Original Article
Genetic analysis of *TNFST15* variants in ankylosing spondylitis

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Abstract: Aims: The purpose of this study was to explore the role of TNF-like ligand 1A (TL1A) gene (*TNFST15*) polymorphisms (rs3810936, rs7848647, and rs6478109) in the generation of ankylosing spondylitis (AS). Methods: Polymerase chain reaction (PCR) and sequencing were used to conduct the genotyping of *TNFSF15* polymorphisms in 113 AS patients and 120 healthy persons as the case and control groups. The frequencies comparison was performed by chi-square or t test between the two groups. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated to represent the correlation between *TNFSF15* polymorphism and AS. Besides, genotypes distribution of the former in controls was checked by Hardy-Weinberg equilibrium (HWE). Results: There was statistically significant difference in AS patients and controls based on family history. Among *TNFSF15* polymorphisms, only TT genotype frequency of rs3810936 in cases was obviously high, compared with the controls (*P*=0.04), the results indicated that TT was a high-risk genotype (OR=2.31, 95% CI=1.03-5.20). However, both of rs6478109, rs7848647 polymorphisms didn't show any association with AS. Conclusion: Rs3810936 of *TNFSF15* were related to the risk of AS and we should pay more attention to the role of *TNFSF15* polymorphisms in the pathogenesis of AS in the future.

Keywords: *TNFSF15*, polymorphism, ankylosing spondylitis

Introduction

Ankylosing spondylitis (AS) is a kind of chronic disease caused by inflammation response and pathological mineralization and usually strikes the males [1-3]. As epidemiology study shows that AS takes the second place in inflammatory arthritis and the number of AS patients in China is about 3,000,000 and similar to Caucasians in Europe or USA [4, 5]. Incipient symptoms of AS appear before 40 years old and is characterized inflammatory back pain and the ankylosis and stiffness of spinal joints [6]. The radiographic progression of AS is determined within the first 10 years of disease, and increasing researches have indicated that initial structural damage is the best predictor of further damage [7-9]. Besides, HLA B27 has been reported to be closely associated with AS and 90% AS patients accompany with the positive HLA B27 [10]. In the meanwhile, AS is also highly heritable and many genetic polymorphisms have showed the correlation with the onset of AS, such as ANTXR2, JMY, JARID1A and PTGER4 [11, 12]. Although many studies focus on the pathogenesis of AS, the functional mechanism of AS is still poorly understood.

TNF-like ligand 1A (TL1A), also called as Vascular endothelial growth inhibitor (VEGI) or TNF superfamily member 15 (*TNFSF15*), is a anti-angiogenic protein and encoded by *TNFSF15* or VEGI gene located on chromosome 9q32 [13]. Unlike the other TL1 protein, TL1A is expressed in endothelial cells and lymphocytes. It not only inhibits the growth of tumors and induces apoptosis, but also possesses the characteristic of TH1 polarization, which plays an important role in immunoregulation and inflammatory reaction [14]. *TNFSF15* is paid attention in various diseases, including colonic fibrosis, rheumatoid arthritis, atherosclerosis, and several cancers, especially its genetic variant [15-19]. However, there are few researches to care for the association between AS and *TNFSF15* polymorphisms.

In this study, we examined 3 single nucleotide polymorphisms (SNPs) (rs3810936, rs7848647, and rs6478109) of *TNFSF15* in both case

Table 1. Clinical characteristics of case and control groups

Characteristics		Case, n (%)	Control, n (%)	P
Age	Mean age ($\overline{x} \pm s$)	29.85±10.15	31.23±11.31	0.08
Sex				0.93
	Male	90 (79.65)	95 (79.17)	
	Female	23 (20.35)	25 (20.83)	
Family history				0.02
	Yes	32 (28.32)	19 (15.83)	
	No	81 (71.68)	101 (84.17)	
Smoking status				0.90
	Yes	65 (57.52)	68 (56.67)	
	No	48 (42.48)	52 (43.33)	
Exercise habit				0.14
	Yes	40 (35.40)	54 (45.00)	
	No	73 (64.60)	66 (55.00)	
Dorsal kyphosis				0.11
	Yes	72 (63.72)	64 (68.33)	
	No	41 (36.28)	56 (31.67)	

and control groups and aimed to assess the association of *TNFSF15* polymorphisms with AS.

Materials and methods

Study objects

This study adopted a case-control design. The case group consisted of 113 AS patients diagnosed by pathology department of Provincial Hospital affiliated to Shandong University from May, 2012 to December, 2013, including 90 males and 23 females. Their age was between 16-45 with the mean age of 29.85 ± 10.15 and they did not receive any hormone and immune-suppressing drug before participating in this study. Excluded patients included those who suffered from tumors, atherosclerosis, and autoimmune diseases, such as rheumatoid disorders or had the histories of these diseases. 120 healthy people as the control group were recruited from the department of physical examination and rehabilitation in the same hospital with the cases at the same time, covering 95 males and 25 females whose average age was 31.23 ± 11.31. The gender and sex of people in control group were frequency-matched with the cases. All subjected were the Han population without blood relationship and the whole study idea was supported by the Ethics Committee of Provincial Hospital affiliated to Shandong University. Before extracting blood

samples, we informed every participant and obtained the informed consents.

