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The genetic association between polymorphisms in lymphotoxin-α gene and ankylosing spondylitis susceptibility in Chinese group

A case-control study

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Abstract

The study was designed to reveal the genetic relationship of lymphotoxin-α (*LTA*) polymorphisms with risk of ankylosing spondylitis (AS) in Chinese Han population.

LTA polymorphisms were genotyped by polymerase chain reaction-direct sequencing (PCR-DS) in 138 AS patients and 141 healthy controls. The genotype distribution in control group was checked the status of Hardy–Weinberg equilibrium (HWE). Odds ratio (OR) with 95% confidence interval (95%CI) calculated by χ^2 test was used to show effects of LTA polymorphisms on AS risk. Logistic regressive analysis was used to calculate the adjusted OR values. Additionally, the linkage disequilibrium of *LTA* polymorphisms was examined by Haploview.

G allele of rs909253 was significantly higher frequency in AS patients ($P = .02$), which was associated with the increased risk of AS (OR = 1.53, 95%CI = 1.07–2.18). The carriages of GG genotype in rs909253 showed a high risk of AS occurrence, compared with AA genotype carriers (OR = 2.46, 95%CI = 1.13–5.35). Multivariate analysis demonstrated that the G allele (OR = 1.52, 95%CI = 1.05–2.15) and GG genotype (OR = 2.36, 95%CI = 1.06–5.24) of rs909253 were still positively associated with AS susceptibility. However, there was no significant association between AS risk and rs2239704 or rs2229094.

LTA rs909253 polymorphism contributes to the occurrence of AS.

Abbreviations: 5′UTR = 5′untranslated region, 95%CI = 95% confidence interval, ARA = American Rheumatism Association, AS = ankylosing spondylitis, HLA = human leukocyte antigen, HWE = Hardy–Weinberg equilibrium, *LTA* = Lymphotoxin-α, NK cells = nature kill cells, OR = odds ratio, PCR = polymerase chain reaction, SNP = single nucleotide polymorphism, TNF-β = tumor necrosis factor-beta.

Keywords: ankylosing spondylitis, haplotype, *LTA*, polymorphism

1. Introduction

Ankylosing spondylitis (AS) is a common chronic rheumatic disorder and mainly affects the spine with the involvement of sacroiliac joints and other organs.[1,2] The clinical symptoms of AS include chronic back pain, ankyloses, and stiffness,[3] which decrease the quality of life in patients. It easily attacks men with the age range of 18 to 22 years old. The prevalence of AS is from 0.7% to 3.2% in the world because of different ethnicity and geographic differences.[4] According to the previous studies, human leukocyte antigen (HLA)-B27 was the firstly identified genetic factor correlated to AS etiology and contributed to individual susceptibility.[5] However, HLA-B27 only accounts for less than 50% of the total AS risk. Actually, the occurrence of AS is a complex multistep and multiple-factor process, with the involvement of genetic and environmental factors.[6–8] Investigation of the AS-related genetic factors may be an effective way to identify the high risk AS population.

Lymphotoxin-α (LTA), also known as tumor necrosis factor-beta (TNF-β), is a member of TNF family. As a proinflammatory cytokines, LTA is mainly produced by lymphocytes in response to tissue injury.[9] LTA is found to significantly influence the function of lymphoid organogenesis.[10] Lymphotoxin (LT)-pathway has been discovered to regulate the production of IL-22 and IL-23 for host defense in adult innate lymphoid cells and nature kill (NK) cells,[11] which are the important inflammatory factors involving in the occurrence of AS.[12,13] What's more, LTA also plays an important role in killing inflammatory cells by activating cytotoxic T cells and macrophages.[14] LTA involves in the pathogenesis of inflammatory diseases.

LTA is a kind of interleukin encoded by *LTA* gene which is located in chromosome 6p21 and is closely linked to TNF-α.[15] With the discovery of single nucleotide polymorphism (SNP), it is used to explore the role in disease risk. However, few reports refer

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to the association of *LTA* polymorphisms with AS susceptibility. Therefore, in the present study, we researched the effect of genetic variants in *LTA* on the occurrence risk of AS in a Chinese Han population and 3 common SNPs were selected, rs2239704, rs909253, and rs2229094.

