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Association of Genetic Variants in Pentraxin 3 Gene with Ankylosing Spondylitis

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Study Design A
Data Collection B
Statistical Analysis C
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Background: Pentraxin 3 is considered to play an important role in immune and inflammatory reaction. This study aimed to detect the effect of pentraxin3 gene (*PTX3*) polymorphisms on ankylosing spondylitis (AS) risk.

Material/Methods: The genotyping of *PTX3* polymorphisms in 101 AS patients and 93 controls was conducted by allelic discrimination assay and the genotype distribution was assessed for Hardy-Weinberg equilibrium (HWE). The differences of genotype, allele, haplotype, and some basic indexes were compared by χ^2 test. Odds ratio (OR) and 95% confidence interval (95%CI) were also calculated by χ^2 test and were used to evaluate the association intensity between gene polymorphisms and disease. Haploview software was used to analyze the linkage disequilibrium (LD) and haplotypes of *PTX3* polymorphisms.

Results: CC genotype of rs3816527 had an obviously higher frequency in cases than in controls and had a positive effect on AS occurrence (OR=3.14, 95%CI=1.04–9.52), and the same was true of the C allele in rs3816527. For rs3845978, CT genotype showed a significant frequency difference between the case and control groups (*P*=0.03) and people with genotypes carrying the T allele developed AS earlier (OR=1.94, 95%CI=1.09–3.47), and the same was found in the analysis of the T allele. G-C-T haplotype dramatically increased the risk of AS, as may A-C-C haplotype.

Conclusions: In *PTX3* polymorphisms rs3816527 and rs3845978 were found to be associated with AS, but rs2305619 was not.

MeSH Keywords: **Ampicillin • Haplotypes • Polymorphism, Genetic • Spondylitis, Ankylosing**

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Background

Ankylosing spondylitis (AS) is a chronic and systemic inflammatory disease with negative seroreaction; it is one of the most serious types of spondyloarthropathy (SpA) [1,2]. It mainly attacks the spine and involves the sacroiliac and surrounding joints. It is characterized by acute episodes, chronicity, disease progression, disability, and pain [3]. The rate of AS is nearly 0.2–1.0% in whites [4,5]. In China, the prevalence of AS is increasing steadily and causes heavy economic and social burdens for patients and their families. The exact pathogenesis of AS still is not known. Studies have reported that AS is a complex polygenetic disease influenced by environmental factors [6–9]. Multiple genes and genetic variants have been explored for effects on AS development.

Pentraxin 3 (PTX3) is a member of the pentraxin superfamily, which participate in the processes of acute inflammation and immune response reaction [10]. The PTX3 protein is encoded by the *PTX3* gene, located on chromosome 3q25 [11]. When the human body suffers from inflammation and injury, the mononuclear phagocytes, fibroblasts, dendritic cells (DCs), and endothelial cells rapidly produce and release PTX3 to respond to the inflammatory signals [12–14]. Previous research reports that PTX3 can regulate leukocyte aggregation to modulate the inflammatory reaction [15]. As an acute-phase protein, PTX3 generally occurs at low levels, but it significantly increases in conditions of inflammation and infection, and the increasing level is positively correlated with the severity of disease. The level of PTX3 in the blood is a biomarker for the diagnosis and prognosis of some diseases [16, 17]. In recent years, increasing research has focused on the association between the single-nucleotide polymorphism (SNP) of *PTX3* and diseases. However, there still few reports about the effect of *PTX3* polymorphism on AS.

In the present study, 3 SNPs of *PTX3* were selected – rs2305619, rs3816527, and rs3845978 – to explore their relationship with AS susceptibility. The roles of single polymorphism and haplotype were analyzed to ensure the association. Through this research, we hoped to find a marker of AS development in the *PTX3* gene.

