04–1.52, $p = 0.021$) models. While, we found that rs4851527 was related to the lower risk of osteoporosis under the allele (OR = 0.78, 95% CI: 0.66–0.94, $p = 0.007$), heterozygote (OR = 0.67, 95% CI: 0.53–0.85, $p = 0.001$), dominant (OR = 0.69, 95% CI: 0.55–0.87, $p = 0.001$), and additive (OR = 0.79, 95% CI: 0.66–0.94, $p = 0.008$) models.

Table 2
The association between *IL-1R2* variants and the occurrence of **osteoporosis**

SNP ID	Model	Genotype	Case	Control	Adjusted by age and gender	
					OR (95%CI)	p
rs11674595	Allele	T	907	963	1	
		C	267	231	1.23 (1.01–1.50)	0.043
	Genotype	TT	361	390	1	
		TC	185	183	1.09 (0.85–1.40)	0.484
		CC	41	24	1.86 (1.10–3.15)	0.020
	Dominant	TT	361	390	1	
		TC-CC	226	207	1.18 (0.93–1.50)	0.169
	Recessive	TT-TC	546	573	1	
		CC	41	24	1.81 (1.08–3.03)	0.025
	Log-additive				1.22 (1.00–1.48)	0.048
rs4851527	Allele	G	877	825	1	
		A	311	373	0.78 (0.66–0.94)	0.007
	Genotype	GG	331	278	1	
		GA	215	269	0.67 (0.53–0.85)	0.001
		AA	48	52	0.78 (0.51–1.19)	0.254
	Dominant	GG	331	278	1	
		GA-AA	263	321	0.69 (0.55–0.87)	0.001
	Recessive	GG-GA	546	547	1	
		AA	48	52	0.93 (0.62–1.41)	0.735
	Log-additive				0.79 (0.66–0.94)	0.008
rs2072472	Allele	A	907	962	1	
		G	281	236	1.26 (1.04–1.54)	0.019
	Genotype	AA	351	388	1	
		AG	205	186	1.22 (0.95–1.56)	0.115
		GG	38	25	1.69 (1.00–2.85)	0.051
	Dominant	AA	351	388	1	
		AG-GG	243	211	1.27 (1.01–1.61)	0.043
	Recessive	AA-AG	556	574	1	
		GG	38	25	1.57 (0.94–2.64)	0.086
	Log-additive				1.26 (1.04–1.52)	0.021
SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.						
<i>p</i> values were calculated by logistic regression analysis with adjustments for age and gender.						
Bold indicate that <i>p</i> < 0.05 respects the data is statistically significant.						

Stratification analysis for the correlation between *IL-1R2* variants and osteoporosis risk

Stratification analysis by age, sex and BMI was performed to explore the correlation between *IL-1R2* variants and osteoporosis risk (Table 3). In the subjects with age > 60 years, rs4851527 was a protective factor for osteoporosis susceptibility (A vs G: OR = 0.68, *p* = 0.002; GA vs GG: OR = 0.61, *p* = 0.003; GA-AA vs GG: OR = 0.60, *p* = 0.001; and additive: OR = 0.68, *p* = 0.002). Rs11674595-CC genotype was associated with the elevated risk of osteoporosis occurrence compared with TT genotype (OR = 2.68, *p* = 0.033) and TT-TC genotype (OR = 2.68, *p* = 0.031) at age ≤ 60 years. In addition, we also found that rs3218977 had a reduced risk of osteoporosis (AG vs AA: OR = 0.66, *p* = 0.023; and AG-GG vs AA: OR = 0.69, *p* = 0.036).

