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ORIGINAL ARTICLE - GENES AND DISEASE

Association study of ankylosing spondylitis and polymorphisms in ERAP1 gene in Zhejiang Han Chinese population

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Abstract The susceptibility loci of *ERAP1* polymorphisms have been found to be strongly associated with ankylosing spondylitis (AS). The researches in multiple ethnic cohorts suggested that the population attributable risk in *ERAP1* polymorphisms is at a high significance level. This study was undertaken to estimate the prevalence and incidence of subsets of AS and investigate the specific variants of *ERAP1* polymorphisms in AS susceptibility, in the Han ethnic Chinese population in Zhejiang Province. AS patients were selected, diagnosed, and confirmed by a qualified rheumatologist. The basal clinical and demographic characteristics were compared with all subjects. Genotypes for eight selected single nucleotide polymorphisms (SNPs) in *ERAP1* gene (rs27038, rs27037, rs27434, rs27980, rs7711564, rs30187, rs10050860, and rs17482078) were determined by using the Sequenom MassARRAY iPLEX platform in Zhejiang Han Chinese population. Association analyses were performed on the whole genotyped data set in 707 unrelated ankylosing spondylitis cases and 837 ethnically matched controls. We observed the strongest association between AS and HLA-B27, which confers over 90 % of ankylosing spondylitis cases. Moreover, we found three loci of *ERAP1* polymorphisms were at a high significance level (rs27037 $P = 0.00451$; rs27434 $P = 0.00012$; rs27980 $P = 0.00682$) with AS in Zhejiang population. We also confirmed polymorphism locus of *ERAP1* previously reported association with AS (rs27434; $P = 5.3 \times 10^{-12}$). Our results indicated a difference in the mechanism of susceptibility loci in subsets of Zhejiang Han Chinese population and provided further evidence that rs27434 is the key polymorphism associated with AS in *ERAP1* gene.

Keywords SNP · Ankylosing spondylitis · Rheumatic diseases

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Introduction

Ankylosing spondylitis (AS) is an obscure and chronic autoimmune disease, which affects predominantly the axial skeleton and characterizes sacroiliitis and enthesitis. As the statistics show, there is a high incidence of AS disease among young adults aged 20–30. AS is highly heritable and occurs in an approximately prevalence of five out of 1000 adults of European descent [1], as well as three out of 1000 individuals in Chinese population [2]. Although AS patients who received tumor necrosis factor blockers therapy on behalf of biologic preparation have obtained effective treatment, no therapy has achieved success in patients

with ankle arthrodesis so far [3]. The pathogenesis of AS has not yet been clarified, which may relate to heredity, immunity, and environment, and further effort is required to understand this disease.

Recently, genetic research has provided the cause of AS with many clues. Ever since the strongest association with AS has been observed between SNPs and *HLA-B27*, a class I major histocompatibility complex (*MHC*) gene, which has been found in about 90 % of Chinese or European patients, however, it only confers 5 % of healthy people. Importantly, the genome-wide association studies (GWAS) screening in ankylosing spondylitis also convincingly targeted several non-MHC genetic susceptibility genes, including *IL23R*, *ERAP1*, *KIF21B*, *EDIL3*, *HAPLN1*, *ANO6*, *ETS1* [1, 4–6]. Burton et al. [7] firstly found *ERAP1* has the second strongest association with a population attributable risk of 26 % in AS and reported two new loci at a high significance level (rs27044 $P = 1.0 \times 10^{-6}$; rs30187 $P = 3.0 \times 10^{-6}$) with AS in a European population. Furthermore, GWAS and follow-up case–control analysis were conducted identifying multiple *ERAP1* polymorphism loci significantly associated with AS in American, Canadian, European Caucasian and Asian population [2, 8–12]. As a result, most studies limited to individuals of Caucasian populations, but gene polymorphism and genetic susceptibility to AS may be various in different human races. Therefore, it is thought that genetic association studies of susceptibility genes in different races could be checked with each other for a high confidence and help to find the real susceptibility genes.