2. Materials and methods

2.1. Subjects

A total of 279 subjects were selected in this study, consisted of 138 AS patients and 141 healthy controls. They were all from The Third Hospital of Hebei Medical University during from December 2014 to December 2015. In the case group, AS patients were diagnosed in clinical according to the diagnosis criteria of American Rheumatism Association (ARA), modified New York criteria.[16] These patients would be excluded who suffered from the other inflammatory diseases and immune diseases. At the same time, the controls were also recruited from the same hospital and they were all healthy with the physical examination. The age and sex were frequency-matched between the control and case groups. The subjects were all Chinese Han population in Shijiazhuang region without blood relationship. This research obtained the support of Research Ethics Committee of The Third Hospital of Hebei Medical University and every subject was informed the objective of this study. All subjects signed written informed consents before collecting blood sample.

2.2. DNA extraction

Firstly, 2 mL peripheral venous blood of every subject was collected in the early morning and was put into 10 mL vacuum tube with anticoagulation EDTA, stored at −80 °C. Then, blood genomic DNA was extracted using TIANamp Genomic DNA Kit purchased by Tiangen Biotech (Beijing) Co., Ltd., according to the manufacturer's instruction. The isolated DNA samples were stored at −20 °C refrigerator.

2.3. Genotyping

Polymerase chain reaction-direct sequencing (PCR-DS) was used to conduct the genotyping of *LTA* three polymorphisms. PCR primers were designed by Primer Premier 5.0 software (Premier Biosoft International, CA) on the basis of the *LTA* gene sequence published on NCBI website. Twenty five microliter PCR system was consisted of 1.0 μL DNA template, each 0.5 μL for forward and reverse primers, 12.5 μL Master Mix and added sterile ddH_2O to the final volume. PCR procedure was conducted as follows: pre-degeneration at 95 °C for 5 minutes, followed by 33 cycles of 94 °C degeneration for 45 seconds, annealing at 60 °C for 30 seconds, 72 °C extension for 30 seconds, and final extension at 72 °C for 7 minutes. The quality of PCR products were detected by 1.0% agarose gel electrophoresis. And then PCR products were sequenced to determine the genotype of every polymorphism in the case and control groups in Shanghai Sangon Biotech Co, Ltd.

2.4. Statistical analysis

In the current study, genotype frequencies were gotten via direct counting and the genotype distribution in the control group was checked by chi-squared test whether conformed to Hardy–Weinberg equilibrium (HWE). The genotype and allele frequencies as well as clinical indexes were compared the different significance between the case and control group by χ^2 test. Odds ratio (OR) with the corresponding 95% confidence interval (95% CI) was used to express the association intensity of AS caused by genetic variants in *LTA*. Logistic regressive analysis was conducted to calculate the adjusted OR and 95%CI. Data processing was conducted by SPSS 18.0 software. What's more, the linkage disequilibrium of *LTA* polymorphisms in this study was explored by Haploview software and haplotype was analyzed in the occurrence risk of AS. $P < .05$ was considered as the significant difference.

Table 1

The characteristics of the cases and controls.

Index	Case (n = 138)	Control (n = 141)	*P*
Age			
The range/year	15–66	21–73	
Mean age/$\bar{x} \pm s$	24.7 ± 12.2	25.3 ± 11.4	>.05
Gender			
Male/female	85/53	76/65	>.05
Family history/%	27/19.6	13/9.2	<.05
Smoking status/%	51/37.0	39/27.7	>.05
Alcohol consumption/%	47/34.1	42/29.8	>.05

3. Results

3.1. The clinical basic information of subjects in the case and control groups

The basic characteristics of subjects in the 2 group in clinical were showed in Table 1. The age range of AS patients was 15 to 66 years old with the mean age of 24.7 ± 12.2, and the controls were from 21 to 73 years old with the average age of 25.3 ± 11.4. The case group included 85 men and 53 women, the number was 76 men and 65 women in the control group. There was no significant difference between the case and control groups in age and sex distribution ($P > .05$ for both). 37.0% of AS patients were smokers and the ratio was 27.7% in the controls, no significant association was showed between AS occurrence and smoking ($P > .05$). The ratio of drinking in the cases was similar to the controls (34.1% vs. 29.8%). Differently, 19.6% of AS patients had the family history of AS and only 9.2% was in the control group ($P < .05$).

3.2. The association analysis of LTA polymorphisms with AS risk

We explored the allele distribution difference of *LTA* polymorphisms between the case and control groups and the results were showed in Table 2. G allele frequency of rs909253 was more in the cases than that in the controls ($P = .02$), compared with A allele, revealing its association with the risk of AS (OR = 1.53, 95%CI = 1.07–2.18). However, the allele of rs2239704 or rs2229094 did not significantly affect the occurrence risk of AS in this study.

Age, sex, smoking, alcohol, and family history were act as integrative factors to adjust the results. Effects of rs909253 G allele for AS susceptibility also had statistical significant ($P = .03$, OR = 1.52, 95%CI = 1.05–2.15).