Table 3
Stratification by age, gender and BMI for the association between *IL-1R2* variants and the occurrence of **osteoporosis**

SNP ID	Genotype	Age stratification				Gender stratification				BMI stratification			
		> 60 years		≤ 60 years		Males		Females		> 24 kg/m ²		≤ 24 kg/m ²	
		OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
rs11674595	T	1		1		1		1		1		1	
	C	1.21 (0.93–1.57)	0.158	1.26 (0.93–1.7)	0.143	1.01 (0.75–1.35)	0.971	1.46 (1.11–1.91)	0.006	0.96 (0.68–1.36)	0.837	1.41 (1.03–1.91)	0.029
	TT	1		1		1		1		1		1	
	TC	1.19 (0.85–1.67)	0.307	0.99 (0.68–1.45)	0.976	0.92 (0.64–1.32)	0.657	1.28 (0.90–1.81)	0.171	1.25 (0.81–1.91)	0.309	1.13 (0.76–1.66)	0.555
	CC	1.52 (0.79–2.93)	0.214	2.68 (1.09–6.61)	0.033	1.28 (0.57–2.88)	0.558	2.51 (1.25–5.03)	0.010	0.23 (0.06–0.87)	0.030	2.38 (1.07–5.27)	0.033
	TC-CC vs TT	1.24 (0.90–1.70)	0.188	1.13 (0.79–1.61)	0.515	0.96 (0.68–1.35)	0.818	1.43 (1.02–1.98)	0.036	1.08 (0.71–1.63)	0.724	1.29 (0.89–1.86)	0.178
	CC vs TT-TC	1.43 (0.75–2.73)	0.280	2.68 (1.10–6.57)	0.031	1.31 (0.58–2.93)	0.515	2.30 (1.16–4.56)	0.017	0.21 (0.06–0.80)	0.022	2.29 (1.04–5.03)	0.039
	Additive	1.21 (0.94–1.56)	0.139	1.23 (0.91–1.66)	0.176	1.01 (0.76–1.34)	0.967	1.43 (1.10–1.86)	0.009	0.91 (0.64–1.30)	0.601	1.33 (0.99–1.77)	0.056
rs4851527	G	1		1		1		1		1		1	
	A	0.68 (0.54–0.87)	0.002	0.93 (0.71–1.21)	0.571	1.01 (0.78–1.29)	0.970	0.61 (0.47–0.78)	1.16·10⁻⁴	0.77 (0.57–1.04)	0.089	0.86 (0.66–1.14)	0.298
	GG	1		1		1		1		1		1	
	GA	0.61 (0.44–0.85)	0.003	0.76 (0.53–1.10)	0.144	0.98 (0.70–1.38)	0.923	0.46 (0.33–0.65)	8.96·10⁻⁶	0.57 (0.37–0.86)	0.008	0.97 (0.67–1.40)	0.867
	AA	0.56 (0.31–1.00)	0.051	1.15 (0.61–2.16)	0.660	1.04 (0.59–1.85)	0.892	0.57 (0.30–1.08)	0.084	0.91 (0.45–1.82)	0.786	0.67 (0.36–1.26)	0.216
	GA-AA vs GG	0.60 (0.44–0.82)	0.001	0.82 (0.59–1.16)	0.261	0.99 (0.72–1.37)	0.970	0.48 (0.34–0.66)	8.67·10⁻⁶	0.62 (0.42–0.92)	0.019	0.90 (0.64–1.28)	0.575
	AA vs GG-GA	0.69 (0.39–1.22)	0.202	1.29 (0.71–2.38)	0.405	1.05 (0.60–1.83)	0.867	0.81 (0.44–1.49)	0.495	1.17 (0.60–2.28)	0.655	0.68 (0.37–1.25)	0.217
	Additive	0.68 (0.54–0.87)	0.002	0.93 (0.72–1.22)	0.613	1.01 (0.79–1.29)	0.964	0.60 (0.46–0.78)	1.47·10⁻⁴	0.78 (0.58–1.06)	0.111	0.88 (0.67–1.15)	0.335
rs3218977	A	1		1		1		1		1		1	
	G	0.99 (0.78–1.27)	0.953	0.80 (0.60–1.05)	0.106	0.98 (0.76–1.27)	0.895	0.82 (0.63–1.07)	0.141	1.09 (0.80–1.48)	0.591	0.76 (0.58–1.01)	0.054
	AA	1		1		1		1		1		1	
	AG	1.26 (0.91–1.75)	0.169	0.66 (0.46–0.94)	0.023	0.86 (0.61–1.21)	0.377	1.02 (0.73–1.43)	0.917	1.20 (0.79–1.82)	0.387	0.86 (0.59–1.26)	0.439

SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

p values were calculated by logistic regression analysis with adjustments for age and gender.

