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Original Article

Association between *HRH4* polymorphisms and ankylosing spondylitis susceptibility

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Abstract: Target: The purpose of the study was to investigate the association between the histamine H4 receptor (HRH4) polymorphisms and the susceptibility to ankylosing spondylitis (AS). Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to analyze the HRH4 rs8088140 and rs657132 polymorphisms. Linkage disequilibrium and haplotype analyses were conducted with Haploview software. The genotypes distributions of HRH4 polymorphisms in the control group were tested by Hardy-Weinberg equilibrium (HWE), allele, genotype and haplotype frequencies between the cases and control groups were compared by χ^2 test. The controls were matched with cases by age and gender. The relative risk of AS with HRH4 polymorphisms was represented by odds ratio (OR) with 95% confidence interval (CI) calculated by χ^2 test. Results: The genotypes distributions of HRH4 rs8088140, rs657132 polymorphisms in controls conformed to HWE. The frequency of rs657132 AA genotype in the case group was obviously higher than that in the control group (P=0.040), and so was the A allele (OR=2.572, 95% CI=1.475-4.486, P=0.022). The frequency differences of A-A haplotype between two groups had statistical significance (P=0.011, OR=2.071, 95% CI=1.172-3.660) through haplotype analysis, indicating A-A might be the susceptible haplotype to AS. Conclusion: The AA genotypes of HRH4 rs657132 polymorphism may be the susceptible factors for AS, and rs657132 plays a role in generation of AS. In addition, A-A haplotype in rs8088140-rs657132 is also increased the risk of AS.

Keywords: *HRH4*, polymorphism, ankylosing spondylitis

Introduction

Histamine is a bioactive amine widespread in the body, and exerts various biological functions through the regulation by specific histamine receptors. There are four members in histamine receptor family, namely histamine H1, H2, H3 and H4 receptors [1, 2]. Among them, histamine H4 receptor, an important active substance of the human body, plays an extensively and momentously physiological role in the pathophysiologic process of many diseases. Widely distributing in body tissues, *HRH4* is a new histamine receptor found by Oda et al. in 2000 with high expression in bone marrow and white blood cells (WBC) [3]. Many studies show that *HRH4* is related to allergy, inflammation and immune response, while some other researchers suggest that *HRH4* may be involved in asthma, rheumatoid arthritis and the processes of some tumors [4]. *HRH4* has been demonstrated to have unique pharmacological properties and special tissue distribution, which may affect the immune response and hematopoiesis of the body [5-8].

Ankylosing spondylitis (AS) is a chronic inflammatory autoimmune disease and mainly affects the spine, sacroiliac joint and surrounding joints in different degrees, which leads to back pain and stiffness and sharply decreases the life quality of patients [9-11]. The generation and development of AS are associated with various factors, including biological, genetic and environmental factors. The genetic factors attribute to 90% of overall AS susceptibility based on twin and family studies [12, 13]. Recently, a number of researchers pay attention to the

Table 1. Primer sequences of the amplified fragments

SNP		Primer sequence	Length
rs8088140	For	5' ATCCCCCTTAGAAGATTATG 3'	356 bp
	Rev	5' ACATAATCTTCTAAGGGGGA 3'	
rs657132	For	5' TAAGATAGTAAAAGAAGGGT 3'	360 bp
	Rev	5' ACCCTTCTTTTACTATCTTA 3'	

effect of gene polymorphism on AS development. So far, multiple genetic variants of genes have been proved to be associated with AS, such as *IL1*, *IL23R*, *ERAP1*, *CYP2D6*, and *TGF-β1*. Even then, the reports about the association between *HRH4* gene polymorphisms and AS occurrence are few.

In present study, two polymorphisms of *HRH4* (rs8088140, rs657132) were selected to investigate the role in the development and progression of AS. 130 patients with AS and 116 healthy persons were conducted the genotyping and statistics of relative indexes. We hoped to provide some evidences for the pathology and etiology of AS.

Materials and methods

Clinical data of study subjects

130 patients (71 males, 59 females) as the control group hospitalized in the rheumatology and orthopedics of Chinese People's Liberation Army General Hospital during Dec 2008 and Jun 2010 were diagnosed with AS by regular examination, X-ray film, CT and MRI and all were in accordance with the AS diagnostic criteria revised in New York in 1984 [14]. All the patients aged 22~45 were not related by blood, and received no radiotherapy or chemotherapy before sampling. The cases did not receive systematical or topical medications before onset, neither did they have history of other system diseases. 116 healthy people undergone physical examination were enrolled in the control group from the hospital during the same period. They were Chinese Han population aged 18~52 and unrelated to each other living in 28 Fuxing Road, Beijing. There was no difference in age or sex between two groups. According to the national ethics guidelines for human genome research, our study was conducted under the permission of the Ethics Committee of Chinese People's Liberation Army General Hospital and the informed consensus of all the subjects.