The genotype difference of *LTA* polymorphisms between the two groups was also analyzed in Table 3. GG genotype of rs909253 had obviously higher frequency in AS patients than that in the controls (16.67% vs. 8.51%, $P = .02$), indicating that the carriage of GG genotype in rs909253 was a risk factor of AS

Table 2

The allele distribution of *LTA* polymorphisms between the case and control groups.

Allele	Case, 2n = 276/%	Control, 2n = 282/%	χ^2/P	OR (95%CI)	P^*	OR (95%CI)*
rs2239704						
C	189/68.48	182/64.54	—	1.00 (Ref.)	—	1.00 (Ref.)
A	87/31.52	100/35.46	0.97/0.32	0.84 (0.59–1.19)	.34	0.85 (0.60–1.21)
rs909253						
A	172/62.32	202/71.63	—	1.00 (Ref.)	—	1.00 (Ref.)
G	104/37.68	80/28.37	5.47/0.02	1.53 (1.07–2.18)	.03	1.52 (1.05–2.15)
rs2229094						
T	206/74.64	220/78.01	—	1.00 (Ref.)	—	1.00 (Ref.)
C	70/25.36	62/21.99	0.88/0.35	1.21 (0.82–1.78)	.37	1.19 (0.79–1.67)

95%CI = 95% confidence interval, OR = odds ratio.
* *P* and OR values were adjusted by age, sex, smoking, alcohol, and family history using Logistic regressive analysis.

Table 3

The genotype frequencies of *LTA* polymorphisms between the 2 groups.

	Case	Control					
Genotype	N = 138/%	N = 141/%	χ^2/P	OR (95%CI)	P^*	OR (95%CI)*	P_{HWE}
rs2239704							0.52
CC	62/44.93	57/40.42	—	1.00 (Ref.)	—	1.00 (Ref.)	
AC	65/47.10	68/48.23	0.26/0.61	0.88 (0.54–1.44)	.65	0.89 (0.54–1.48)	
AA	11/7.97	16/11.35	1.14/0.29	0.63 (0.27–1.48)	.25	0.60 (0.25–1.43)	
rs909253							0.79
AA	57/41.30	73/51.77	—	1.00 (Ref.)	—	1.00 (Ref.)	
AG	58/42.03	56/39.72	1.21/0.27	1.33 (0.80–2.20)	.39	1.26 (0.75–2.13)	
GG	23/16.67	12/8.51	5.28/0.02	2.46 (1.13–5.35)	.04	2.36 (1.06–5.24)	
rs2229094							0.56
TT	79/57.25	87/61.70	–	1.00 (Ref.)	—	1.00 (Ref.)	
CT	48/34.78	46/32.63	0.29/0.59	1.15 (0.69–1.91)	.85	1.05 (0.62–1.78)	
CC	11/7.97	8/5.67	0.73/0.40	1.51 (0.58–3.96)	.30	1.68 (0.63–4.49)	

95%CI = 95% confidence interval, OR = odds ratio, HWE = Hardy–Weinberg equilibrium.
* *P* and OR values were adjusted by age, sex, smoking, alcohol and family history using Logistic regressive analysis.

(OR = 2.46, 95%CI = 1.13–5.35). After adjusted by age, sex, smoking, alcohol consumption and family history, association between rs909253 GG genotype and AS susceptibility also had statistical significance ($P = .04$, OR = 2.36, 95%CI = 1.06–5.24). Unfortunately, there was not independently correlation between AS occurrence and rs2239704 or rs2229094 in genotype.

3.3. The role analysis of haplotype among LTA polymorphisms in AS occurrence

In the present study, the strong linkage disequilibrium of *LTA* three polymorphisms was found, and three haplotypes were analyzed, that is, C-G-T, A-A-T and C-A-C (rs2239704-rs909253-rs2229094). The detailed information of haplotype was listed in Table 4. A-A-T haplotype frequency was lower in AS patients than that of the controls, compared with haplotype C-G-T. It was suggested that A-A-T might be a protective factor against the occurrence of AS ($P = .05$, OR = 0.67, 95%CI = 0.44–1.01), despite the association was not significant. After adjusted by confounding factors, the association was still not significant ($P = .05$).

4. Discussion

AS is a frequently diagnosed inflammatory disease, which significantly decreases the quality of life of the cases. Unfortunately, there are no effective methods for AS treatment. Until now, management of AS mainly dependents on prevention and early diagnosis. Growing evidences have demonstrated that genetic factors play an important role in etiology of AS. In the

Table 4

The haplotype analysis of *LTA* polymorphisms in the occurrence of AS.

