Int J Clin Exp Pathol 2014;7(12):8958-8961
www.ijcep.com /ISSN:1936-2625/IJCEP0003268

Original Article

TNFAIP3 rs2230926 polymorphisms in rheumatoid arthritis of southern Chinese Han population: a case-control study

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Received October 20, 2014; Accepted December 1, 2014; Epub December 1, 2014; Published December 15, 2014

Abstract: Polymorphism of tumor necrosis factor alpha-induced protein 3 (TNFAIP3) has been be related to various auto-immune diseases. Based on previous studies that the single nucleotide polymorphism (SNP) of rs2230926 was association with rheumatoid arthritis (RA) of Japanese, Caucasian population and the northern Chinese Han population, we tested the alleles and geno-type frequencies of rs2230926 in TNFAIP3 to investigate whether rs2230926 is susceptible to RA of southern Chinese Han population. In our case-control association study, 207 RA patients fulfilling the American College of Rheumatology (ACR) 1987 criteria were compared with 199 unrelated healthy subjects. After testing the alleles and genotype frequencies of rs2230926, the airwise linkage disequilibrium (LD) was computed and odd ration (OR) and 95% confidence intervals (95% CI) were used for evaluating the susceptibility to RA. The SNP of rs2230926 of the cases and control subjects were conformed to the Hardy-Weinberg equilibrium (P = 0.02257). The significantly statistical differences in alleles of T, G were founded in the cases and controls (P = 0.0027, OR 0.417, 95% CI 0.232-0.749); the genetic types of rs2230926 were associated with a susceptibility to RA, with OR 0.375 (95% CI 0.198-0.707, P = 0.0018). In the present study, our results indicated that the genetic polymorphism of rs2230926 in TNFAIP3 may be a susceptible factor conferring risk for RA in southern Chinese Han population.

Keywords: Rheumatoid arthritis, TNFAIP3, single nucleotide polymorphism

Introduction

RA is one of the most common autoimmune diseases. Although RA is multifactorial nature and the etiology is incompletely known [1], the genetic factors playing a major role in the development of RA is better understood since the recent advent of genome-wide association studies (GWAS). A growing number of genes discovered are associated with RA [2], Such as the human leukocyte antigen (HLA) locus accounting for approximately 30% of overall genetic risk, and the non-HLA genes which have also been studied for association with RA [3]. However, environmental factors are also related to the pathogenesis of RA, such as climate or geographical environment, as well as specific behavioural factors such as air pollution, smoking or foods [4]. In addition, the gene-environmental interaction plays an important role in the development of RA [5] and [6]. Therefore, the same gene polymorphism may be different susceptibility to RA across the same ethnic groups in different geographic areas of countries.

TNFAIP3, known as A20, plays a negative role in T cell activation and inflammatory signalling and may be involved in the pathogenesis of RA [7], and studies have identified that the SNPs in the region in TNFAIP3 are related to RA [8, 9]. Recently, The SNP of rs2230926 in TNFAIP3 has been reported that it was associated with RA both in the Japanese and Caucasian population [10] and [11], as well as in the northern Chinese Han population [12, 13] and [14].

However, the same gene polymorphism variant and ethnic group may be implicated different

Table 1. Allele Distribution in south Chinese Han population with RA and Controls

Gene allele	Controls N = 200 n (%)	RA[b] N = 200			
		n (%)	value	OR	95% CI
TNFAIP3 rs2230926					
T	381 (0.957)	374 (0.903)			
G	17 (0.043)	40 (0.097)	0.0027	0.417	0.232-0.749

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

Table 2. Genotypes in RA Cases and Controls and Their Association with Risk of RA

Gene allele	Controls N = 200 n (%)	RA[b] N = 200			
		n (%)	value	OR	95% CI
TNFAIP3 rs2230926					
TT	184 (0.925)	170 (0.821)			
GG	2 (0.010)	3 (0.014)			
GT	13 (0.065)	34 (0.164)	0.0068		
GG+GT	15 (0.075)	37 (0.179)	0.0018	0.375	0.198-0.707

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

susceptibility for RA in different geographic areas because of geneticheterogeneity of diseases and gene-environmental interaction in the development of the disease. In the current study, we conducted a case-control association study of the SNP in the TNFAIP3 region to identify whether rs2230926 is associated with RA also in southern Chinese Han population.

Materials and methods

Patients and controls

The case-control study was conducted in a total of 207 unrelated Han Chinese RA patients (range 25-73 years, mean age 48.3 years) and 199 unrelated healthy controls (mean age 45.4 years, range 32-71 years), recruited from May 2011 to August 2013 in Hangzhou, Zhejiang, south of China. All RA patients met the diagnostic criteria of ACR 1987. Furthermore, the control was without any symptoms and indications of RA. The protocol of the present study was approved by the Ethical Review Committee at Zhejiang Provincial People's Hospital. Informed consent was obtained from all participants. All participants were of the southern Chinese Han nationality without kinship. The cases and controls donated 2 ml venous blood in the morning. The SNP of rs2230926 in TNFAIP3 was analyzed in the present study.