Material and Methods

Study Population

We recruited 194 unrelated subjects, including 101 patients with AS and 93 healthy volunteers as the case and control groups, respectively, from April 2012 to December 2013. The cases, diagnosed clinically and by pathobiology in the Affiliated Hospital of WeiFang Medical University, consisted of 59 males and 42 females, with an age range of 17–45 years old. We excluded patients with other immune or inflammatory diseases, tumors, or cardiovascular disease. The controls underwent physical examination in the same hospital as the case group, showing that the control subjects were healthy. The controls were required to match the cases in sex and age, so there were 61 males and 32 females in the control group and their age range was 15–49 years with a mean age of 26.88±9.31. The study was approved by the Research Ethics Committee of our hospital. Participants were informed about the entire experiment and provided written consent before collection of samples.

In this study, the detailed clinical information of all subjects was surveyed and recorded, including age, sex, body mass index (BMI), cigarette and alcohol consumption, and the expression level of PTX3. Smokers were defined as people who smoked more than 1 cigarette every day for at least 2 years, and drinkers were defined as people who drank liquor or beer at least twice a week.

ELISA assay and DNA extraction

We drew 3 ml of venous blood from every subject on an empty stomach in the morning; 1 ml of the blood sample was centrifuged to separate plasma at 4000 rpm for 10 min. Plasma PTX3 was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Aviscera Bioscience Inc., USA) according to the manufacturer's instructions. The other 2 ml of the blood sample was used to extract genomic DNA by use of the TIANGEN blood genomic DNA extraction Kit (BEIJING TIANGEN BIOTECH CO., LTD) and stored at –20°C for genotyping in the next step.

Genotyping

Polymorphisms with global minor allele frequencies (MAF) of more than 0.05 in the Chinese Han population were selected in our study. Finally, the present study included 3 *PTX3* polymorphisms (rs2305619, rs3816527, and rs3845978).

The genotyping of these 3 polymorphisms were conducted using the method of Barbati et al. [18] in which allelic discrimination assay was used based on an ABI 7900 sequence detection system. Fluorescent data were analyzed by Sequence Detection System 3.2 (Applied Biosystem, ABI) and TaqMan Assay Kits in real-time polymerase chain reaction (RT-PCR) purchased from ABI. All steps were performed according to the manufacturer's instructions. To ensure the allelic discrimination accuracy, all samples were measured in 3 replicates.

Statistical analysis

All data are shown by $\bar{\chi}\pm s$ or%. Hardy-Weinberg disequilibrium in controls was evaluated using the chi-square test based

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Table 1. Demographic characteristics of the cases and controls.

Indexes	Cases (n=101)	Control (n=93)	*P*
Age (years)	27.10±11.29	26.88±9.31	0.10
Sex (male/female), n (%)	59(58.42)/42(41.58)	61(65.59)/32(34.41)	0.30
Body mass index (kg/m^2)	25.31±3.56	23.54±3.14	0.01
Smoking, n (%)	38 (37.62)	27 (29.03)	0.21
Drinking, n (%)	23 (22.77)	16 (17.20)	0.33
PTX3, ng/mL	3.24±0.76	2.26±0.11	0

on 3 *PTX3* polymorphisms. To assess the association of *PTX3* SNPs with AS risk, odds ratio (OR) and 95% confidence interval (CI) were calculated with the χ^2 test. The *t* test was used to assess age distribution and PTX3 expression differences between the cases and controls. The comparison among genotype, allele, haplotype, sex, cigarette smoking, and alcohol consumption were measured by the χ^2 test. Differences in BMI and plasma PTX3 level between cases and controls were assessed by *t* test or Mann-Whitney test. The above data analyses were conducted using PASW Statistics 18.0 software and the statistical significance was set at $P<0.05$. The linkage disequilibrium (LD) was detected by Haploview.

Results

Patient characteristic

The characteristics of the subjects are listed in Table 1. In this study population, there were more males – 58.42% in cases and 65.59% in controls – but there were no significant differences in age and sex between AS patients and healthy controls ($P>0.05$). Similarly, cigarette and alcohol consumption were also not associated with the onset of AS ($P>0.05$). However, in AS patients BMI was significantly higher than in controls and overweight people were at greater risk of AS ($P=0.01$). It was worth noting that the expression level of PTX3 protein in cases also remarkably increased compared to the controls ($P=0.01$).