The detailed clinical characteristics of all subjects were collected and recorded through the way of questionnaire, including age, family history, cigarette consumption, excise habit, dorsal kyphosis. The relative data were displayed in **Table 1**.

DNA extraction and genotyping

Genome DNA was extracted from peripheral blood of every subject by the conventional chloroform/iso-amyl alcohol extraction and then stored at -20°C. Genotypes were determined by polymerase chain reaction (PCR) and sequencing. Rs-3810936, rs7848647, and rs64-78109 polymorphisms of *TNFSF15* were selected according to previous studies. Amplified fragment length of rs3810936 was 438 bp and the primers were 5'-TGAACTAGGCCTGG-CCTTCA-3' (forward), 5'-AACTCGGTGGCAGAG-GACTT-3' (reverse); the target fragment of rs-6478109 and rs7848647 was 884 bp and the primers were 5'-AATCCTGGCTACCACTTG-3' (forward), 5'-ATGCACCGCCTACAACAGAA-3' (reverse). For each sample, PCR was carried out in 20 µl reaction mixture which contained 1 µl genomic DNA template, 1 µl 10 × loading buffer (Mg^{2+}), 0.4 µl dNTP, 0.3 µl Taq DNA polymerase, 0.8 µl of each primer (forward and reverse primers) and 15.7 µl sterile ddH_2O. The reaction conditions: initial denaturation at 94°C for 5 min, and then 35 cycles of denaturation at 94°C for 50 s, annealing at 60°C for 30 s, extension at 72°C for 50 s, and final extension at 72°C for 7 min.

After that, the quality and concentration of amplified PCR products were checked using 1% agarose gel electrophoresis (AGE) and Na-noDrop 2000 superfine spectrophotometric instrument. And then eligible PCR products were sequenced to identify the different genotypes in Shanghai Sangon Biotech Co Ltd.

Statistical analysis

All data in this article were represented with $\overline{x}$ ± s or n (%). In the first, the genotypes distribu-

Table 2. Genotype and allele frequencies comparison of *TNFSF15* polymorphisms between the case and control groups

Genotype/ allele	Genotype				Allele			
	Case n (%)	Control n (%)	P	OR (95% CI)		Case, 2n (%)	Control, 2n (%)	P
rs3810936								0.06
CC	27 (23.89)	39 (32.50)	-	1.000	C	116 (51.33)	144 (60.00)	
CT	62 (54.87)	66 (55.00)	0.32	1.36 (0.74-2.47)	T	110 (48.67)	96 (40.00)	
TT	24 (21.24)	15 (12.50)	0.04	2.31 (1.03-5.20)				
rs7848647								0.97
CC	55 (48.67)	56 (46.67)	-	1.000	C	152 (67.26)	161 (67.08)	
CT	42 (37.17)	49 (40.83)	0.63	0.87 (0.50-1.52)	T	74 (32.74)	79 (32.92)	
TT	16 (14.16)	15 (12.50)	0.84	1.09 (0.49-2.41)				
rs6478109								0.78
GG	56 (49.56)	59 (49.17)	-	1.000	G	160 (70.80)	167 (69.58)	
GA	48 (42.48)	49 (40.83)	0.91	1.03 (0.60-1.77)	A	66 (29.20)	73 (30.42)	
AA	9 (7.96)	12 (10.00)	0.62	0.79 (0.31-2.02)				

tion of these three polymorphisms was tested the Hardy-Weinberg equilibrium (HWE). Clinical characteristics of all subjects and genotype frequencies of *TNFSF15* three polymorphisms in the case and control groups were compared by the chi-square or t test. Odds ratio (OR) and 95% confidence interval (CI) were used to decide the risk factors of AS. The whole statistical analysis was performed by SPSS 18.0 software and *P*<0.05 indicated significant difference.

Results

Clinical characteristics of case and control groups

As was shown in **Table 1**, a total of 233 subjects were enrolled in this study, containing 113 cases and 120 controls. The clinical characteristics such as age, sex, smoking status, exercise habits, and dorsal kyphosis had no significant difference between the case and control groups. However, the obvious difference was showed between the two groups based on family history, the results suggested that AS had a family heredity and this is similar to the conclusion of others researches.

Genotypes and alleles comparisons of TNFSF15 polymorphisms in case and control group and the association with AS

All genotypes distribution of *TNFSF15* rs3810-936, rs7848647, and rs6478109 polymorphisms conformed to HWE in controls, which indi-

cated that our study population possessed a regional representativeness.