Bold indicate that *p* < 0.05 respects the data is statistically significant.

	GG	0.66 (0.36– 1.20)	0.171	0.92 (0.46– 1.86)	0.819	1.17 (0.64– 2.11)	0.613	0.38 (0.18– 0.82)	0.013	1.08 (0.49– 2.37)	0.850	0.53 (0.28– 1.00)	0.048
	AG-GG vs AA	1.12 (0.83– 1.53)	0.460	0.69 (0.49– 0.98)	0.036	0.91 (0.66– 1.26)	0.564	0.90 (0.65– 1.25)	0.524	1.18 (0.80– 1.75)	0.410	0.78 (0.55– 1.11)	0.175
	GG vs AA-AG	0.60 (0.33– 1.08)	0.090	1.09 (0.55– 2.17)	0.807	1.24 (0.69– 2.20)	0.471	0.38 (0.18– 0.80)	0.011	1.00 (0.46– 2.15)	0.997	0.56 (0.30– 1.04)	0.064
	Additive	0.98 (0.77– 1.25)	0.889	0.80 (0.61– 1.05)	0.112	0.98 (0.77– 1.26)	0.896	0.81 (0.62– 1.06)	0.124	1.11 (0.81– 1.52)	0.512	0.78 (0.59– 1.02)	0.067
rs2072472	A	1		1		1		1		1		1	
	G	1.25 (0.97– 1.62)	0.091	1.28 (0.95– 1.73)	0.102	1.08 (0.81– 1.44)	0.593	1.46 (1.11– 1.91)	0.006	1.10 (0.79– 1.54)	0.574	1.33 (0.98– 1.79)	0.066
	AA	1		1		1		1		1		1	
	AG	1.29 (0.92– 1.79)	0.135	1.14 (0.79– 1.65)	0.491	0.98 (0.69– 1.40)	0.921	1.48 (1.05– 2.08)	0.027	1.21 (0.79– 1.86)	0.389	1.24 (0.85– 1.81)	0.261
	GG	1.46 (0.76– 2.79)	0.253	2.38 (0.94– 6.02)	0.066	1.44 (0.67– 3.10)	0.349	1.92 (0.93– 3.97)	0.077	0.79 (0.32– 1.95)	0.605	1.84 (0.81– 4.15)	0.144
	AG-GG vs AA	1.31 (0.96– 1.80)	0.089	1.23 (0.87– 1.76)	0.245	1.04 (0.74– 1.45)	0.840	1.53 (1.10– 2.13)	0.011	1.14 (0.75– 1.71)	0.539	1.31 (0.92– 1.88)	0.139
	GG vs AA-AG	1.33 (0.70– 2.52)	0.376	2.29 (0.91– 5.73)	0.078	1.45 (0.68– 3.09)	0.336	1.66 (0.81– 3.39)	0.163	0.74 (0.30– 1.81)	0.508	1.70 (0.76– 3.80)	0.196
	Additive	1.25 (0.97– 1.60)	0.087	1.28 (0.95– 1.73)	0.107	1.08 (0.82– 1.42)	0.600	1.43 (1.09– 1.88)	0.009	1.04 (0.75– 1.46)	0.803	1.29 (0.96– 1.74)	0.089
SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.													
<i>p</i> values were calculated by logistic regression analysis with adjustments for age and gender.													
Bold indicate that <i>p</i> < 0.05 respects the data is statistically significant.													

Stratified by sex, we found that rs11674595 and rs2072472 were associated with a higher occurrence of osteoporosis, while rs4851527 and rs3218977 were with a lower osteoporosis risk in female. For rs11674595, the allele (OR = 1.46, *p* = 0.006), homozygote (OR = 2.51, *p* = 0.010), dominant (OR = 1.43, *p* = 0.036), recessive (OR = 2.30, *p* = 0.017) and additive (OR = 1.43, *p* = 0.009) models were found. For rs2072472, the allele (OR = 1.46, *p* = 0.006), heterozygote (OR = 1.48, *p* = 0.027), dominant (OR = 1.53, *p* = 0.011) and additive (OR = 1.43, *p* = 0.009) models were observed. For rs4851527, the allele (OR = 0.61, *p* = 1.16·10⁻⁴), heterozygote (OR = 0.46, *p* = 8.96·10⁻⁶), dominant (OR = 0.48, *p* = 8.67·10⁻⁶) and additive (OR = 0.60, *p* = 1.47·10⁻⁴) models were found. And for rs3218977, homozygote (OR = 0.38, *p* = 0.013) and recessive (OR = 0.38, *p* = 0.011) models were observed.

