Original Paper

The Interaction Between Obesity and RAGE Polymorphisms on the Risk of Knee Osteoarthritis in Chinese Population

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Key Words

Receptor for advanced glycation end products • Osteoarthritis • Polymorphisms • Obesity

Abstract

Background: The receptor for advanced glycation end products (RAGE) has been reported to relate to osteoarthritis (OA), however, the role of RAGE genetic variants in OA remains unknown.

Method: A total of 233 patients with primary knee OA and 255 healthy volunteer were recruited. Three RAGE gene polymorphisms, namely, Gly82Ser (rs2070600), -374T/A (rs1800624) and -429T/C (rs1800625) were genotyped. **Results:** Of all three RAGE gene polymorphisms, only the genotype distributions and alleles frequencies of 82G/S polymorphisms significantly differed between knee OA and control subjects. The presence of SS genotype and S allele of 82G/S were significantly higher in knee OA subjects than in controls (34.76% vs. 19.61%, P for trend = 0.004; 57.64% vs. 48.59%, P for trend < 0.001, respectively). Multivariate logistic regression analysis showed a significantly increased risk for knee OA for the SS genotype compared with the AA genotype (OR = 1.984, 95% CI: 1.238-3.181; P = 0.004). The adjusted OR for S allele carriage was significantly higher than G allele carriage (OR = 1.440, 95% CI: 1.137-1.8231, P = 0.002). Moreover, a significant multiplicative interaction was observed between 82G/S polymorphisms with obesity (Pinteraction = 0.028). Taking the non-obese 82GG genotype as references, the OR for OA in non-obese SS carriers was 2.537 (95% CI 1.241-5.189, P = 0.001). Notably, the OR in obese GS carriers was 2.304 (95% CI: 1.218-4.357, P = 0.009) and in obese SS was 3.392 (95% CI: 1.672-6.885, P = 0.001). The -374T/A and -429T/C did not show positive interaction with obesity and smoking status. **Conclusion:** The AGE 82G/S polymorphisms, in interaction with obesity, may determine the susceptibility of OA in Chinese population.

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Introduction

Osteoarthritis (OA) is a common musculoskeletal disease among the elderly, characterized by the degradation of articular cartilage and formation of abnormal bone (osteophyte) [1-3]. OA is a multifactorial disorder in which aging, hormonal and mechanical factors contribute to its onset and progression [4-6]. Recently, several studies have demonstrated the polymorphism in certain genes may be related to the pathogenesis of OA [7-10].

The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface molecules and a receptor for advanced glycation end products (AGEs) [11, 12]. AGE-RAGE interaction alters several cell functions through modulation of multiple intracellular signaling pathways, thus contribute in a variety of diseases, including inflammation and aging [13, 14]. RAGE has been implicated in the pathogenesis of arthritis. RAGE is markedly expressed in joints with rheumatoid arthritis (RA) and amplifies the immune/inflammatory response in animal model; blockade of RAGE suppressed the histologic evidence of arthritis [15]. A previous study showed upregulated chondrocyte expression of the RAGE in OA cartilage, and RAGE signaling promote inflammation-associated chondrocyte hypertrophy, suggesting that RAGE signaling has a potential to contribute to OA [16].

To date, several functional single nucleotide polymorphisms (SNPs) have been identified in RAGE gene, among which the Gly82Ser (rs2070600, 244G>A), -374T/A (rs1800624) and -429T/C (rs1800625) were mostly studied. The functional Gly82Ser occurs in the ligand-binding V domain of RAGE and affects ligand affinity while the -374T/A and -429T/C are located in the promoter region and have been shown to increase reporter gene expression [17-20]. Previous studies reported positive associations between the variants of RAGE gene and chronic inflammation [21, 22]. However, no study regarding the role of RAGE genetic variants in OA was reported. In present study, we performed a case-control study in knee OA subjects in Chinese cohorts to explore the association of RAGE polymorphisms and risk of knee OA. Meanwhile, we studied the potential interaction between RAGE gene polymorphisms and other factors associated with OA, such as obesity and smoking status, in determining the susceptibility of OA.

Materials and Methods

Patients

A total of 233 patients with primary knee OA were recruited from Feb 2007 to Dec 2011. The diagnosis of knee OA was based on the American College of Rheumatology criteria [23], 255 healthy volunteer were enrolled as controls. Both OA and control groups were interviewed to obtain demographic data and all of established risk factors. In the study, other etiologies causing knee diseases such as inflammatory arthritis (rheumatoid, polyarthritic, or autoimmune disease), posttraumatic or post-septic arthritis, skeletal dysplasia or developmental dysplasia were excluded from OA group. All the control never had any signs or symptoms of arthritis or joint diseases (pain, swelling, tenderness, or restriction of movement). The clinical characteristics of all enrolled subjects, including age, sex, body mass index (BMI), diabetes mellitus (DM) smoke status, bone fracture history, knee activity and regular exercise were recorded. Obesity was defined as BMI > 30 kg/M². The study was approved by the ethics review committee of our hospital and written informed consent was obtained from all participants.