It has profound pathogenetic implications that considerable genetic association studies of susceptibility genes ERAP1 with AS, which is restricted to HLA-B27 positive cases, because the SNPs in AS patients are important for the mechanism involving of HLA-B27 and ERAP1 interaction. ERAP1 has been found to service as a major determinant of the abundance of many peptides, with function of N-terminal trimming peptide antigens to optimal length for binding to MHC class I molecules [13–15]. Moreover, peptide analyses have suggested that the balance of HLA-B27 between epitope generation and destruction is determined by ERAP1 variants [16]. Therefore, ERAP1 variants not only affect the stability and processing of HLA-B27 but also influence the peptide repertoire presented to the immune system, which could have secondary effects on specific adaptive or autoimmune responses in AS. ERAP1 polymorphisms associated with reduced endopeptidase activity appear to be protective against AS, and raising the strong protective effect of ERAP1 could represent a future treatment strategy in AS, at least in HLA-B27-positive disease [1].

In a sense, AS is genetically heterogeneous disease with widely disparate cause [17]. Later, more and more researches proved it has not only a greater prevalence of the familial aggregation, but also the relative action of genetic and environmental factors [18]. Many researchers have investigated specific variants for particular genetic loci in different populations and made those associated polymorphisms with AS to be confirmed. Whether the known *ERAP1* polymorphism loci play the same role in the AS patient in regional district is still unknown. The aims of this paper are to estimate the prevalence and incidence of AS and to investigate the genetic basis of AS to determine whether polymorphisms in *ERAP1* contribute to AS susceptibility in the Han ethnic Chinese population in Zhejiang province.

Materials and methods

Subjects

Unrelated AS patients ($n = 707$) were recruited from outpatient clinics at the First Affiliated Hospital of Wenzhou Medical University; healthy controls ($n = 837$) were enrolled and interviewed to exclude any history of AS disorder. All subjects in both groups ensured from Han Chinese population voluntarily joined the current study with informed consents and donated their blood cells for this study.

Ethics

The design of the work and experimental procedures were approved by Ethical Committee First Affiliated Hospital Wenzhou Medical University (Protocol number 2012-092). All had been diagnosed as having AS, which was confirmed by a qualified rheumatologist.

Clinical assessment

All patients underwent a complete physical examination and clinical assessment before treatment at each visit until the end of the study. We analyzed data from AS patients, who were registered in the First Affiliated Hospital of Wenzhou Medical University and assessed disease activity based on self-reporting using the visual analog scale (VAS) in persons with AS, including patient's global assessment of disease activity [patient's global VAS), VAS of night pain, disease activity (Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)], and functional impairment [Bath Ankylosing Spondylitis Functional Index (BASFI)]. The global pain intensity was measured on a 0- to 100-mm VAS, while the BASDAI scored to normalize to 0–10 scores, where 0 is no activity and 10 is maximum activity, and the BASFI scores was normalized to 0–10, where higher values of BASFI indicate worse functional ability.

Reagent

Genomic DNA was extracted from whole blood samples with AxyPrep Blood Genomic DNA Miniprep Kit

Table 1 Basal characteristics of patients with ankylosing spondylitis and controls

Characteristics	AS patients	Healthy controls	OR (95 % CI)
Number of subjects	707	837	
Male	480 (67.9 %)	652 (78.0 %)	
Age (years)	39.1 ± 11.2	39 ± 11.6	
Range	17–82	17–77	
HLA-B27+	647 (91.7 %)	19 (2.27 %)	34.4 (25.0–47.4)
Age at onset (years)	24.15 ± 3.43	NA	
Disease duration (years)	7.68 ± 1.59	NA	
VAS patient's global (mm)	38.03 ± 24.64	NA	
VAS of night pain (mm)	34.75 ± 22.56	NA	
BASDAI	3.84 ± 2.17	NA	
BASFI	3.52 ± 2.43	NA	

Values are presented by percent or mean ± SD

NA not available

(Axygen, UnionCity, CA) according to the manufacturer's instruction. The concentration of genomic DNA was detected by QuantiTPicoGreen dsDNA assay (Invitrogen, Carlsbad, CA) and adjusted to 50 ng/mL. DNA samples were stored in TE buffer at −20 °C before genotyping.