	Haplotype/%					
Site1-site2-site3	Case	Control	χ^2/P	OR (95%CI)	P^*	OR (95%CI)*
C-G-T	104/39.85	80/33.06	—	1.00 (Ref.)	—	1.00 (Ref.)
A-A-T	87/33.33	100/41.32	3.71/.05	0.67 (0.44–1.01)	.05	0.68 (0.45–1.01)
C-A-C	70/26.82	62/25.62	0.38/.54	0.87 (0.55–1.36)	.55	0.87 (0.55–1.36)

Site1 = rs2239704; site2 = rs909253; site3 = rs2229094; OR = odds ratio; 95%CI = 95% confidence interval.
* *P* and OR values were adjusted by age, sex, smoking, alcohol, and family history using Logistic regressive analysis.

current study, we also found that family history was significantly different between AS cases and healthy individuals. The individuals with family history of AS were more likely to present AS than those without family history. To investigate the AS-related genetic factors may provide a new insight into the prevention and detection of AS. Thus, in the current study, we explored the genetic association of *LTA* rs2239704, rs909253, and rs2229094 polymorphisms with the occurrence of AS based on a Chinese Han population.

LTA is located on HLA-III gene region of chromosome 6p, closely linked to *TNF-α* and is consisted of 4 exons and 3 introns. Like *TNF-α*, *LTA* plays an important role in immune activation, inflammatory regulation, and anti-virus response.[17,18] LTA signaling is necessary for the activation of NK cells and may participate in the maturation and recruitment of NK cells.[19] NK cells play an important role in inflammatory response, including AS.[20]

In normal, LTA protein can induce cytokines and cell adhesion molecules from some cells, including vascular smooth-muscle cells, vascular endothelial cells and several kinds of leukocytes, so as to confer to the inflammatory process.[21] However, some mutations in *LTA* influence the inflammatory biological activities. Rs2239704 is a mutation in 5′untranslated region (5′UTR) of *LTA* with the base substitution of C/A and may influence LTA protein production.[22] Rs909253 is located on intron1 region of *LTA* with the mutation of A/G and is associated with high LTA expression.[23] Rs2229094 is a missense mutation with the replacement of Cys/Arg in exon region of *LTA*, which may be correlated to the alteration of *LTA* expression and the increased vascular- and autoimmune-mediated inflammation.[24] Therefore, it is well-founded to investigate the association of *LTA* polymorphisms with the occurrence risk of AS.

In the present study, for the allele of polymorphisms, only G allele in rs909253 showed the significantly higher frequency in AS patients than that of the controls, and showed positive association with increased risk of AS. There was no obviously association with AS in the allele distribution of rs2239704 or rs2229094. What's more, the carriage of the homozygous mutant genotype in rs909253 obviously increased the risk of AS, compared with the homozygous common genotype carriers. However, the genotype distribution of neither rs2239704 nor rs2229094 was significantly different between the case and control groups. Additionally, the strong linkage disequilibrium among *LTA* three polymorphisms was found and haplotype A-A-T marginally associated with the negative susceptibility for AS. In the precious study, Chen et al[25] also explored the role of *LTA* polymorphisms in the occurrence of AS in Ningxia population, 7 SNPs were selected including rs2239704, rs909253, and rs2229094, only rs909253 in *LTA* was significantly associated with the elevated risk of AS. Our results consisted with these results. However, in the study of Wang et al,[26] there was no significant association between *LTA* rs909253 polymorphism with the risk of AS in Jilin population. Ji et al[27] also report that the genotype and allele of *LTA* rs909253 (+252 G>A) were not significantly different between AS patients and healthy controls in Xinjiang population. This inconsistent result in these studies may result from regional divergence, because people living in different regions have different genetic background and the influence of environment. Inconsistent sample size is also the important cause for different results in these studies, additionally, sampling criterion may also affect the final results. Therefore, the exact effect of *LTA* polymorphisms in the occurrence of AS needs to be verified with large samples, multiple populations.

In conclusion, only rs909253 in *LTA* is identified to be significantly associated with AS susceptibility in this study population, revealing the functional roles of *LTA* in the pathogenesis of AS. What's more, the linkage disequilibrium analysis is also verified this view. However, some limitations were found to disturb the veracity of our results, mainly including small sample size, single study population with only one race and interaction analysis. In addition, the specific mechanisms of rs909253 in *LTA* in etiology of AS still remain unclear. Therefore, well-designed studies with large sample size are required to address the above issues.

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