MUTYH and *ORAI1* polymorphisms are associated with susceptibility to osteoarthritis in the Chinese Han population

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Abstract

Background: This study analyzed the associations between single nucleotide polymorphisms (SNPs) in the mutY homolog gene (*MUTYH*) and the calcium release-activated calcium channel gene (*ORAI1*) with susceptibility to osteoarthritis in the Chinese Han population.

Methods: A total of 350 patients diagnosed with osteoarthritis from October 2013 to May 2016 were selected as the study group, together with 350 age- and gender-matched healthy controls. *MUTYH* SNP rs3219463 and *ORAI1* SNPs rs712853, rs12313273, rs6486795, rs12320939, and rs7135617 were analyzed by Sanger sequencing. Serum MUTYH levels were measured by enzyme-linked immunosorbent assay. The relationship between SNPs in *MUTYH* and *ORAI1* and osteoarthritis susceptibility was analyzed and compared with the level of serum MUTYH in the osteoarthritis and control groups.

Results: *MUTYH* rs3219463 G allele carriers (GG or GA genotypes) and *ORAI1* rs7135617 T allele carriers had a higher risk of osteoarthritis than patients with other genotypes. The level of serum MUTYH in the study group was significantly higher than in the control group (22.05 ± 19.14 ng/mL vs. 14.15 ± 13.54 ng/mL).

Conclusions: *MUTYH* and *ORAI1* SNPs are associated with osteoarthritis susceptibility in the Chinese Han population.

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Introduction

Osteoarthritis (OA) is a joint disease with various manifestations including articular cartilage fibrosis, ulcers, pain, stiffness, and attrition. The etiology of OA is still largely unknown, but various studies have indicated that its occurrence is associated with age, obesity, joint injury, and genetic factors.[1–3]

Human MutY homolog (MUTYH) is a unique DNA glycosylase that excises adenine bases that have mispaired with 8-oxo-G during DNA replication. Dysfunctional *MUTYH* variants may lead to G:C to A:T transversions in the genome.[4] Previous studies revealed that MUTYH inactivation caused by genetic polymorphisms plays a crucial role in cancer-associated inflammatory responses.[5,6]

Calcium release-activated calcium channel protein 1 (ORAI1), located on the plasma membrane, is an essential component of store-operated calcium channels (SOCCs). The interaction between ORAI1 and stromal interaction molecular 1 regulates intracellular calcium ion homeostasis through activating SOCCs and mediating their calcium entry.[7,8] Additionally, the haplotypes of *ORAI1* single nucleotide polymorphisms (SNPs) rs12313273 and rs7135617 are associated with the risk of developing HLA-B27-positive ankylosing spondylitis (AS).[9] Kung et al. previously showed that *MUTYH* SNPs rs3219463 and rs3219476 were associated with susceptibility to rheumatoid arthritis (RA) in Taiwanese patients.[10] Based on these results, we speculated that polymorphisms of *MUTYH* and *ORAI1* might affect the risk of OA in the Chinese Han population, but little is known of this potential association. Therefore, we carried out a case–control study to examine the association between *MUTYH* and *ORAI1* polymorphisms and susceptibility to OA in the Chinese Han population. Specifically, we examined five tagged SNPs (tSNPs) of *ORAI1* (rs712853, rs12313273, rs6486795, rs12320939, and rs7135617) with minor allele frequencies (MAFs) >10% from the HapMap Han database, and the *MUTYH* SNP rs3219463.

Materials and methods

Subject selection

Patients with OA diagnosed at the General Hospital of Ningxia Medical University (Ningxia, China) from October 2013 to May 2016 were included in the study. Patients were diagnosed with OA according to published guidelines.[11] The severity of OA was assessed using the Kallgren–Lawrence grading system; other types of arthritis were excluded from the study. Age- and gender-matched healthy control subjects were also recruited. Informed consent was obtained from all subjects and the study was approved by the Ethical Committee of the General Hospital of Ningxia Medical University.

Genomic DNA extraction and MUTYH and ORAI1 genotyping

A total of 10 mL of venous blood was collected from each subject. Genomic DNA was extracted from 2 mL blood using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). *MUTYH* and *ORAI1* genotyping was performed by Sanger sequencing.[12] Sequencing primers were synthesized as previously described.[10,12]

Quantitative determination of MUTYH in the serum

The remaining 8 mL of blood was used as a serum sample and diluted 200-fold in dilution buffer (5% skim milk, w/v). MUTYH levels were then quantified by enzyme-linked immunosorbent assay (ELISA) (USCN Life[TM] Science & Technology, Wuhan, China). Recombinant MUTYH protein (PeproTech Inc., Rocky Hill, NJ, USA) was used as the standard. The amount of MUTYH in the serum was determined according to the standard curve and dilution ratio.

