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Original Article

Association of HLA-DQA1 (rs9272219) with susceptibility to rheumatoid arthritis in a Han Chinese population

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Abstract: HLA-DQA1 (rs9272219) has been previously reported that it is a susceptibility locus in rheumatoid arthritis (RA) of UK Caucasian population and North American; however, it has not reported in RA of Chinese population. Our study was to identify whether or not this relationship is reside between rs9272219 and RA in a Han Chinese population. 207 patients with RA and 199 control subjects were recruited. The single nucleotide polymorphism (SNP) of rs9272219 was tested in alleles and genotype frequencies and the data was analyzed by doing the statistic analysis of odds ratio (OR) and 95% confidence interval (CI) from multivariate unconditional logistic regression analyses after pairwise linkage disequilibrium (LD) was estimated. Finally, the Alleles and genotype frequencies distribution of rs9272219 locus among RA patients and control subjects were in accordance with Hardy-Weinberg equilibrium. We found significant association between rs9272219 and RA of Chinese population (OR 0.494, 95% confidence interval [95% CI] 0.354-0.688, $P = 0$ and OR 2.541, 95% CI 1.695-3.808, $P = 0$, respectively). In this study, we found that the SNP of rs9272219 in HLA-DQA1 is a potential susceptibility locus in RA of Han Chinese population; the results suggest that HLA-DQA1 may be related to the development of RA.

Keywords: Rheumatoid arthritis, HLA-DQA1, single nucleotide polymorphism

Introduction

RA is an autoimmune disease that affects 0.5-1% of the population worldwide and is characterized by chronic destructive inflammation in synovial joints and women take greater risks than men of developing the disease. It is known that genetic factors play an important role in the disease, and that a growing number of genes have been identified that they are associated with RA in genome-wide association studies (GWAS) [1-3]. Although the precise contribution of genetic factors has yet to be determined and only a small number of genetic risk factors have been identified, and the genetics of susceptibility to RA has been considerably boosted, largely because of the findings of GWAS. Of all the multiple genes contributing to RA susceptibility, the Human Leukocyte Antigen (HLA) locus accounts for 30% to 50% of overall genetic risk. Over the past decade, several of HLA genes have been extremely successful in picking up RA association, such as HLA- DRB1 consistently shown to have strong association with RA [4].

A recent study has shown a SNP of rs9272219 in HLA-DQA1 was associated with RA both in UK Caucasian populations and North American [5], and the SNP might act together with other genes in biological processes to determine RA susceptibility and be driving the pathway association signals in RA. However, the same gene polymorphism variant may be implicated different susceptibility for RA across different ethnic groups because of genetic heterogeneity of diseases. So, we conducted a matched case-control study to examine whether or not this relationship is resided in a Han Chinese population.

Materials and methods

Patients and controls

We recruited 207 Han Chinese RA patients (mean age 48.3 years, range 25-73 years) and 199 healthy (mean age 45.4 years ,range 32-71 years) controls to analyze the SNP from May 2011 to August 2013. All RA cases were selected following the diagnostic criteria of the

Table 1. Allele distribution in a Han Chinese with RA and controls

Gene allele	Controls N = 200	RA[b] N = 200			
	n (%)	n (%)	*P* value	OR	95% CI
HLA-DQA1 rs9272219					
T	119 (0.299)	72 (0.174)			
G	279 (0.701)	342 (0.826)	0	0.494	0.354-0.688

RA = Rheumatoid Arthritis; [b]compared with controls; OR = odds ratio; 95% CI = 95% confidence interval.

Table 2. Genotypes in RA cases and controls and their association with risk of RA

Gene allele	Controls N = 200	RA[b] N = 200			
	n (%)	n (%)	*P* value	OR	95% CI
HLA-DQA1 rs9272219					
TT	12 (0.060)	7 (0.034)			
GG	92 (0.462)	142 (0.686)			
GT	95 (0.477)	58 (0.280)	0		
TT+GT	107 (0.538)	65 (0.314)	0	2.541	1.695-3.808

RA = Rheumatoid Arthritis; [b]compared with controls; OR = odds ratio; 95% CI = 95% confidence interval.

American College of Rheumatology (ACR 1987). The controls and the case were matched in age and sex. At the same time, unrelated healthy control subjects without any indication of RA were selected. Informed consent was provided by all subjects. The study protocol was approved by the Ethical Review Committee at Zhejiang Provincial People's Hospital. All participants were of the Chinese Han nationality without kinship. 2 ml venous blood was donated by each subject in the morning. The SNP of rs9272219 in HLA-DQA1 was analyzed in our study.