Genotyping

RAGE genotyping. The protocol for genomic DNA extraction was described in a previous study. A PCR-RFLP assay was used to determine the RAGE polymorphisms. PCR was done in 20 µL reaction mixtures containing 1.625 mmol/L MgCl₂, 0.14 mmol/L deoxynucleotide triphosphates, 1 unit of Taq polymerase (MBI Fermentas), 2 µL of 10× PCR buffer (MBI Fermentas), 200 ng genomic DNA and 0.25 µmol/L of each primer (forward, 5'-GTAAGCGGGGCTCTGTTGCA-3'; reverse, 5'-GGCCAAGGCTGGGGTTGAAGG-3' [15]), (forward, 5'-GTAAGCGGGGCTCTGTTGCA-3'; reverse, 5'-GGCCAAGGCTGGGGTTGAAGG-3' [15]), (forward,

Table 1. The demographic and clinical characteristics of all subjects in the study.

Variables	Cases (n = 275)	Control (n =283)	P
Age (years)	63.5±6.4	63.6±6.1	0.871
Female (%)	169(61.5%)	177(62.5%)	0.429
Obesity	174(62.27%)	147(51.94%)	0.003
DM	98(35.63%)	69(24.38%)	0.002
Smoking (%)	125(45.45%)	130(45.93%)	0.43
OA family history	109(39.1%)	114(40.3%)	0.472
History of labor work (%)	114(41.4%)	120(42.4%)	0.444

5'-GTAAGCGGGGCTCCTGTTGCA-3'; reverse, 5'-GGCCAAGGCTGGGGTTGAAGG-3' [15]). After an initial denaturation at 95°C for 5 min, the DNA was amplified by 35 cycles of 94°C for 30s, 62°C for 40 s, and 72°C for 45s, with a final elongation at 72°C for 10 min. The 397-bp PCR products were digested by the restriction enzyme *AluI*, 5 units for 16 h at 37°C, followed by electrophoresis on a 2% agarose gel. The digestion revealed fragments of length 249, 123, and 26 bp for the wild-type Gly82 allele and 181, 123, 67, and 26 bp for the variant Ser82 allele. About 10% of the samples were randomly selected to do the repeated assays, and the results were 100% concordant. Two researchers, blinded to the clinical data, scored the genotypes independently.

Statistical analyses

Quantitative variables e.g. age between OA subjects and controls were shown as compared using Student's t test, and qualitative variables, e.g. genotype and allele frequencies, et al., were compared using the chi-square test or Fisher's exact test. The power calculations were performed as previously described [24]. Logistic regression was used to test the associations between disease status and genetic polymorphisms of OPN gene after adjusting for relevant covariates. The D' value and r² for the studied 3 SNPs were calculated with the SHEsis software [25]. The interaction between smoking and RAGE SNPs were estimated via multiplicative interaction term and the stratified analysis of the effect of SNPs on OA by smoking and obesity status. The Statistical Package of the Social Sciences software version 16.0 (SPSS Inc., Chicago, IL) was used for statistical analyses. A 2-sided P < 0.05 was considered to be significant.

Results

Table 1 shows demographic and clinical characteristics of all subjects in the study. There were no significant differences in sex, age, Smoking, OA family history, History of labor work between knee OA cases and controls. Obesity, which is one of the risk factor of knee OA, was significantly higher in the OA patient group than in controls (P<0.001).

Table 2 described the genotype distributions and allele frequencies of RAGE polymorphisms in knee OA and control subjects. The genotype frequencies for all polymorphisms in controls did not differ significantly from those expected under Hardy-Weinberg equilibrium (all P>0.05). There were no significant differences of genotype distributions and alleles frequencies of -429T/C and -374 T/A between knee OA subjects and control subjects (P for trend=0.347 and 0.460, respectively, Table 2). However, genotype frequencies and allele frequencies at 82G/S polymorphisms significantly differed between OA and control subjects. The presence of SS genotype of 82G/S was significantly higher in knee OA subjects than in controls (35.27% vs. 22.61%, P for trend =0.002). Accordingly, the 82S allele frequency was higher in OA patients than controls (57.64% vs. 48.59%, P<0.001). Multivariate logistic regression analysis showed a significantly increased risk for knee OA for the SS genotype of 82G/S compared with the AA genotype (OR= 1.984, 95% CI:

Table 2. Described the genotype distributions and allele frequencies of RAGE polymorphisms in knee OA and control subjects.

