Gene 646 (2018) 8–11

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Association of polymorphisms in ERAP1 and risk of ankylosing spondylitis in a Chinese population

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ARTICLE INFO

Keywords:
ERAP1
Polymorphism
Ankylosing spondylitis (AS)
Susceptibility

ABSTRACT

To explore the association between five polymorphisms in endoplasmic reticulum associated aminopeptidase 1 (*ERAP1*) gene and risk of ankylosing spondylitis (AS) in a Chinese population. A case-control study enrolled 250 AS patients and 250 healthy controls was carried out. The genotypes of involved polymorphisms (rs27037, rs27038, rs469876, rs27044 and rs27980) in *ERAP1* were detected by Sequenom Mass-Array platform. There were significant differences of the level of WBC (white blood cell), Platelets, CRP (C-reactive protein) and ESR (erythrocyte sedimentation rate) between AS patients and controls ($P_{all} < 0.05$). There was statistically association between *ERAP1* rs27044 polymorphism and risk of AS, and the carriers with rs27044 CG genotype have an increased the risk for AS (CG *versus* GG, OR = 1.70, 95% CI = 1.10–2.62, $P = 0.015$). However, we found no evidence for the association of rs27037, rs469876, and rs27980 polymorphisms in *ERAP1* with AS risk. Our findings indicated that *ERAP1* rs27044 polymorphism was associated with the susceptibility of AS.

1. Introduction

Ankylosing spondylitis (AS) is an autoimmune disease with highly disabling and onset age of 18–22 years old usually (Braun and Sieper, 2007; Zvyagin et al., 2010), and the incidence of AS ranged 0.1%–0.9% on a global scale and about 0.06%–0.54% in China (Zeng et al., 2008). Genetic factors have been regarded as a risk of AS. It has been identified that the incidence of AS is closely related to the human leukocyte antigen (HLA) B27 which belongs to major histocompatibility complex (MHC) (Brown, 2009). However, increasing evidence indicates that more genetic factors other than the MHC contribute to AS susceptibility in the past decade (Karaderi et al., 2014; Meng et al., 2015; Kucuksahin et al., 2016). Genome-wide association studies revealed that non-MHC genes include anthrax toxin receptor 2 (*ANTXR2*), endoplasmic reticulum aminopeptidase 1 (*ERAP1*), interleukin-23 receptor (*IL-23R*) and *IL12B* are responsible for AS susceptibility in Europe population (Australo-Anglo-American Spondyloarthritis et al., 2010).

ERAP1 is a member of the zinc metallopeptidase M1 family and responsible for shearing the N-terminus peptides of major histocompatibility complex class I (MHC-I) molecule (Saric et al., 2002). In addition, ERAP1 can cleave proinflammatory cytokine including tumor necrosis factor receptor (TNFR) (Cui et al., 2002), IL6Rα (Cui et al., 2002) and IL1R2 (Cui et al., 2003), resulting in the downregulation of their signal intensity on the cell surface. Moreover, functional loss caused by genetic variation of *ERAP1* results in a proinflammatory state, indicating genetic variations in *ERAP1* were associated with risk of AS (Wang et al., 2015; Lee and Song, 2016). However, previous published result about the susceptibility genetic variations in *ERAP1* to AS risk was inconsistent among different ethnic groups (Cai et al., 2015; Nossent et al., 2016).

In this study, we performed a population based case-control study to investigate the relationship between *ERAP1* polymorphisms (rs27037, rs27038, rs469876, rs27044 and rs27980) and risk of AS in a Chinese population.

2. Subjects and methods

2.1. Subjects

Two hundred and fifty unrelated AS patients fulfilling the 1984 modified New York Criteria(van der Linden et al., 1984) and an equal number of unrelated healthy individuals without history of autoimmune disorders were recruited mainly from Zhangjiagang Hospital of Traditional Chinese Medicine and Jinhu People's Hospital from January

Abbreviations: ERAP1, endoplasmic reticulum associated aminopeptidase 1; AS, ankylosing spondylitis; HLA, human leukocyte antigen; MHC, major histocompatibility complex; *ANTXR2*, anthrax toxin receptor 2; *IL-23R*, interleukin-23 receptor; TNFR, tumor necrosis factor receptor; WBC, white blood cell count; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HWE, Hardy-Weinberg equilibrium; OR, odds ratio; CI, confidence interval

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https://doi.org/10.1016/j.gene.2017.12.050
Received 30 October 2017; Received in revised form 4 December 2017; Accepted 22 December 2017
Available online 24 December 2017
0378-1119/

Table 1
Clinical characteristics of patients with ankylosing spondylitis and controls.

Clinical characteristics	Cases (n = 250)	Controls (n = 250)	P value
	n (%)	n (%)	
Gender			0.082
Male	166 (66.4)	150 (60.0)	
Female	84 (33.6)	100 (40.0)	
Age (yeas), mean ± SD	34.32 ± 11.25	33.28 ± 9.35	0.262
Laboratory test[a]			
WBC (10^9)	6.66 ± 1.91	5.99 ± 1.58	0.000
Platelets (10^9)	224.62 ± 52.72	205.26 ± 47.18	0.000
CRP (mg/L)	10.80 ± 13.57	4.87 ± 2.53	0.000
ESR (mm)	21.62 ± 18.26	11.59 ± 6.87	0.000

[a] WBC: white blood cell count; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

2008 to December 2016. Demographic and clinical data was obtained from laboratory investigations including white blood cell count (WBC), platelets, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) of all participants. Written informed consent was obtained from each participant before study. The study was approved by the regional ethics committee. Clinical characteristics of patients with AS and controls are shown in Table 1.