The result of stratification analysis based on BMI displayed that rs11674595 (CC vs TT: OR = 0.23, *p* = 0.030; and CC vs TT-TC: OR = 0.21, *p* = 0.022) and rs4851527 (GA vs GG: OR = 0.57, *p* = 0.008; and GA-AA vs GG: OR = 0.62, *p* = 0.019) were related to the reduced occurrence of osteoporosis among the subjects with BMI > 24 kg/m². In the subgroup of ≤ 24 kg/m², we found that the C allele (C vs T: OR = 1.41, *p* = 0.029) and CC genotype (CC vs TT: OR = 2.38, *p* = 0.033; and CC vs TT-TC: OR = 2.29, *p* = 0.039) of rs11674595 conferred to the increased risk of osteoporosis; whereas GG genotype of rs3218977 (OR = 0.53, *p* = 0.048) might be a marginal protective factor for osteoporosis susceptibility compared to AA genotype.

Association of IL-1R2 haplotype with osteoporosis risk

Three pairs of SNPs (rs11674595 and rs4851527, rs719250 and rs3218896, rs3218977 and rs2072472) with the strong linkage disequilibrium (*D'* > 0.9) were analyzed by Haploview v4.2 software (Fig. 1). The results of association analyses of *IL-1R2* haplotype with osteoporosis risk showed that the frequencies of T_{rs11674595}A_{rs4851527}, C_{rs11674595}G_{rs4851527} and A_{rs3218977}G_{rs2072472} haplotypes were significant difference in the case and control groups (*p* = 0.015, *p* = 0.043, and *p* = 0.022, respectively), as shown in Table 4. We also found that T_{rs11674595}A_{rs4851527} (OR = 0.80, 95% CI: 0.67–0.96, *p* = 0.015) haplotype reduced the occurrence of osteoporosis, while C_{rs11674595}G_{rs4851527} (OR = 1.22, 95% CI: 1.01–1.48, *p* = 0.043) and A_{rs3218977}G_{rs2072472} (OR = 1.25, 95% CI: 1.03–1.52, *p* = 0.022) haplotypes had a high risk of osteoporosis.

Table 4
Haplotype frequencies of *IL-1R2* gene and their association with osteoporosis occurrence

Block	SNP	Haplotype	Frequency		χ^2	p^a	Adjusted by age and gender	
			Case	Control			OR (95% CI)	p^b
Block 1	rs11674595 rs4851527	TA	0.265	0.311	6.059	0.014	0.80 (0.67–0.96)	0.015
	rs11674595 rs4851527	CG	0.227	0.193	4.322	0.038	1.22 (1.01–1.48)	0.043
	rs11674595 rs4851527	TG	0.492	0.504	0.333	0.564	0.96 (0.82–1.12)	0.602
Block 2	rs719250 rs3218896	TC	0.153	0.136	1.423	0.233	1.15 (0.91–1.45)	0.234
	rs719250 rs3218896	TT	0.154	0.171	1.158	0.282	0.88 (0.70–1.10)	0.272
	rs719250 rs3218896	CT	0.308	0.308	$1.18 \cdot 10^{-5}$	0.997	1.00 (0.84–1.20)	1.000
Block 3	rs3218977 rs2072472	AG	0.237	0.197	5.395	0.020	1.25 (1.03–1.52)	0.022
	rs3218977 rs2072472	GA	0.246	0.266	1.264	0.261	0.90 (0.75–1.08)	0.261
	rs3218977 rs2072472	AA	0.482	0.463	0.873	0.350	1.08 (0.92–1.27)	0.350
SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.								
p values were calculated by logistic regression analysis with adjustments for age and gender.								
Bold indicate that $p < 0.05$ respects the data is statistically significant.								

