Connection for TESPA1 Polymorphisms to ankylosing spondylitis incidence in the Chinese Population

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Abstract

Background

The aim of this study was to investigate whether thymocyte-expressed, positive selection-associated 1 (*TESPA1*) gene polymorphisms were associated with increased risk of developing ankylosing spondylitis(AS) in a Chinese Han population.

Methods

A total of 99 AS patients were recruited as case group and 96 healthy individuals were collected as control group. *TESPA1* polymorphisms were genotyped by polymerase chain reaction (PCR) and sequencing methods. The genotype distribution of *TESPA1* gene rs4758993 and rs4758994 polymorphism was detected by Hardy-Weinberg equilibrium (HWE). The genotype and allele distributions of each polymorphism were also compared between groups. Moreover, odds ratios (OR) with 95% confidence intervals (95%CI) were calculated using the χ^2 test to evaluate the association between AS susceptibility and *TESPA1* polymorphisms.

Results

rs4758993 and rs4758994 polymorphisms were conformed to be in HWE in genotypes distribution of the control group (P > 0.05 for both). A remarkable decrease trend of rs4758993 AG genotype and A allele were detected in AS patients than in healthy controls (*P* = 0.01 and 0.02, respectively), indicating that they obviously decreased the risk of AS in a Chinese Han population (OR = 0.303, 95%CI = 0.144–0.637; OR = 0.002, 95%CI = 0.173–0.703). However, No significant differences were detected for *TESPA1* gene rs4758994 polymorphism in both genotype and allele distributions between case and control groups (*P* > 0.05).

Conclusions

Our findings suggest that *TESPA1* gene rs4758993 polymorphism was significantly associated with AS susceptibility in the Chinese Han population and the mutant A allele severed as a protect factor for the development of AS.

Background

Ankylosing spondylitis (AS) is the most common chronic inflammatory disorder of spondyloarthropathy (SpA) involving the axial skeleton, sacroiliac joint, and periphery joints[1–3]. AS is generally characterised by sacroiliac joint and spine attachment point inflammation, which can cause spine fibrosis and poker spine, leading to damage of muscle, skeleton, and lung function[4, 5]. The increasing prevalence of AS

lead to heavy economic and social burdens for patients and their families in China. Growing evidence indicates that AS is caused by genetic and environmental factors. And genetic factors play the leading role in the onset of AS[6, 7]. Moreover, with the development of molecular biology and modern genetics many AS-associated genes have been discovered, such as pentraxin3 gene (PTX3), protein tyrosine phosphatase non-receptor type 22 (PTPN22) and IL-23R[8–10]. But these efforts fail to completely explain the etiology of AS, and more related factors need to be discovered.

TESPA1, a component of the T cell receptor (TCR) signalosome, is essential for T cell selection and maturation through the regulation of TCR signaling during T cell development demonstrated by the team of Wang D[11]. Another study reported that *TESPA1* can as a novel binding partner of IP3R in the T and B lymphocytes, which shed light on the molecular mechanism underlying calcium signaling through the regulation of inositol 1, 4, 5-trisphosphate receptor (IP3R) in the immune system[12]. The IP3R is a calcium channel expressed on ER membranes, which also can be regulated by many intracellular modulators such as Ca^{2+}, phospho rylation, and associated proteins [13–15]. These studies provide new evidence for mechanisms of *TESPA1* gene related diseases in diagnosis and treatment. The study carried out by Wang C et al. reported that changes in the Th1/Th2 and Th17/Treg ratios are evidence for the suffering of AS[16]. In a recent study, Yao Y et al. found that *TESPA1* may be associated with B cell function and the onset of rheumatoid arthritis(RA)[17]. Few studies have explored the exact function of *TESPA1* in AS. Therefore, in this study our aim was to analyze whether *TESPA1* gene polymorphisms are associated with risk of AS.

In the present study, we selected the common SNPs (rs4758993 and rs4758994) of *TESPA1* to investigate their influence on AS development. And we explored the relationship between the genetic polymorphisms of the *TESPA1* gene and the risk of AS in Chinese Han population.

Methods

Subjects

In this study, a total of 99 patients (47 men, 42 women; mean age: 40.09 ± 18.36 years) with AS without other immune-related diseases were enrolled, along with 96 unrelated healthy volunteers without a family history of AS or other immune-related diseases, who were matched for age and gender. All subjects were all from Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University from March 2014 to March 2016 and they had no blood relation with other study subjects. We excluded patients with other immune or inflammatory diseases, tumors, or cardiovascular disease. The controls underwent physical examination in the same hospital.