Statistical analysis

IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Statistical differences in genotypes and allelic frequencies between cases and controls were assessed using the chi-squared test. The association of each SNP with OA was assessed using multivariate logistic regression analysis. The linkage disequilibrium (LD) map of the five tSNPs in *ORAI1* was constructed using Haploview software (version 4.2; http://www.broad.mit.edu/mpg/haploview/). The odds ratios (ORs) of genetic loci were calculated from genotype and allele frequencies with a 95% confidence interval (CI). Results were considered statistically significant when P values were less than 0.05.

Results

Subject characteristics

This study included a total of 350 patients with OA and 350 healthy controls. Subject characteristics are shown in Table 1. There was no significant difference in basal characteristics between the patients and controls.

Association between ORAI1 rs7135617, MUTYH rs3219463, and the susceptibility of OA

We selected five tSNPs of *ORAI1* (rs712853, rs12313273, rs6486795, rs12320939, and

Table 1 Basal characteristics of patients with osteoarthritis and controls.

Characteristics	Patients with OA (n = 350)	Healthy controls (n = 350)	t/χ^2 value	P value
Age (years)	63.80 ± 11.52	64.10 ± 12.24	0.334	0.739
Gender: male, n (%)	50 (14.29%)	55 (15.71%)	0.280	0.597
Course (years)	6.10 ± 2.14	–		
Education: n (%)			0.625	0.731
Primary school or below	75 (21.43%)	71 (20.29%)		
Middle school	212 (60.57%)	208 (59.43%)		
Graduate and above	63 (18.00%)	71 (20.29%)		
Obesity: n (%)	189 (54.00%)	191 (54.57%)	0.023	0.879
Heavy physical labor: n (%)	98 (28.00%)	120 (34.29%)	3.224	0.073
Smoking history: n (%)	91 (26.00%)	114 (32.57%)	3.649	0.056
Drinking history: n (%)	134 (38.29%)	158 (45.14%)	3.384	0.066

rs7135617) with MAFs >10% from the HapMap Han database. Genotype and allele frequencies were consistent with the Hardy–Weinberg equilibrium (HWE). A comparison of the difference in genotype and allele frequencies of SNPs between patients and controls showed that individuals carrying the rs7135617 TT genotype had a significantly higher risk of developing OA than individuals with other genotypes ($OR = 1.332$, 95%CI = 1.069–1.661, $P = 0.01$) (Table 2).

The genotype and allele frequency distribution of *MUTYH* SNP rs3219463 is shown in Table 2. The frequencies were consistent with the HWE. Individuals with the rs3219463 GA or GG genotype had a significantly higher risk of developing OA than individuals with other genotypes ($OR = 1.506$, 95%CI = 1.204–1.830, $P = 0.000$).

Haplotype analysis of *ORAI1* SNPs in OA susceptibility

To further confirm the existence of a correlation between *ORAI1* haplotypes and OA, we constructed an LD map (Figure 1) and estimated the differences of haplotype frequencies between OA patients and controls.

As shown in Table 3, the haplotype analysis revealed that there was no significant correlation in rs12313273/rs7135617 or rs7135617/rs6486795 pairwise allelic comparisons.

MUTYH expression levels in the serum

Serum levels of MUTYH protein from 350 OA patients and 350 controls were detected by ELISA. The mean MUTYH level of OA patients was significantly higher than that of healthy controls (Figure 2, $P < 0.005$). Individuals carrying mutant rs3219463 (GA+GG) genotypes also had significantly higher serum MUTYH levels than those with the wild-type genotype (AA) (Figure 2, $P < 0.05$).

Discussion

OA is a chronic disease that can lead to the total degeneration of articular cartilage structure and function. The development of OA may also result in changes to other soft tissue parts of the body.[13–15] The most common symptoms include joint pain, swelling, stiffness, and functional limitations.[16] OA can be induced by a number of predisposing causes, including both occupational and non-occupational factors. Coggon et al.[17] showed that individuals who kneeled or squatted for prolonged periods had a higher risk of developing knee OA, which was also associated with obesity.[18] Moreover, Valdes et al.[19] analyzed the genotypes of 36 SNPs in 17 candidate genes, and found that multiple genetic variants could predict the risk of knee OA. Thus, genetic factors appear to be critical for the development of OA.