DNA isolation and genotyping

The peripheral blood samples were collected from the individuals in the morning, and the genomic DNA was extracted using Ayres Blood Genomic DNA Maniple kit (Oxygen Biosciences, Union City, CA, USA), following the manufacturer's instructions. Genotyping of rs2230926 was carried out using the SEQUENOM Mass ARRAY MALDI-TOF mass spectrometry platform (Sequined, San Diego, CA, USA), according to the manufacturer's instructions.

The sequences of primers synthesized by Shanghai Ben gene Biotechnology Co., Ltd. (Shanghai, China) are listed as follows: rs2230926, forward primer: ACGTTGGATGGCCAGCGGAATTTAAAGTTG; reverse primer: ACGTTGGATGACACAGACTTGGTACTGAGG; extension: ctccGTTTCCTTGAGCGTGCTG. The analysis of SNP was carried out by TaqMan assays.

Statistical analysis

The Allele frequency difference of rs2230926 was performed using Pearson's chi-square test. Hardy-Weinberg equilibrium (HWE) test was performed using Microsoft Excel macro PHARE version 2.1. We tested the Single marker differences and genotypes of the subjects by Pearson's chi-square test. We also performed an unconditional logistic regression analysis to calculate the OR with 95% CI and evaluate the relation between alleles and genotypes of TNFAIP3 rs2230926 in the cases and controls. The interaction was evaluated by Logistic regression models. We performed the statistical analyses with the SPSS (v16.0) and the statistical significances were considered as $P < 0.05$.

Results

The association of TNFAIP3 (rs2230926) with RA was examined in southern Chinese Han

population. Departure from HWE of the allele and genotype frequencies of the SNP was evaluated neither in the cases nor in the controls (P = 0.02257). The alleles of TNFAIP3 (rs2230926) were "T" and "G" and the genetic types were "T/T", "G/G" and "G/T". As shown in **Table 1**, rs2230926 G allele was significantly increased in RA patients of southern Chinese Han population (0.097%) compared with controls (0.043%, P = 0.0027, OR 0.417, 95% CI 0.232-0.749). Furthermore, the comparison of genotype frequencies between cases and controls in TNFAIP3 (rs2230926) was a significantly different (OR 0.375, 95% CI 0.198-0.707, P = 0.0018) (**Table 2**). Importantly, The obtained results implied that the SNP has a significant contribution to RA pathogenesis in southern Chinese Han population, confirming the association in the Caucasians, Japanese and northern Chinese Han population.

Discussion

RA is a chronic autoimmune disease of unknown etiology. Although the genetic contribution to the pathogenetics of RA are still not fully understand, a increasing number of genes have been indicated in the development of RA and the understanding of the etiology and pathogenetics of RA has been exactly enhanced since GWAS has been applied. More than 30 genomic risk loci on patients with RA have been revealed, such as PTPN22, STAT4, CD244, CTLA4, et al [15]. As with other complex diseases, besides the inherited genetic architecture, environmental factors are significantly related to the etiology of RA [16]. In addition, studies has showed that the development of RA can be influenced by genetic and environmental factors [17], as well as the gene-environmental interactions, which are likely to drive the development of RA-related autoimmunity long before the appearance of the first characteristic symptoms of RA [18]. In other words, genes of different ethnicities or countries even different regions of a country may have different susceptibilities to the patients of RA because of the variant environmental factors and gene-environmental interactions.

The HLA region is subdivided into DR, DP and DQ and includes the family of *04. The associated alleles to RA are *401, *404, *405 and *408 in western countries, while the *01 and *10 are related to Indians, Jews and Europeans [19]. Meanwhile, a meta-analysis was performed to confer the susceptibility of TNFAIP3 polymorphisms to RA in ethnically different populations, the results showed that the two allele of rs6920220 was significantly associated with RA in Europeans only and there was no association between the two allele of the rs10499194 polymorphism and RA in Europeans, but a significant association was found in Asians [20].

In this study, we replicated the association of TNFAIP3 rs2230926 with RA in a southern Chinese Han population, which showed that TNFAIP3 is a susceptibility gene to RA shared by the Caucasian, Japanese and northern Chinese Han population.

In the association analysis with the present data, there is a statistical significance in alleles and genotype frequencies of TNFAIP3 rs2230926 between the cases and the controls (P < 0.05). The results further confirmed that rs2230926 is susceptibile to patients of RA in a southern Chinese Han population. Thus, taken together with previous studies, the result considered TNFAIP3 as a susceptibility gene to RA common to Caucasian, Japanese, northern and southern Chinese Han population.

We acknowledge that the modest RA sample size of our study was insufficient to identify the role of TNFAIP3 in southern Chinese Han population. A larger sample size is required to replicate these findings in multiple populations and different regions.

Acknowledgements

All authors have contributed to this article, we thank for their kind help and the technical support of Hailong Liu and Junli Li.

Disclosure of conflict of interest

None.

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