MDR analysis for association of IL-1R2 SNPs with osteoporosis risk

Further, MDR method was performed to investigate the high-order interactions of six SNPs in osteoporosis occurrence (Table 5 and Fig. 2). As was shown in Fig. 1, the dendrogram plot described the interactions between these SNPs. MDR analyses found that rs4851527 was the most influential attributor for osteoporosis risk in the single-locus model. Moreover, the three-locus interaction model (testing accuracy = 0.5505 and CVC = 9/10) and six-locus interaction (testing accuracy = 0.5783 and CVC = 10/10) were found to be better in the multilocus model, indicating a potential accumulated effect of *IL-1R2* SNPs contributed to osteoporosis susceptibility.

Table 5
MDR analysis for SNP–SNP interaction of the *IL-1R2* genes with osteoporosis susceptibility

Locus number	Best combination	Training Bal. Acc.	Testing Bal. Acc.	CVC	p
1	rs4851527	0.5474	0.5345	10/10	0.0012
2	rs4851527, rs3218977	0.5609	0.5202	6/10	< 0.0001
3	rs4851527, rs719250, rs3218977	0.5791	0.5505	9/10	< 0.0001
4	rs4851527, rs719250, rs3218896, rs3218977	0.5902	0.5488	10/10	< 0.0001
5	rs11674595, rs4851527, rs719250, rs3218977, rs2072472	0.6008	0.5429	6/10	< 0.0001
6	rs11674595, rs4851527, rs719250, rs3218896, rs3218977, rs2072472	0.6128	0.5783	10/10	< 0.0001
MDR, multifactor dimensionality reduction; Bal. Acc., balanced accuracy; CVC, cross-validation consistency; OR, odds ratio; CI, confidence interval.					
p values were calculated using χ^2 tests for whole dataset statistics.					
Bold indicate that $p < 0.05$ indicates statistical significance.					

Discussion

In this study, we performed a case-control study including 594 osteoporosis patients and 599 healthy controls to understand the genetic correlation between *IL-1R2* polymorphisms and osteoporosis susceptibility. Our result revealed that rs11674595, rs2072472 and rs4851527 were related to the risk of osteoporosis. Interestingly, the effect of *IL-1R2* variants on osteoporosis susceptibility presented age, sex and BMI difference. We also found that $T_{rs11674595}A_{rs4851527}$, $C_{rs11674595}G_{rs4851527}$ and $A_{rs3218977}G_{rs2072472}$ haplotypes were related to the risk of osteoporosis. SNP-SNP interaction analysis indicated a potential accumulated effect of *IL-1R2* SNPs on osteoporosis susceptibility. As far as know this is the first study to investigate the relationship between *IL-1R2* variants and osteoporosis risk occurrence in a Chinese Han population.

IL-1, as “osteoclast activating factor,” can regulate osteoclastogenesis by increasing osteoclast viability and resorptive capacity and enhancing RANKL-induced multinucleation of osteoclast precursors [22]. IL-1 receptors expressed on osteoclasts can activate RANKL-RANK signaling pathway, resulting in the further activation of NF- κ B and induction of marked osteoclast specific genes [23]. These studies provided an explanation for the role of IL-1 on the pathogenesis of osteoporosis. IL-1R2, as the suppressive receptor, has a higher affinity for IL-1 β , which could be related to the proliferation and

differentiation of osteoclast precursors into mature osteoclasts [24]. IL-1R2 expressed on osteoclasts in the normal healthy bone, but markedly suppressed expression level of IL-1R2 in severe bone destruction was found [15]. IL-1R2, serves as a negative regulator of IL-1 signaling, is involved in the pathogenesis of osteoporosis. Therefore, *IL-1R2* gene was selected as the candidate gene in our study. Some studies reported the relation of *IL-1R2* genetic variants to various inflammatory diseases, such as rheumatoid arthritis and aggressive periodontitis [25, 26], but not osteoporosis. Here, our study firstly explored the correlation of *IL-1R2* variants with osteoporosis susceptibility. Our result revealed that rs11674595 and rs2072472 were risk factors for osteoporosis occurrence, while rs4851527 was related to the lower risk of osteoporosis in the whole sample. These polymorphisms are located in the intron sequence of *IL-1R2* gene, but the function is still unknown. Several studies supported that intronic SNPs might contribute to the susceptibilities of diseases by affecting the binding of transcription factor bind and/or RNA splicing to influence gene expression [27, 28]. However, the biological function of these SNPs in the *IL-1R2* gene needs to further explore.