The results of comparison between the case and control groups were stated in **Table 2**. There was no significant difference in genotype frequencies of rs7848647 or rs6478109 between the two groups (*P*>0.05). Similarly, the allele frequencies of all three polymorphisms (rs3810936, rs7848647, rs6478109) between the case and control groups didn't show any obvious difference (*P*=0.06, 0.97, 0.78). However, referring to the genotypes of rs3810936 polymorphism, the frequencies were 23.89%, 54.87%, 21.24% in cases and 32.50%, 55.00%, 12.50% in controls for CC, CT, TT respectively. TT genotype frequency in two groups had the statistically significant difference (*P*=0.04), the result indicated that rs3810936 might be as candidate susceptibility polymorphism in AS development (OR=2.31, 95% CI=1.03-5.20). Unfortunately, the other two polymorphisms were not the independent risk factors for AS.

Discussion

AS has the obviously clinical characteristics of inflammatory and is a complex autoimmune disease [20]. The etiology of AS may have relationship with many factors, such as heredity, immunity, chronic infection, endocrine dyscrasia, and environment. Many researches also demonstrate that AS closely links to the hereditary, biological and environmental factors. Researchers have found HLA-B27 is closely con-

nected with the nosogenesis of AS by untiring efforts since 1973 [21-23]. More than 90% of AS patients are positive based on HLA-B27 antigen test, but less than 10% of normal persons who antigen test are positive will become AS patients finally [24]. O'Dwyer et al. explored that the relationship between health-related physical fitness and AS in adults, the results demonstrated that decreased body fat, muscular fitness and flexibility, higher aerobic capacity improved the function of health-related physical fitness and benefited to reduce AS occurrence [25]. Zhang et al. drew an conclusion that IL-12B rs6871626 polymorphism showed an significant correlation to the risk of AS and increased the BASDAI, BASFI activity which were the AS activity indexes [26]. In addition, many other genes can also influence the occurrence of AS, such as IL-23, FCRL4b, KIR3DS1 [27-29].

Human TL1A protein consists of 251 amino acids: 35 in the cytoplasmic domain, 24 in transmembrane region, and 192 in the extracellular domain. There are two potential N-linked glycosylation sites in the TL1A amino acid sequence, specifically Asn residues at 133 and 229 amino acids [14]. *TNFSF15* plays an important role in immunoregulation and inflammatory diseases. TL1A not only inhibits the growth of tumors and induces apoptosis, but also combines with special death receptor 3 (DR3), which is a TNF receptor family member containing a cytoplasmic death domain to affect the immunoregulation, to induce the activation of nuclear factor Kappa B (NF-κB), which activate T cell and promote the secretion of inflammatory factors. Konsta et al. found that TL1A was up-related in AS patients and might be associated with disease activity [30]. In the study of Aiba et al., TL1A was also detected the increased expression in serum and inflamed tissues of AS patients and the regulation of TL1A-DR3 interaction might serve as the potential therapeutic goal of AS [31]. Meanwhile, genetic variants have been proved to influence the gene expression. However, TNTSF15 polymorphisms were rarely studied based on AS in either home or abroad.

In this research, three polymorphisms of *TNFSF15* gene were selected to detect the relevance with AS in Chinese Han population. The clinical characteristics were compared between cases and controls and the results revealed that smoking, exercise habit, dorsal kyphosis had no obvious association with AS in our population, but family history was a independent influence factor for AS, which suggested that AS was a familial inherited disease. Only TT genotype frequency of rs3810936 polymorphism was significantly different between two groups. It might be a virulence gene polymorphism locus which could promote the occurrence of AS. Additionally, all of genotype frequencies in rs7848647 and rs6478109 had no statistically significance difference between the two groups. This was the first time to report the correlation between *TNFSF15* rs3810936, rs6478109 rs7848647 polymorphisms and AS susceptibility. This article provided the theoretical evidence for further research.

We obtain several achievements about the role of *TNFSF15* polymorphisms in AS, but some restriction factors should not be avoided to ensure accurate conclusion. Firstly, the sample size is small which only includes the Han population in a finite region. Secondly, beside several genetic variants of *TNFSF15*, environmental and the other factors are not been considered and even the interaction analysis of polymorphisms is avoided. Finally, AS is a complicated and heredo familial disease, single gene is not enough to determine its pathology and etiology.

In summary, *TNFSF15* rs3810936 polymorphism can affect the occurrence of AS. The results of this article may help us understand the genetic and molecular pathogenesis of AS. *TNFSF15* polymorphisms can be used to guide genetic analysis and surgical treatment options. In order to get more accurate conclusion, further studies with larger sample size are required to elucidate the pathogenesis of AS.

Disclosure of conflict of interest

None.

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