		OA		Control		adjusted OR	95%CI		X ²	adjusted P
RAGE		N	%	N	%					
-374T/A	TT	84	30.55%	71	25.09%	1.000				
	TA	120	43.64%	131	46.29%	0.774	0.518	1.157	1.563	0.211
	AA	71	25.82%	81	28.62%	0.741	0.473	1.160	1.719	0.190
	T	288	52.36%	273	48.23%	1.000				
	A	262	47.64%	293	51.77%	0.848	0.670	1.072	1.904	0.168
-429T/C	TT	59	25.00%	69	24.38%	1.000				
	TC	118	50.00%	155	54.77%	0.890	0.584	1.358	0.291	0.589
	CC	59	25.00%	59	20.85%	1.169	0.709	1.930	0.375	0.540
	T	236	50.00%	293	51.77%	1.000				
	C	236	50.00%	273	48.23%	1.073	0.841	1.370	0.321	0.571
82G/S	GG	55	20.00%	72	25.44%	1.000				
	GS	123	44.73%	147	51.94%	1.095	0.716	1.675	0.177	0.674
	SS	97	35.27%	64	22.61%	1.984	1.238	3.181	8.176	0.004
	G	233	42.36%	291	51.41%	1.000				
	S	317	57.64%	275	48.59%	1.440	1.137	1.823	9.172	0.002

Table 3. The associations between the RAGE haplotypes and knee OA risk.

-374T/A	-429T/C	82G/S	Case(freq)	Control(freq)	Chi ²	P	Odds Ratio [95%CI]
A	C	S	102.98(0.201)	172.28(0.176)	1.59	0.234	1.195 [0.901~1.674]
A	C	G	33.92(0.064)	106.49(0.143)	7.53	0.0061	0.572 [0.251~0.895]
A	T	G	62.5(0.127)	115.45(0.224)	0.31	0.647	1.094 [0.777~1.598]
T	C	G	70.11(0.146)	15.53(0.142)	0.38	0.539	0.909 [0.611~1.529]
T	T	G	49.14(0.113)	85.75(0.093)	0.006	0.972	0.985 [0.683~1.412]
T	C	S	115.27(0.223)	107.83(0.107)	0.735	0.0232	2.176 [1.252~2.671]

1.238-3.181; $P=0.004$) after adjustment with sex, age, BMI, smoke status, history of labor work, regular exercise and knee activity. The adjusted OR for S allele carriage was significantly higher than G allele carriage (OR=1.440, 95% CI: 1.137-1.8231, $P=0.002$). In order to testify this difference is real or just by chance, we performed power calculation. Our data showed that the difference of 82G/S between OA and controls had acceptable power values (power values =0.85 between GG and SS genotype carriers and =0.86 between G and S allele carriers). Besides 82G/S polymorphisms, multivariate logistic regression analysis also showed that obesity and DM were risk factor for the occurrence of OA in this study (OR=1.754, 95% CI:1.215-3.154, $P=0.014$ and OR=1.584, 95% CI:1.132-3.545, $P=0.022$, respectively).

The associations between the RAGE haplotypes and knee OA risk were analyzed in this study. The D' value and r^2 for the studied 3 SNPs were calculated with the SHEsis software [21]. All 3 SNPs were in strong LD (all $D'>0.8$). The estimated haplotype frequencies of the RAGE SNPs are shown in Table 3. The haplotype A₋₃₇₄C₋₄₂₉G₈₂ represented protective effect for developing knee OA (OR=0.572, $P=0.0061$). In contrast, the T₋₃₇₄C₋₄₂₉S₈₂ showed a higher risk for developing OA (OR= 2.176, $P=0.0129$, Table 3).

We further analyzed the interaction between the 82G/S RAGE polymorphisms and smoking status in determining the risk of OA. Table 4 showed the 82G/S polymorphisms did not have a positive interaction with smoke status (all FDR- $P_{\text{interaction}}=0.236$, table 4) for OA risk. We next analyzed the interaction between RAGE and obesity status with the risk

Table 4. The interaction between 82G/S polymorphisms with smoking status and obesity for the susceptibility to OA.