Osteoporosis is age-related degenerative changes, which accelerate with age [29]. Stratified by age, we found that rs4851527 was a protective factor for osteoporosis susceptibility in the subjects aged > 60 years; while rs11674595 had the elevated risk of osteoporosis and rs3218977 had a reduced risk at age ≤ 60 years. Considering the pathogenesis of osteoporosis might differ by sex [30], stratification analysis based on sex was performed. In female, we found that rs11674595 and rs2072472 were associated with a higher occurrence of osteoporosis, while rs4851527 and rs3218977 were with a lower osteoporosis risk. Some authors have proposed that high BMI is a protective factor for osteoporosis [31, 32]. The result of stratification analysis by BMI displayed that rs11674595 and rs4851527 were related to the reduced occurrence of osteoporosis among the subjects with BMI > 24 kg/m², while rs11674595 conferred to the increased risk of osteoporosis at BMI ≤ 24 kg/m². These results displayed that the effect of *IL-1R2* variants on osteoporosis risk presented age, gender and BMI difference. In addition, we also investigated the correlation between combined SNPs and osteoporosis risk using haplotype and MDR analysis. We found the relationship of T_{rs11674595}A_{rs4851527}, C_{rs11674595}G_{rs4851527} and A_{rs3218977}G_{rs2072472} haplotypes to osteoporosis occurrence, and a potential accumulated effect of *IL-1R2* SNPs on osteoporosis susceptibility. Our data suggest that the interaction of polymorphisms in the risk of osteoporosis should be paid attention.

However, this study has some weaknesses. Firstly, the relatively small sample size may limit the power and generalization of our study. Secondly, due to lack of information, gene–environment interactions were not evaluated. Thirdly, the expression of IL-1R2 was not detected, so the effect of these SNPs on the expression of IL-1R2 is still unknown. Despite these limitations, we provide evidence for the first time that *IL-1R2* variants are related to osteoporosis occurrence in a Chinese Han population.

Conclusion

In conclusion, *IL-1R2* polymorphisms (rs11674595, rs4851527, rs2072472 and rs3218977) might contribute to the susceptibility of osteoporosis among Chinese Han population. We also found the association of TA, CG and AG haplotypes with osteoporosis occurrence, and a potential accumulated effect of *IL-1R2* SNPs on osteoporosis susceptibility. Our finding may increase our understanding of the effects of *IL-1R2* genetic polymorphisms on osteoporosis risk and may provide important clues for a risk assessment for osteoporosis occurrence in the Chinese Han population.

List Of Abbreviations

SNP, single-nucleotide polymorphisms; OR, odds ratio; CI, confidence intervals; MAFs, minor allele frequencies; TLC, total leukocyte count.

Declarations

Ethics approval and consent to participate

All subjects signed a written consent before participating in the study. The research protocol was in accordance with the Research and Ethical Committee of the Xi'an 630 hospital and with the 1964 Helsinki Declaration.

Consent for publication

Not applicable.

Availability of data and material

All the data regarding the findings are available within the manuscript. Anyone who is interested in the information should contact the corresponding author.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

The work presented here was carried out in collaboration between all authors. Kai Rong carried out the molecular genetic studies and drafted the manuscript. Zhiquan Liang designed the methods and experiments, performed the statistical analyses and interpreted the results. Wenyan Xiang designed primers and performed the SNP genotyping experiments. Zhan Wang and Fengli Wen collected clinical information about patients and performed the SNP genotyping experiments. Laijin Lu conceived of the study, worked on associated data collection and their interpretation, participated in the design and coordination of the study, and funded the study. All authors read and approved the final manuscript.

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33. Legends.

Figures

Figure 1

Haplotype block map for the linkage disequilibrium of six SNPs in IL-1R2. The numbers inside the diamonds indicate the D' for pairwise analyses

Figure 2

The interaction dendrogram derived from MDR for the SNP-SNP interaction in IL-1R2. Short connections among nodes represent stronger synergistic (orange) or redundant (green and blue) interactions.

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