DNA isolation and genotyping

We collected the blood samples from the cases and controls in the morning, and The DNA of blood samples was extracted by the AxyPrep Blood Genomic DNA Miniprep kit (Axygen Biosciences, Union City, CA, USA). HLA-DQA1 (rs9272219) was genotyped by the SEQUENOM MassARRAY MALDI-TOF mass spectrometry platform (Sequenom, San Diego, CA, USA). Primers were synthesized by Shanghai Benegene Biotechnology Co., Ltd. (Shanghai, China). The sequences of primers are listed as follows: rs9272219, forward primer: ACGTTGGATGTCATTTGCCAAAGT-CCTAAG, reverse pri mer:ACGTTGGATGCTGTATCCAGACTTACCTGC, Extension: cccccAGTCCTAAGGAATTTCCAT. TaqMan assays were used for the analysis of SNP.

Statistical analysis

The comparison of the distribution of rs9272219 alleles and genotypes between cases and controls was analyzed by Pearson's chi-square test. Pearson's chi-squared was used for testing Single marker differences and the genotypes in cases and controls. Unconditional logistic regression analysis was used to calculate the odds ratios (OR) with 95% confidence intervals (CI), estimating the association between alleles and genotypes of HLA-DQA1 (rs9272219) in the subjects. Logistic regression models were carried out for the evaluation of the interactions. The statistical analyses were performed with the SPSS (v16.0). Statistical significance of the tests was defined as $P < 0.05$.

Results

The associations between the HLA-DQA1 (rs9272219) alleles and genotype frequencies and RA patients were analyzed in the cases and controls for observing whether or not the alleles and genotype frequencies of HLA-DQA1 (rs9272219) are correlated with RA. In our study, we genotyped the SNP of all the 406 subjects (207 with RA, 199 healthy controls) in a Han Chinese population. Allele and genotype frequencies of the SNP among the cases and controls were compatible with Hardy-Weinberg equilibrium (HWE Pearson's P 0.33929). The alleles were "T" and "G" in loci of rs9272219 in HLA-DQA1. The genetic types were "G/G", "T/T" and "G/T" in rs9272219. Importantly, the data indicates that the rs9272219 alleles and genotypes were strongly associated with RA (**Tables 1** and **2**). There was a significant statistical difference between cases and controls in allele and genotype frequencies (especially the homozygous Major alleles frequencies are compared with other genotypes) in HLA-DQA1 rs9272219 (OR 0.494, 95% confidence inter-

val [95% CI] 0.354-0.688, $P = 0$ and OR 2.541, 95% CI 1.695-3.808, $P = 0$, respectively).

Discussion

RA is a chronic autoimmune disease of unknown etiology with genetic and environmental predisposition. Moreover, many familial studies have provided lots of evidence that the multiple genetic factors play an important role in the development of RA [6]. Many studies to do for the arthritis causing genes, however, only a few of the genetic loci have been identified to be associated with RA. At present, the Human Leukocyte Antigen (HLA) or Major Histocompatibility Complex (MHC) region and non-HLA genes have been identified as susceptible genes of RA, located across the entire genome in different loci of different ethnic groups. In fact, these genes have been reported in different ethnicities and the polymorphisms of them have been shown to predispose to RA. TNFAIP3 (also known as the A20 protein) is a negative regulator of the NF-κB signaling pathway that is essential in the pathogennesis of RA [7]. In a Japanese population study of 3446 RA patients and 2344 unrelated control subjects revealed that TNFAIP3 rs10499194 was associated with RA and protective against it [8], while it was a risk allele for RA in aucasians [9, 10]. HLA-DRB1 shared epitope alleles are the important genetic determinants for autoantibody positive RA and a study from four European Caucasian populations (6649 RA cases and 5118 controls) tested that the independent of the major risk for RA from the MHC class II transactivator locus is from HLA-DRB1 shared epitope alleles [11]. In addition to the HLA-DRB1 was the major MHC risk locus in RA, HLA-C was considered as a second susceptibility locus in ACPA-positive RA in Norwegians [12]. Meanwhile, recent studies suggest that the HLA-DRB1 locus, which has been shown to be associated with RA, is only associated with the presence of anti-CCP antibodies, and that this association is independent of both RA development and the presence of RFs in the Netherlands [13]. Over the past, most of the researches considered that HLA-DQA1 (rs9272219) was strongly associated with schizophrenia and/or bipolar disorder in the MHC region [14]; but recent studies showed the gene is closely related with immune response in function [15] and associated with RA in UK Caucasian populations and North American [5]. However, the associations of HLA-DQA1 (rs9272219) and RA has not been reported in a Han Chinese population.

In the present study, the association of rs9272219 with Chinese RA susceptibility was analyzed and the present data suggests that there is a significant difference in alleles and genotype frequencies between the case and the controls ($P = 0$). In conclusion, we further confirmed that rs9272219 is closely related with RA susceptibility in a Han Chinese population. In agreement with previous reports, our results strongly support the importance of the HLA-DQA1 (rs9272219) alleles and genotypes in the Susceptibility of RA. In other words, the candidate gene may play the same role in different ethnic groups. However, since the genotypes frequencies are different between different ethnic groups for polymorphic variants and we partly chosen Chinese Han subjects as the study population. So, a search of larger sample sizes is required to replicate these findings and clarify the associations of the SNPs and gene-environment interactions with RA indifferent populations.

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Disclosure of conflict of interest

None.

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