This study was approved by the Research Ethics Committee of Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University and Informed consent was obtained from all of the patients. All individuals are from the Han Chinese population.

Sample collecting

5 ml fasting peripheral venous blood was collected from each subject using a blood collection tube. Blood samples were immediately centrifuged at 3000 rpm for 10 min, and the supernatant was kept at -80°C for further analyses.

DNA extraction and genotyping

The genomic DNA was extracted by TaKaRa Genome DNA Extraction Kit (Dalian Biological Engineering CO., LTD, China), according to the manufacturer's instructions, and was stored at -20℃. Polymerase chain reaction (PCR) was conducted for genotyping of *TESPA1* polymorphisms. The primer sequences for *TESPA1* gene rs4758993 and rs4758994 polymorphisms were designed by Primer Premier 5.0 software, and were synthesized by Sangon Biothch (Shanghai, China) (**Table 1**). The PCR procedure was run according the following steps: 95℃ pre-degeneration for 5 min, 30 cycles of degeneration at 95℃ for 30 s, 57℃ annealing for 30 s, 72℃ extension for 30 s, and final extension at 72℃ for 5 min. Genotyping of *TESPA1* gene rs4758993 and rs4758994 was completed by sequencing these PCR products using the genomic DNA as templates.

Statistical analysis

All data showed was conducted using PASW Statistics 18.0 statistical software. Hardy-Weinberg disequilibrium in controls was evaluated using the chi-square test based on 2 *TESPA1* polymorphisms. Relative risk of AS based on *TESPA1* polymorphism is represented with odds ratios (OR) and 95% confidence intervals (95%CI). The comparison among genotype and allele were measured by the χ^2 test. The *P* value less than 0.05 was considered as statistically significant.

Results

HWE test

The genotype distributions of both *TESPA1* rs4758993 and rs4758994 polymorphisms in both case and control groups. The genotype distributions of each SNP all had non-significant *P* values ($P > 0.05$), which showed that this study group was a representative Mendelian population.

Distributions of TESPA1 gene polymorphisms between groups

Genotype, allele frequencies of *TESPA1* polymorphisms, and their effects on AS risk were shown in Table 2. The distribution of genotypes and alleles of rs4758993 was significantly different between the case and control groups. Compared with control groups, the AG genotype frequency in the case was remarkable decreased (31.25% VS 12.12% $P = 0.01$) and it showed a significant association with the risk of AS (OR = 0.303, 95%CI = 0.144–0.637). However, the frequencies of rs4758993 GG and AA genotypes were respectively 87.88%, 0% in AS patients and 68.75%, 0% in controls respectively. Data showed that

the GG and AA genotypes frequencies were no differences between groups ($P > 0.05$). Meanwhile, lower frequency of A allele was also found in case group compared with control groups (6.06% vs 15.62%), and the results also indicated that the A allele of rs4758993 could meaningfully decrease AS incidence (OR = 0.348, 95%CI = 0.173–0.703, $P = 0.02$). All results suggested that *TESPA1* gene rs4758993 polymorphism was associated with AS susceptibility in the Chinese Han population, and the AG genotype and A allele were protect factor for the onset of AS. Frequencies of rs4758994 GG, GA and AA genotypes were respectively 13.13%, 38.38%, 48.49% in AS patients and 12.5%, 33.33%, 54.17% in controls. And the A and G allele frequencies were 32.32%, 67.68% in case group, and 29.17%, 70.83% in control group respectively. Data showed that neither genotype nor allele of rs4758994 polymorphism were associated with the risk of AS ($P > 0.05$).

Discussion

AS, a common autoimmune disease, has a complicated pathogenesis mechanism that is closely related to the body's immune functions[18]. AS is one of the seronegative spondyloarthropathies, which can give rise to varying degrees of complications including eyes, lungs, muscle and bone lesions[19]. The etiology of AS is complex, but a lot of work has confirmed that genetic and environmental factors contribute to its etiology. It has been indicated that T helper cells (Th cells), a type of T cell, play an important role in the regulation of immune response and the balance of Th1/Th2 cells determines the response of the immune system[20]. The preferred treatment of AS is through a multidisciplinary approach that involves exercise, physiotherapy, and drug therapy[21, 22]. It is believed that AS is a polygenic disease caused by the combined influence of environmental and genetic factors. In recent years, it has become the most promising molecular genetics research method to investigate the genetic polymorphisms of candidate genes of AS onset.