OA is traditionally thought to be associated with degeneration and trauma. However, increasing evidence suggests that it is not a single disease caused by aging or biological stress, but rather the accumulation of multiple factors such as metabolic disorders and inflammatory senescence. Lipid metabolism and body fluids are also involved in the initiation and progression of disease. Because the systemic inflammatory response associated with RA is believed to play an important role in the development of AS, it appears that OA, RA, and AS may share common features regarding the inflammatory response, while the incidence of OA and RA may be related to calcium signaling. Moreover, *MUYTH a*nd *ORAI1* SNPs have been significantly associated with the risk of chronic RA.[6,12] However, few studies have investigated the role of *MUYTH* and *ORAI1* SNPs in the susceptibility to OA in the Chinese Han population.

Here, we performed a case–control association study of *MUTYH* SNP rs3219463 and five *ORAI1* tSNPs (rs712853, rs12313273, rs6486795, rs12320939, and rs7135617).

Table 2 Genotype and allele frequencies of *ORAI1* and *MUTYH* and the risk of OA.

ORAI1	Genotype	Patients with OA (n = 350)	Healthy controls (n = 350)	OR (95% CI)	P value	Adjusted P value
rs712853						
	CC	34	37	0.875 (0.507–1.509)	0.609	0.704
	CT	150	155	0.921 (0.665–1.275)	0.607	0.663
	TT	166	158	ref		
Allele						
	C	218	229	0.93 (0.738–1.172)	0.528	0.566
	T	482	471	ref		
rs12313273						
	CC	27	30	0.890 (0.490–1.614)	0.683	0.791
	CT	142	141	0.894 (0.487–1.640)	0.699	0.809
	TT	181	179	ref		
Allele						
	C	196	202	0.959 (0.755–1.218)	0.722	0.767
	T	504	498	ref		
rs6486795						
	CC	52	47	1.090 (0.670–1.775)	0.714	0.804
	CT	163	170	0.945 (0.676–1320)	0.729	0.791
	TT	135	133	ref		
Allele						
	C	267	264	1.018 (0.816–1.271)	0.869	0.912
	T	433	436	ref		
rs12320939						
	TT	85	82	1.003 (0.644–1.562)	0.990	1
	TG	173	179	0.935 (0.643–1.360)	0.713	0.782
	GG	92	89	ref		
Allele						
	T	344	342	1.011 (0.816–1.254)	0.915	0.957
	G	356	358	ref		
rs7135617						
	TT	79	54	1.728 (1.112–2.686)	0.010	0.014
	TG	144	146	1.165 (0.826–1.643)	0.364	0.411
	GG	127	150	ref		
Allele						
	T	302	254	1.332 (1.069–1.661)	0.009	0.010
	G	398	446	ref		
MUTYH rs3219463						
	AA	51	45	1.792 (1.091–2.945)	0.014	0.020
	AG	194	139	2.207 (1.569–3.104)	0.000	0.000
	GG	105	166	ref		
Allele						
	A	297	230	1.506 (1.204–1.83)	0.000	0.000
	G	403	470	ref		

ref, reference.

Gender was previously shown to be one of the key factors affecting the risk of OA,[20] so the 350 controls were age- and gender-matched to the 350 OA patients.

Other than the association between *MUTYH* SNPs and RA risk, *MUTYH* was also chosen as a target gene in this study because of the reported correlation between its polymorphisms and cancer-linked inflammatory responses.[21,22] Indeed, tumor inflammatory responses including tumor necrosis factor-α and interleukin-1β

are important factors during OA development.[23,24]

The Yen group previously reported that only the *MUTYH* SNP rs3219463 was relevant to the development of RA, so we focused on the association between rs3219463 and OA. As shown in Table 2, individuals with the rs3219463 G allele (GA or GG genotypes) had a higher risk of developing OA than those with other genotypes. We also found that individuals with GA or GG rs3219463 genotypes had higher serum MUTYH levels than wild-type individuals with the AA genotype. Although it is not obvious why MUTYH accumulates
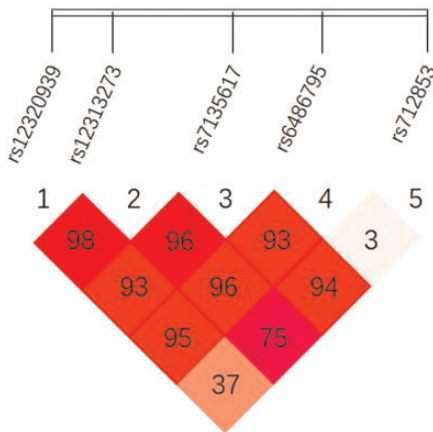