2.2. DNA isolation

Whole blood samples were stored in tubes containing EDTA at − 80 °C before DNA extraction. Genomic DNA was extracted from whole blood samples containing EDTA using a DNA extraction kit (Axygen Biosciences, CA, USA) according to the manual instructions. The concentration of genomic DNA was measured by NanoDrop 2000c spectrophotometer (Thermo Scientific, DE, USA).

2.3. Polymorphism selection and genotyping

Five polymorphisms in the intron of *ERAP1*, locate at the chromosome 5:96758990–5:96785702, were included in this study. Criteria were used for polymorphism selection as previous study described. (Zhu et al., 2016). Genotypes of polymorphisms were detected using Sequenom Mass-Array platform.

2.4. Statistics analysis

First of all, Hardy-Weinberg equilibrium (HWE) testing based on χ^2 test was carried out to evaluate the representativeness of the control group. The difference of age, WBC, Platelets, CRP and ESR between cases and controls was calculated by *t*-test. The frequency differences of *ERAP1* genotypes among groups were tested by χ^2 test. The relative risk of AS susceptibility was estimated by odds ratio (OR) and 95% confidence intervals (95% CI) calculated with logistic regression method. The SPSS 20.0 program and SAS 9.3 software was used for the statistical analysis. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Subject characteristics

There was no significant difference between cases (34.32 ± 11.25 years) and healthy controls (33.28 ± 9.35 years) in average age ($P > 0.05$). Similarly, there were no significant differences in gender, GT, and Hemoglobin between cases and controls ($P > 0.05$). However, we found that the value of WBC, platelets, CRP and ESR was significantly higher in cases than those in controls ($P_{all} < 0.05$) shown in Table 1.

3.2. Correlation analysis of ERAP1 polymorphisms and AS susceptibility

The genotype frequencies of all the five SNPs met Hardy-Weinberg

Table 2
Genotype distribution of ERAP1 in control subjects and patients with ankylosing spondylitis.

Genotypes	Cases, n (%)	Controls, n (%)	OR[a] (95% CI)	OR[b] (95% CI)	P value
rs27037					
GG	87 (34.80)	88 (35.20)	1.00 (reference)	1.00 (reference)	
GT	128 (51.20)	118 (47.20)	1.10 (0.75,1.62)	1.16 (0.78,1.72)	0.639
TT	35 (14.00)	44 (17.60)	0.81 (0.47,1.37)	0.87 (0.51,1.50)	0.424
GT/TT	163 (65.20)	162 (64.80)	1.02 (0.71,1.47)	1.04 (0.72,1.51)	0.925
rs27038					
GG	77 (30.80)	84 (33.60)	1.00 (reference)	1.00 (reference)	
GA	140 (56.00)	115 (46.00)	0.71 (0.41,1.21)	0.78 (0.45,1.36)	0.202
AA	33 (13.20)	51 (20.40)	1.33 (0.89,1.97)	1.41 (0.94,2.11)	0.159
GA/AA	173 (69.20)	166 (66.40)	1.14 (0.78,1.66)	1.17 (0.80,1.71)	0.503
rs469876					
AA	125 (50.00)	138 (55.20)	1.00 (reference)	1.00 (reference)	
AG	109 (43.60)	88 (35.20)	1.37 (0.94,1.98)	1.39 (0.95,2.01)	0.098
GG	16 (6.40)	24 (9.60)	0.74 (0.37,1.45)	0.69 (0.35,1.37)	0.374
AG/GG	125 (50.00)	112 (44.80)	1.23 (0.87,1.75)	1.24 (0.87,1.77)	0.244
rs27044					
GG	53 (21.20)	70 (28.00)	1.00 (reference)	1.00 (reference)	
CG	147 (58.80)	114 (45.60)	1.70 (1.11,2.63)	1.70 (1.10,2.62)	**0.015**
CC	50 (20.00)	66 (26.40)	1.00 (0.60,1.67)	0.99 (0.59,1.67)	0.998
CG/CC	197 (78.80)	180 (72.00)	1.45 (0.96,2.18)	1.42 (0.94,2.15)	0.078
rs27980					
TT	67 (26.80)	70 (28.00)	1.00 (reference)	1.00 (reference)	
GT	125 (50.00)	111 (44.40)	1.18 (0.77,1.79)	1.16 (0.76,1.77)	0.449
GG	58 (23.20)	69 (27.60)	0.88 (0.54,1.43)	0.80 (0.49,1.32)	0.599
GT/GG	183 (73.20)	180 (72.00)	1.06 (0.72,1.57)	1.01 (0.68,1.50)	0.764

[a] ORs were calculated using logistic regression to measure the ORs for patients of a specific genotype (e. g. rs27037 GT/TT genotypes versus GG genotype).
[b] Adjusted for age, sex.
Significant results are in bold.