The association between *PTX3* SNPs and AS

The genotype and allele information of *PTX3* gene polymorphisms are summarized in Table 2. The distribution of genotypes and alleles of rs2305619 was not significantly different between the case and control groups ($P>0.05$). However, in rs3816527, compared with the common genotype AA, the CC genotype frequency in the 2 groups was significantly different ($P=0.04$) and it showed a significant association with the risk of AS (OR=3.14, 95%CI=1.04-9.52). C allele carriers were at greater risk of AS (OR=1.65, 95%CI=1.06-2.56). In the analysis of rs3845978, CT genotype, but not TT genotype, was revealed to be associated with increased risk of AS, (OR=1.92, 95%CI=1.05–3.51). Carrying T allele in the genotype was also a risk factor for AS development (OR=1.94, 95%CI=1.09–3.47). T allele itself also conferred a 1.68 times greater risk of AS in our study population (OR=1.68, 95%CI=1.04–2.70).

The LD status and haplotype analysis

Rs2305619, rs3816527, and rs3845978 polymorphisms were involved in a block of *PTX3* gene in Haploview and a strong LD existed among these 3 polymorphisms. They made up 5 haplotypes in this population and the detailed information is shown in Table 3. Among them, G-C-T haplotype frequency was obviously higher in AS patients than in healthy people ($P=0.02$) and it was closely correlated with AS risk (OR=2.86, 95%CI=1.12–7.26). In addition, haplotype A-C-C might be a risk factor in the onset of AS (OR=1.63, 95%CI=0.97–2.73).

Discussion

As a rheumatic inflammatory disease, AS mainly involves the axial skeleton. A clinical symptom of AS is inflammatory back pain, and over time patients may develop spine rigidity and loss of mobility [19]. Studies proved that AS mostly attacks people 20–30 years old, especially men [20], so it causes a heavy burden on society and the economy. Due to its unclear pathogenesis, the prevention and treatment of AS are not adequate. Recently, several hypotheses about the pathology of AS have been proposed, such as the genetic hypothesis, the unfolded protein response (UPR) hypothesis, and the immune, bacterial infection, and endocrine factor hypotheses, and TNF-α is also considered to be involved in the onset of AS, but these are rather one-sided approached to the problem.

Two phenomena have been verified in the pathogenesis of AS: inflammation and ossification [21,22]. They are independent of each other in terms of the body's reaction, but they are also linked to each other. Entheseal stress caused by infection,

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Table 2. The relationship between genotype distribution of *PTX3* polymorphisms and AS susceptibility

Polymorphism	Genotypes/alleles		Case/control	OR (95%CI)	*P*	P_{HWE}
rs2305619	Genotype	GG	39/37	1.00 (Ref.)	–	0.09
		AG	54/49	1.05 (0.58–1.89)	0.88	
		AA	8/7	1.08 (0.36–3.29)	0.89	
		AG+AA	62/56	1.05 (0.59–1.87)	0.87	
	Allele	G	132/123	1.00 (Ref.)	–	
		A	70/63	1.04 (0.68–1.58)	0.87	
rs3816527	Genotype	AA	43/52	1.00 (Ref.)	–	0.70
		AC	45/36	1.51 (0.83–2.74)	0.17	
		CC	13/5	3.14 (1.04–9.52)	0.04	
		AC+CC	58/41	1.71 (0.97–3.02)	0.06	
	Allele	A	131/140	1.00 (Ref.)	–	
		C	71/46	1.65 (1.06–2.56)	0.03	
rs3845978	Genotype	CC	50/61	1.00 (Ref.)	–	0.73
		CT	44/28	1.92 (1.05–3.51)	0.03	
		TT	7/4	2.14 (0.59–7.71)	0.24	
		CT+TT	51/32	1.94 (1.09–3.47)	0.02	
	Allele	C	144/150	1.00 (Ref.)	–	
		T	58/36	1.68 (1.04–2.70)	0.03	

HWE – Hardy-Weinberg equilibrium.