Collection of DNA

2 mL fasting venous blood was collected in the morning from every participant and conducted anticoagulation with EDTA. Genome DNA was extracted with the chloroform/isoamyl alcohol extraction and preserved at -20°C for later.

Polymerase chain reaction (PCR) amplification and the genotyping

PCR-restriction fragment length polymorphism (PCR-RFLP) was operated for the genotyping of *HRH4* rs8088140, rs657132 polymorphisms. The PCR primer was designed by Primer 5.0 software and the sequences were synthesized by Shanghai Sangon Biotech Co., Ltd (**Table 1**). The PCR reaction was performed in a total volume of 15 μL, including 1 μL DNA template, 0.5 μL forward primer, 0.5 μL reverse primer, 7.5 μL Master Mix and 5.5 μL ddH_2O. PCR reaction conditions were as follows: initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 95°C for 30 s, at 60°C, 30 s for annealing and at 72°C, 30 s for extension; and a final extension at 72°C for 10 min. The PCR products were digested respectively with restriction enzymes *MspI*, *HinfI* and determined the genotypes of every genetic variants by 2% agarose gel electrophoresis.

Statistical analysis

The χ^2 test was used to compare the differences of genotype and allele frequencies between two groups with PASW Statistics 18 software and the difference had statistical significance if $P<0.05$. The Haploview software was employed to perform the linkage disequilibrium (LD) and haplotype analyses. PLINK1.07 software was utilized to check the Hardy-Weinberg equilibrium (HWE) deviation in controls. Odds ratio (OR) and 95% confidence interval (95% CI) represented the relative risk (RR) of AS in *HRH4* rs8088140, rs657132 polymorphisms.

Results

Comparison of basic information between two groups

The control group contained 62 males and 54 females with a mean age of 40.61±8.25. The case group had 130 patients (71 males, 59 females) with a median age of 37.89±10.92.

Table 2. Comparison of the genotype and allele distributions of *HRH4* rs8088140 and rs657132

Genotype/ Allele	Cases (n=130)	Controls (n=116)	χ^2	*P*	OR (95% CI)
rs8088140					
CC	61 (46.9)	57 (49.1)	-	-	1.000 (Ref.)
CA	57 (43.9)	49 (42.2)	0.097	0.756	1.087 (0.643-1.838)
AA	12 (9.2)	10 (8.7)	0.060	0.806	1.121 (0.450-2.796)
C	179 (68.8)	163 (70.3)	-	-	1.000 (Ref.)
A	81 (31.2)	69 (29.7)	0.115	0.734	1.069 (0.727-1.571)
rs657132					
GG	54 (41.5)	63 (54.3)	-	-	1.000 (Ref.)
GA	60 (46.2)	46 (39.7)	2.430	0.119	1.522 (0.897-2.582)
AA	16 (12.3)	7 (6.0)	4.214	0.040	2.667 (1.021-6.962)
G	168 (64.6)	172 (74.1)	-	-	1.000 (Ref.)
A	92 (35.4)	60 (25.9)	5.207	0.022	1.570 (1.064-2.315)

Table 3. The haplotype analysis of rs8088140and rs657132 polymorphisms based on AS

Haplotype site1-site2	Cases (2n=260) (%)	Controls (2n=232) (%)	χ^2	*P*	OR (95% CI)
C-G	134 (51.5)	124 (53.4)	-	-	1.000 (Ref.)
C-A	45 (17.3)	39 (16.8)	0.068	0.795	1.068 (0.652-1.749)
A-A	47 (18.1)	21 (9.1)	6.432	0.011	2.071 (1.172-3.660)
A-G	34 (13.1)	48 (20.7)	2.731	0.098	0.655 (0.397-1.084)

Note: site1: rs8088140; site2: rs657132.

No significantly statistical difference existed in age and sex between two groups (*P*>0.05). Two groups with equilibrium distribution are comparable. In addition, the genotypes distributions of *HRH4* polymorphisms in controls also had no significant deviation in HWE and suggested that our study population possessed the representativeness.