TESPA1 gene interacts with Lat signalosome that formed in the TCR signaling, which play a key role in the regulation of T cell development. *TESPA1* is located on human chromosome 12 with 36729 bp in size[23]. Liu S et al.'s work found no association between the four polymorphisms (rs1801876, rs2171497, rs4758994, and rs997173) of the *TESPA1* gene and AS in Chinese adults, but may play an important role in the clinical characteristics of AS[24]. However, recent investigations have indicated that the correlation between the allele (G) in rs4758993 and familial RA is relatively significant, suggesting that *TESPA1* may play an important role in RA patients[17]. Therefore, in this study we evaluated the link between Tespa1 polymorphisms with the occurrence and disease activity of AS.

In this research, we investigated the effects of *TESPA1* rs4758993 and rs4758994 polymorphisms on AS susceptibility. The results showed that rs4758993 was significantly associated with the individual susceptibility to AS, but independent association was not detected based on genotypes or alleles of rs4758994 for the risk of AS. In single nucleotide polymorphism analysis, the data showed that people with rs4758993 GA genotype showed a significant lower risk to suffer from AS, compared with the AS with GG and AA genotypes. Besides, the major allele (G) inrs4758993 may be a risk allele associated with AS. A allele of rs4758993 had distinctly low frequency in AS patients. It suggested that A allele was

distinctly correlated with reduced AS risk. Present results were conformed with previous study. A major study has presented that *TESPA1* rs4758993 SNP shows significant relationship with RA development in the Zhejiang Han population[17]. In the present study, we examined the association between rs4758994 polymorphism and susceptibility to AS in a Chinese Han population. The allele and genotype frequencies of rs4758994 in patients and healthy controls are shown in Table 2. Although no significant association of rs4758994 with AS susceptibility in this paper, it should be replicated in more other studies to verify the results.

Conclusions

In conclusion, results from this case-control study revealed that *TESPA1* rs4758993 polymorphism, but not rs4758994, significantly decreases the risk of AS in the Chinese Han population. However, many limitations in present study should not be ignored. Further genetic studies of *TESPA1* gene polymorphisms in more haplotype blocks and various populations are necessary.

Abbreviations

thymocyte-expressed, positive selection-associated 1 (*TESPA1*)

ankylosing spondylitis(AS)

polymerase chain reaction (PCR)

Hardy-Weinberg equilibrium (HWE)

odds ratios (OR)

95% confidence intervals (95%CI)

spondyloarthropathy (SpA)

protein tyrosine phosphatase non-receptor type 22 (PTPN22)

T cell receptor (TCR)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Beijing Tsinghua Changgung Hospital, School of Clinical Medicine, Tsinghua University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

H.L., D.W., D.H. design of the work; J.D., J.Z., K.X. the acquisition, analysis, D.H., J.L., W.H. interpretation of data; Y.W., S.X. the creation of new software used in the work; L.F., H.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Tables

Table 1. Primer sequences of *TESPA1* gene two polymorphisms rs4758993 and rs4758994

SNP		Primer sequences	Annealing temperature (°C)
rs4758993	Sense	5'-GCT GAG GGTAAG AAG TTG C-3'	56.5
	Reverse	5'-CGT ATT GGT GGC TGG TAG- 3'	
rs4758994	Sense	5'-CAGGCTGGAAGGCTCAGGATCT-3'	57.7
	Reverse	5'-GACCAAGCTCCTTTGGGCAGAC- 3'	

Table 2. Genotype and allele distributions of *TESPA1* gene rs4758993 and rs4758994 polymorphisms in case and control groups

Genotype/ Allele	Case *n*=99(%)	Control *n*=96(%)	χ^2	P	OR(95% CI)
rs4758993					
GG	87(87.88)	66(68.75)	-	-	1
AG	12(12.12)	30(31.25)	10.553	0.001	0.303(0.144-0.637)
AA	0(0)	0(0)	-	-	-
G	186(93.93)	162(84.38)	-	-	1
A	12(6.06)	30(15.62)	9.279	0.002	0.348(0.173-0.703)
P_{HWE}	0.521	0.070			
rs4758994					
GG	13(13.13)	12(12.5)	-	-	1
GA	38(38.38)	32(33.33)	0.039	0.844	1.096(0.439-2.736)
AA	48(48.49)	52(54.17)	0.128	0.720	0.852(0.354-2.049)
G	64(32.32)	56(29.17)	-	-	1
A	198(67.68)	136(70.83)	1.280	0.258	1.274(0.837-1.939)
P_{HWE}	0.222	0.058			