Table 3. The distribution of haplotypes in *PTX3* polymorphisms between the case and control groups in this study population.

Haplotype		Case, 2n=202	Control, 2n=186	*P*	OR (95%CI)
rs2305619-rs3816527-rs3845978	G-A-C	74	87	–	1.00 (Ref.)
	A-C-C	54	39	0.06	1.63 (0.97–2.73)
	G-A-T	41	29	0.08	1.66 (0.94–2.93)
	A-A-C	16	24	0.50	0.78 (0.39–1.59)
	G-C-T	17	7	0.02	2.86 (1.12–7.26)

micro-lesions, and biomechanics leads to acute inflammatory reaction and the activation of progenitor cells. In some circumstances, these acute events subside as soon as they restore the homeostasis, but in particular cases, acute events develop into chronic events that show inflammation and ossification. Genetic factors may regulate the chronic inflammation and participate in the alterations of joints and surrounding tissues in AS patients. For example, Xu et al. analyzed the studies of many scholars and found that interleukin receptor gene polymorphisms (rs7517847 and rs2201841) are significantly correlated with AS [23].

PTX3 gene encodes the prototypic long pentraxin PTX3 first identified in the early 1990s [24]. It is induced by some proinflammatory factors, such as leukocyte-specific protein (LSP), interleukin-1 (IL-1), and tumor necrosis factor-α (TNF-α) in endothelial cells, monocytes, and myocardial cells. As a dissoluble model recognition receptor, PTX3 plays an important role in innate immune reaction and is closely correlated with immune function, infectious diseases, and atherosclerosis of the coronary artery. It has been reported that PTX3 expression level was elevated in patients with systemic lupus erythematosus (SLE), which is a chronic inflammatory disease, and was

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associated with the disease activity [25]. Mabrouk et al. and Luchetti et al. found that increased PTX3 level regulates the inflammatory processes involved in cardiovascular disease in rheumatoid arthritis patients [26,27]. Similarly, as a rheumatic inflammatory disease, AS occurrence may be associated with PTX3 level. Furthermore, genetic polymorphism influences the gene and relative protein expression level, which is not rare. Therefore, exploring the association of *PTX3* polymorphisms with AS susceptibility is feasible and essential.

In the basic clinical information of subjects, we found that BMI index was significantly higher in AS patients than in healthy people and the content of PTX3 protein remarkably increased in the case group, so they may be the risk factors for AS, but not cigarette or alcohol consumption. These results are consistent with those of Deniz et al. [28]. Patients with inflammatory diseases have higher PTX3 concentration compared with normal controls [29]. Another study indicated that *PTX3* polymorphisms are associated with plasma PTX3 levels [18], showing that individuals with AA genotype of rs2305619 and CC genotype of rs3816527 have high plasma levels of PTX3. Therefore, the *PTX3* polymorphisms might play roles in the development of AS. Investigation of the association between *PTX3* single polymorphism and AS susceptibility shows that rs2305619 does not have a direct association with the risk of AS development. However, the CC genotype and C allele frequencies in rs3816527 were remarkably higher in AS patients than in controls, and increased the risk of AS in our study group. Similarly, in rs3845978, CT genotype increased the risk of AS development, and carrying T allele of rs3845978 in the genotype was also a risk factor. In the combined analysis of the 3 *PTX3* SNPs, a strong LD was detected; therefore, haplotype analysis was part of our design. G-C-T haplotype carriers had a higher risk of developing AS than G-A-C haplotype carriers, and A-C-C haplotype may also be a susceptible haplotype in our study population.

Conclusions

We found that *PTX3* rs3816527 and rs3845978 polymorphisms were associated with the generation and development of AS. However, our study has some limitations. Firstly, this result was established only in our study population, so we cannot generalize our results to other populations. Secondly, the small sample size reduces the power of our study. Thirdly, other factors that may contribute to the occurrence of AS were not included in the present study. Considering the possible environmental factors involved, larger samples from different populations and countries are needed to verify the present conclusions.

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