Individual SNP genotyping

Participants were genotyped for *ERAP1* gene polymorphisms in this study. Eight SNPs, rs27038(A/G), rs27037(G/T), rs27434(A/G), rs27980(A/C), rs7711564(C/G), rs30187(C/T), rs10050860(C/T), and rs17482078(C/T), were selected for genotyping. According to the reference widely reported, they were found to be associated with AS [15].

The genotyping was performed at Wenzhou Medical University using the Sequenom iPLEX platform (Sequenom, San Diego, California, USA) and MassARRAY Typer 4.0 software.

Statistical analysis

All eligible volunteers including AS patients and healthy controls were recruited and questionnaire on physical examination and clinical assessment. The clinical and demographic characteristics of all subjects are presented by number and mean ± SD. The minor allele frequency (MAF) in our study is 0.05, an overall missing rate >10 % among the subjects. The Hardy–Weinberg equilibrium (HWE) at individual loci was carried out online using http://analysis.bio-x.cn [19]. The test of odds ratios (ORs) associated with each SNP and 95 % confidence intervals (CIs) were all implemented for alleles and genotypes on single locus using unconditional logistic regression. A standard case–control association analysis was then performed using the Cochrane-Armitage trend test as implemented in PLINK [20, 21]. Differences were considered statistically significant for $P < 0.05$.

Results

Clinical and demographic characteristics of all subjects in both groups are presented in Table 1. The number of subjects, sex ratio, age, and range were similar between the AS patient and healthy control groups. A total of 707 AS patients (480 males and 227 females, mean age 39.1 ± 11.2 years, mean onset age 24.15 ± 3.43 years, and mean disease duration 7.68 ± 1.59 years) were evaluated. The percentage of AS patients who were positive for HLA-B27 was 91.7 %, and that of healthy control group was 2.27 %.

As shown in Table 1, the clinical assessment of AS disease activity revealed that the value of patient's global VAS (38.03 ± 24.64) was higher than that of VAS of night pain (34.75 ± 22.56). The score of BASDAI (disease activity level) was 3.84 ± 2.17, which indicated AS patients with a moderate pain. Another assessment showed that the score of BASFI (disability level) was 3.52 ± 2.43, which indicated AS patients with moderate degree of functional impairment.

The well-known strong association between HLA-B27 and AS was also confirmed in our cohort study. From the 707 cases and 837 controls, 647 (91.7 %) and 19 (2.27 %), respectively, were found to be HLA-B27 positive (Table 1). Additionally, ERAP1 polymorphisms have significant and complex functions to alter the immunological and pathogenetic features with ankylosing spondylitis among HLA-B27-positive individuals. Therefore, we genotyped the *ERAP1* polymorphisms using the Sequenom MassARRAY iPLEX platform with genomic DNA isolated from blood of all subjects.

To validate the elevated risks associated with *ERAP1* gene, covered by a large number of SNP markers, we selected eight of discovered SNPs, rs27038(A/G), rs27037(G/T), rs27434(A/G), rs27980(A/C), rs7711564(C/G), rs30187(C/T), rs10050860(C/T), and rs17482078(C/T), all mapping to a 35-kb region of chromosome 5 (Table 2). The subjects included in the control group presented an overall missing rate >10 %. The genotype frequencies in AS patients and healthy controls were in Hardy–Weinberg equilibrium.