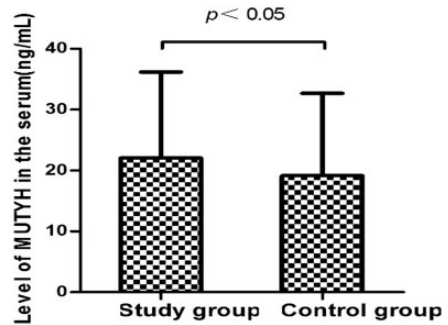**Figure 1.** Linkage disequilibrium map of five *ORAI1* tSNPs.

Figure 2. MUTYH expression levels in the serum of OA patients and controls. $P < 0.05$ represents statistical significance.

Table 3 Haplotype frequency of the *ORAI1* gene in patients with OA and normal controls.

Haplotype	Patients with OA (n=350)	Healthy controls (n=350)	OR (95% CI)	P value
rs12313273/rs7135617				
T/T	147 (42.00%)	143 (40.86%)	1.093 (0.748–1.597)	0.698
T/G	106 (30.29%)	106 (30.29%)	1.063 (0.707–1.598)	0.834
C/G	95 (27.14%)	101 (28.86%)	ref	
rs7135617/rs6486795				
T/T	144 (41.14%)	142 (40.57%)	1.006 (0.710–1.427)	0.971
G/T	69 (19.71%)	75 (21.43%)	0.913 (0.596–1.400)	0.661
G/C	132 (37.71%)	131 (37.43%)	ref	

Haplotype frequencies <1% were excluded. ref, reference.

in the serum, we propose that this might reflect the intracellular function of MUTYH.[25] We believe that SNP rs3219463 affects the expression level of MUTYH, but more *in vitro* studies will be needed to confirm this hypothesis.

The ORAI1-mediated calcium signaling pathway is associated with a range of diseases. *ORAI1* mutations may lead to disruption of the store-operated Ca^{2+} influx and the suppression of immune responses.[26] A number of *ORAI1* SNPs have been reported to be associated with human disease, including the correlation of rs7135617 with AS,[9] while *ORAI1* polymorphisms were shown to influence calcium signal transduction by affecting protein splicing.[27] We found that individuals with the rs7135617 TT genotype had a higher risk of developing OA. This may be because rs7135617 polymorphisms affect calcium signal transduction, causing Ca^{2+} signaling abnormalities during OA development.[28] Wen *et al.*[29] revealed differences in the characteristics of Ca^{2+} signals in different layers of normal cartilage tissue, which are regulated by extracellular Ca^{2+} concentrations. Moreover, the Ca^{2+} signal in OA chondrocytes differs from that in normal cells. Therefore, abnormalities of OA cartilage tissue may be linked to Ca^{2+} signal abnormalities, which could reflect differences in the expression of genes such as *MUTYH* and *ORAI1*. In our study, *ORAl1* rs7135617 T allele carriers and *MUTYH* rs3219463 G allele carriers had abnormal cartilage tissue Ca^{2+} signal responses, which would likely result in the dysfunction of OA cartilage cell voltage-regulated calcium channels and calcium-release activated channels. Additionally, Berenbaum *et al.*[30] showed that inflammatory cytokines play an important role in the pathogenesis of OA.

Our study has a number of limitations. First, we were unable to collect clinical biochemical data from OA patients, such as ORAI1 expression levels, so we only focused on the association between SNPs and the risk of OA. Second, we did not perform an *in vitro* analysis of the mechanisms affecting OA-associated risk factors. Finally, the small sample size of this study meant that no distinction could be made among individuals of different nationalities in China. Therefore, further study is required to confirm whether these SNPs are affected by different ethnic groups or sites of OA.

In conclusion, *MUTYH* SNP rs3219463 and *ORAI1* SNP rs7135617 appear to be associated with susceptibility to OA in the Chinese Han population. The SNPs may affect protein expression and function, which in turn affect the Ca^{2+} signaling pathway, resulting in disease development.

Declaration of conflicting interest

Funding

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