Genotype	Factor	OA	Controls	OR	95%CI		P	FDR-P _{interaction}
82G/S	GG	Non-Smokers	35	36	1			
	GS	Non-Smokers	60	76	0.81203	0.456766	1.443611	0.477879
	SS	Non-Smokers	55	41	1.379791	0.744887	2.555854	0.305475
								0.236
	GG	Smokers	20	36	0.571429	0.278752	1.171403	0.125117
	GS	Smokers	63	71	0.912676	0.513152	1.623257	0.755752
	SS	Smokers	42	23	1.878261	0.942867	3.741634	0.071757
82G/S	GG	Non-obese	19	37	1.00			
	GS	Non-obese	39	66	1.151	0.583	2.272	0.686
	SS	Non-obese	43	33	2.537	1.241	5.189	0.010

of OA. We found that there was a positive interaction only between the 82 G/S and obesity (FDR- $P_{\text{interaction}} = 0.0122$). Taking the non-obese 82GG genotype as references, the OR for OA in non-obese SS carriers was 2.537, 95CI 1.241-5.189, $P=0.001$). Notably, the OR in obese GS carriers was 2.019 ($P=0.028$); the OR in obese SS carriers was 3.392 (95% CI: 1.671-6.885, $P=0.001$). The -374T/A and -429T/C did not show positive interaction with obesity and smoking status (data not shown).

Discussion

In this study, we investigated whether 3 RAGE SNPs, interacting with smoking status and obesity, influence the susceptibility of OA in a Chinese cohort. Our results showed that only the 82G/S had a closely association with OA risk by multiple regression analyses. The 82SS carriage tends to have a 2.3 times higher risk to develop OA. There was no interaction between RAGE and smoke in determining the OA risk. In contrast, obesity status significantly influenced the effect of RAGE gene polymorphisms on the susceptibility of OA. With non-obese 82GG genotype as references, the OR for OA in obese 82GG carriers was 3.432 ($P=0.005$); in obese 82GS was 4.141 and in obese 82SS reached 6.812 ($P<0.001$). To the best of knowledge, this is the first study regarding the interaction between RAGE SNPs and obesity status in OA susceptibility. Our finding highlights the importance of obesity in determining the genetic risk for OA.

Recent studies reported the RAGE 82G/S polymorphism results in the enhancement of pro-inflammatory mechanisms in immune/inflammatory diseases [26]. Soluble RAGE (sRAGE) is well accepted as a protective factor to inflammation [27]. A previous study showed the sRAGE concentrations were highest in subjects with the G/G genotype, medium in those with the G/S and lowest in S/S genotype carriers [22]. sRAGE levels were reported to be significantly lower in OA patients compared with controls, and sRAGE levels in plasma and synovial fluid also decreased significantly as the disease severity increased [28]. A remarkable association of the G82S variant with elevated serum CRP levels in the Chinese Han population with coronary heart disease, implying that the prevalence of RAGE 82S allelic variation may influence vascular inflammation level [29]. In this study, we found had higher presence of 82SS genotype in OA subjects than in controls, we postulated that might be due to the higher inflammation level in OA patients than controls. Unfortunately, in this study, we did not detect the plasma sRAGE level (ideally, the sRAGE level in the synovial fluid) to

better document the association between the RAGE polymorphisms and OA. This is a major limitation of this study.

Two studies reported higher GG distribution (between 47%-63%GG prevalence) in Chinese cohorts [18, 30]. However, our study showed a lower GG frequencies (between 20%-26%). In contrast, the SS genotype rate in our study was higher in our study. Our data showed that the difference of 82G/S between OA and controls had acceptable power values (>0.8). Although all the participants were Chinese, their geographic living area and environmental factors were quite different. We postulate the disparities of gene distribution might be due to the geographic and environmental factors.

Obesity is closely associated with low-grade inflammation. The Gly82Ser polymorphism was reported to be related to RAGE expression and is also involved in inflammatory response. [31] Particularly in obese subjects, S/S carriers showed significantly higher concentrations of AGEs and C reaction protein (CRP) than G allele carriers. RAGE is involved in the development of obesity and insulin resistance [32]. The association between obesity and insulin resistance as well as DM has been documented previously [33]. In our study, the OA patients had a higher rate of DM and obesity. Multiple-variants analysis showed that both DM and obesity were risk factors for OA.

The association between obesity and knee osteoarthritis, and specifically the role of obesity as a risk factor for knee osteoarthritis has been well documented [34-36]. A systematic review and meta-analysis examined 36 papers reporting on BMI and found that all studies demonstrated obesity and being overweight to be risk factors for knee osteoarthritis [37]. The potential mechanisms to link obesity and knee osteoarthritis, as both a biomechanical and metabolic condition are strongly linked. It has been established that weight loss for obese patients with knee OA is clinically beneficial. In present study, the mean BMI was significantly higher in OA patient than in controls. The positive interaction between RAGE SNPs and obesity suggest that reduction of body weight might reduce the genetically determined risk for OA.

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