The association of the genotypes and alleles of HRH4 with the onset of AS

The genotype distribution of *HRH4* rs8088140 and rs657132 polymorphisms were listed in **Table 2**, the results showed that the frequencies of rs8088140 genotypes were no obviously difference in case than in control group (*P*>0.05), indicating *HRH4* rs8088140 might not be an independent risk factor for AS. Differently, the frequency of rs657132 AA genotype in case group was significantly higher than that of in control group and it increased 1.667 times risk for AS, compared with the common genotype GG (*P*=0.040, OR=2.667, 95% CI=1.021-6.962) and so was for the A allele (OR=1.570, 95% CI =1.064-2.315, *P*=0.022). In the generation and development of AS, rs657132 might be an independent susceptibility factor.

LD test and haplotype analysis

Haplotype analysis with online Haploview software showed that LD parameters of *HRH4* rs8088140 and rs657132 polymorphisms were D'=0.94 and r^2=0.755, which indicated high LD in these two polymorphisms. The analysis result of haplotypes manifested that the frequency of A-A haplotype in rs8088140-rs657132 was significantly statistical difference between two groups (χ^2=6.432, *P*=0.011, OR= 2.071, 95% CI=1.172-3.660), revealing that A-A was the susceptible haplotype to AS (**Table 3**).

Discussion

HRH4 is a membrane protein receptor that belongs to G-protein-coupled receptor family. It consists of 390 amino acid residues (NP0-67637) and 7 transmembrane domains. *HRH4* is located in chromosome 18 ql1.2 and contains 2 introns (>7 kbp) and 3 exons with its corresponding mRNA of 3.689 kb (NM 021624) [15]. So far, Nakamura et al. have certified with RT-PCR method that *HRH4* mRNA expresses in multiple peripheral tissues [16]. *HRH4* preferentially is activated on immune organs and hematopoietic cells with high expression in many inflammatory-response-related positions, such as bone marrow, peripheral blood cells, spleen, lung and small intestine [17, 18]. Therefore, *HRH4* is deduced to have great influence on immune diseases like anaphylaxis and asthma as well as on the process of cancer treatment [19, 20].

AS is an autoimmune inflammatory disease with the main clinical characteristic of back

pain and stiffness [21]. It is generally believed that genetic, environmental and immune factors play important roles in the development of AS [22, 23]. Young and middle-aged adults are more likely to suffer from AS, and some patients have complicated lesions of eye, lung, cardiovascular and renal in different degrees. The incidence rate of AS in China is about 0.2% and the ratio of female to male is 4:10.1 [24]. Recently, studying the polymorphisms of the candidate genes causing AS has become the most promising method in molecular genetics research. In a biological groups often appear two or more discontinuous variants, genotypes or alleles simultaneously, known as genetic polymorphism, and it consists of DNA repeat sequence polymorphism, DNA length polymorphism and single nucleotide polymorphism [25, 26]. Great progress has been made in the study of AS pathogenesis with regard to genetic polymorphism. Scientists have found and identified many candidate genes encoding AS [13, 27-29].

Our study used PCR-RFLP method to detect two polymorphisms of *HRH4* rs8088140 and rs657132. The calculation showed that *HRH4* rs8088140 CC genotype and C allele had no significantly frequency difference in case and control groups, which meant that it might be not an independent risk factor for the onset of AS, while the frequencies of AA genotype and A allele at rs657132 polymorphism were higher in cases than in controls, which indicated that AA genotype and A allele were associated with the increased susceptibility to the generation of AS. Rs657132 polymorphism had an influence on the AS susceptibility through charging the expression of *HRH4* in lesion tissues possibly.

In recent years, LD phenomenon has been found among different allele polymorphisms in some special groups, and it only exists in a small section of chromosome which formed haplotypes based on these polymorphisms. Therefore, the haplotypes analysis is also as the evidence which explores the correlation between gene polymorphisms and disease. In the present study, the haplotype analysis and LD test on rs8088140 and rs657132 polymorphisms in *HRH4* gene demonstrated that different alleles between two polymorphisms were not randomly combined, and LD of gene polymorphisms existed in human beings widely. A-A haplotype in rs8088140-rs657132 increased the risk of AS generation and development, compared with the common haplotype C-G, indicating A-A haplotype might be the susceptible haplotype to AS.

In conclusion, *HRH4* rs657132 polymorphism has certain correlation with AS in Chinese Han population. But due to the small sample size and the restriction of region, our results may have some bias. In addition, as for *HRH4*, its specific mechanism taking part in the pathogenesis of AS and the role of its polymorphisms are still not clear. Therefore, further research needs to be carried out with more races and larger sample size, considering the interaction among various factors.

Disclosure of conflict of interest

None.

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