Table 2 Association analysis results for *ERAP1* SNPs and AS outcome

rs	Chr	Position	Base	*P* value	ORs	(95 % CIs)	HWE
rs27434	5	96155268	A/G	0.00012	1.29	(1.08–1.53)	0.1267
rs27037	5	96120450	G/T	0.00451	0.76	(0.62–0.94)	0.2571
rs27980	5	96123651	A/C	0.00682	0.65	(0.46–0.92)	0.6641
rs27038	5	96138710	A/G	0.03453	0.72	(0.52–1.01)	0.8977
rs17482078	5	96144622	C/T	0.03569	1.17	(0.99–1.38)	0.4599
rs7711564	5	96121975	C/G	0.08761	1.16	(0.98–1.38)	0.1344
rs30187	5	96150086	C/T	0.14643	1.16	(0.97–1.38)	0.4571
rs10050860	5	96147966	C/T	0.17891	1.15	(0.97–1.36)	0.0983

ORs odds ratios, *CIs* confidence intervals *HWE* Hardy–Weinberg equilibrium

* Significant associations ($P < 0.05$)

As shown in Table 2, the genotype of eight SNPs spanning the *ERAP1* gene and association analyses with AS were performed. Three of these SNPs in the *ERAP1* gene (rs27037, rs27434, and rs27980) were in strong association ($P < 0.05$) (Table 2). The smallest *P* value was observed for SNP rs27434 in the *ERAP1* gene region ($P = 0.00012$; OR G allele = 1.29, 95 % CI 1.08–1.53). Though these selected SNPs have been demonstrated to associate with AS in various populations, we distinguished certain SNPs of *ERAP1* gene are mainly involved in AS susceptibility in a Zhejiang population.

Discussion

In the present study, we recruited and questionnaired volunteers including AS patients and healthy controls and presented the significant association of SNPs in the *ERAP1* gene with AS susceptibility for the first time in a Zhejiang population. We also demonstrated a strong relationship between HLA-B27 antigen and AS susceptibility, which has been widely confirmed over the past forty years [22]. Although a dominant prevalence has been found, there are still a few ratio of HLA-B27 carrier among the healthy controls (Table 1). Therefore, it is to be sure that some new susceptibility loci for AS disease need to be identified.

The presence of AS may not be noticeable in early stages, such as the high percentage HLA-B27 carrier among our healthy controls; in this case, the accurate diagnosis needs to be performed to find out the possibility of AS. The clinical assessment of AS disease activity, including patient's global VAS, night pain VAS, BASDAI, and BASFI, is an effective and necessary diagnosis to be determined by the symptoms of the individual [23].

As the technology advances in recent years, the genome-wide association studies provide a much better understanding of disease pathogenesis, such as *ERAP1*, *IL23R*, *EDIL3* genes [4, 7, 24]. Furthermore, the association between *ERAP1* and AS has been replicated in multiple ethnic cohorts [15]. We then genotyped a total of eight SNPs in a cohort of 707 Han Chinese ankylosing spondylitis cases and 837 unselected Han Chinese controls from Wenzhou Medical College. The SNPs genotyped included four ancestry-informative SNPs for Han Chinese, and four SNPs for other ethnic groups were selected to investigate *ERAP1* polymorphisms involving into Zhejiang population. Interestingly, these findings suggest that three markers in *ERAP1*, rs27037, rs27434, and rs27980, associated with AS were observed at *P* values of ≤ 0.01 (Table 2), indicating a difference in the mechanism of disease pathogenesis in Zhejiang Han Chinese population. The strongest association between *ERAP1* polymorphisms and AS was observed for rs27434, which plays a key role in disease pathogenesis in other ethnic groups from the European, Australian, British, US, UK population, etc. [15].

Further study with larger sample sizes will therefore be useful and likely to identify more accurately gene polymorphisms associated with AS. The replicable identification of three genetic loci associated with AS in Zhejiang Han Chinese population extends our understanding of the genetic etiology of AS. These findings may provide an important foundation for research on the potential functions of *ERAP1* polymorphisms for this highly genetic disease in physiological and pathological processes.

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Compliance with ethical standards

Conflict of interest We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work; there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled "Association Study of Ankylosing Spondylitis and Polymorphisms in ERAP1 Gene in Zhejiang Han Chinese Population".

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