ERAP1/ERAP2 and RUNX3 polymorphisms are not associated with ankylosing spondylitis susceptibility in Chinese Han

W. Su,* L. Du,* S. Liu,† J. Deng,* Q. Cao,* G. Yuan,* A. Kijlstra‡ and P. Yang

*The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Ophthalmology and Chongqing Eye Institute, Chongqing, China, †Rheumatology Department of the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China, and ‡University Eye Clinic, Maastricht, Maastricht, the Netherlands

Accepted for publication 9 February 2018
Correspondence: P. Yang, The First Affiliated Hospital of Chongqing Medical University, Chongqing Key Laboratory of Ophthalmology and Chongqing Eye Institute, Chongqing, 1 You Yi Road, Yu Zhong District, Chongqing 400016, China.
E-mail: peizengyrcmu@126.com

Summary

Previous studies show that endoplasmic reticulum-associated aminopeptidase (ERAP1/ERAP2) and runt-related transcription factor 3 (RUNX3) gene polymorphisms are associated with AS (ankylosing spondylitis) in European Caucasians. However, contradictory results were reported in different Asian populations. The purpose of this study was to determine whether eleven candidate single nucleotide polymorphisms (SNPs) in ERAP1/ERAP2 and six in RUNX3 genes confer susceptibility to AS with or without acute anterior uveitis (AAU) [AS+AAU+ or AS+AAU+] in Chinese Han. Therefore, a case–control association study was performed in 882 AS+AAU–, 884 AS+AAU+ and 1727 healthy controls. Genotyping was performed using the iPLEXGold genotyping assay. A meta-analysis was performed to assess the association of polymorphisms of ERAP1 with AS susceptibility in Asian populations. No association was found between SNPs of ERAP1/ERAP2/RUNX3 and susceptibility of AS with or without AAU. A case–control study between patients with human leucocyte antigen HLA-B27-positive and healthy controls also failed to demonstrate an association of the tested SNP with AS with or without AAU. Moreover, a meta-analysis showed that there was no association of rs30187, rs27037, rs27980, rs27434 and rs27582 in ERAP1 with AS in Chinese Han. Taken together, 17 SNPs in ERAP1/ERAP2 and RUNX3 genes did not confer disease susceptibility to AS in Chinese Han.

Keywords: ankylosing spondylitis, Chinese Han, ERAP1/ERAP2, gene polymorphism, RUNX3

Introduction

Ankylosing spondylitis (AS), a particular subtype of spondyloarthritides, is a progressive chronic disorder primarily affecting the spine. Peripheral arthritis, enthesitis and iritis are the common complications of AS [1]. The incidence of AS is 0.24% in Chinese Han [2] and 0.55% in Europeans [3]. Acute anterior uveitis (AAU), an inflammation usually affecting the iris and ciliary body of the eye, is the most common form of ocular involvement. Earlier studies show that 12% of patients experience AAU when diagnosed with AS, and the incidence can increase up to 25% after 20 years and 60% after more than 50 years follow-up [4,5]. Our previous study showed that AAU patients with AS show a more common bilateral eye involvement and a higher frequency of fibrinous exudates in the anterior chamber compared with AAU without AS patients in human leucocyte antigen HLA-B27 positive carriers [6]. The pathological mechanisms leading to AS remain uncertain. Twin and family studies showed that genetic factors play a very important role in the occurrence of AS [3,7]. Recently, genomewide association studies (GWAS) in European Caucasians show that gene polymorphisms in endoplasmic reticulum-associated aminopeptidase (ERAP1/ERAP2) and runt-related transcription factor 3 (RUNX3) are associated significantly with AS susceptibility [8–10].

The ERAP1 gene is the second most important gene that has been shown to be related closely to AS after HLA-B27. ERAP1 and ERAP2, which are localized in the endoplasmic reticulum, play an important role in trimming peptides prior to or after their binding onto major
histocompatibility complex (MHC) class I molecules [11]. The MHC–peptide complex is presented by antigen-presenting cells and can be recognized by specific T cell receptors as well as natural killer cell receptors [12,13]. RUNX3, a member of the RUNX transcription factor family, plays a key role in T cell line development and in regulation of the transforming growth factor (TGF)-β signalling pathway in dendritic cells [14]. RUNX3 affects multiple types of immune cells [15], including regulatory T cells and natural killer (NK) cells, but seems to be particularly involved in the differentiation and development of CD8$^+$ T cells [16,17]. This latter lymphocyte subpopulation is presumed to play a vital role in the development of AS [18–20]. ERAP1/ERAP2 and RUNX3 interact with each other in the formation of peptide repertoires and T cell development and differentiation, respectively [21,22]. The peptide spectrum formed after ERAP1/ERAP2 cleavage affects the type and quantity of activated CD8$^+$ T cells directly [23], and it was therefore likely that both ERAP1/ERAP2 and RUNX3 could be involved in the development of AS.

Contradictory results have, however, been reported in several studies on the genetic association of gene polymorphisms in ERAP1/ERAP2 and RUNX3 with AS susceptibility worldwide [9,10,24–26]. The aim of our study was therefore to re-evaluate the association of polymorphisms in ERAP1/ERAP2 and RUNX3 with AS susceptibility in a large cohort of Chinese Han patients.

Materials and methods

Case and control cohorts

For this case–control study, 882 unrelated Chinese Han patients with AS without AAU and 884 AS with AAU were recruited from the Rheumatology Department of the First Affiliated Hospital of Zhengzhou University (Zhengzhou, China) and the Ophthalmic Center of the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) from March 2010 to May 2017. At the same time, 1727 geographically and ethnically matched Chinese Han healthy individuals were also enrolled for the study. Patients and controls were sex- and age-matched. All AS patients recruited for the study were diagnosed following the 1984 modified New York criteria [27]. The study complied with the Declaration of Helsinki and was supported by the local ethical research committee of the Chongqing Medical University. Informed consent was obtained from all patients.

Single nucleotide polymorphism (SNP) selection and genotyping statistical analysis

Genomic DNA was collected from peripheral whole venous blood of healthy individuals and patients using the QIAamp DNA Blood Mini Kit (250) (Qiagen, Valencia, CA, USA). DNA concentration was measured with Nanodrop 2000 equipment (Thermo Fisher Scientific, Wilmington, DE, USA), standardized, quality-checked and stored at −40°C until used. Seventeen candidate SNPs were selected, based on reported susceptibility loci in ERAP1/ERAP2 and RUNX3 as identified in GWAS results in European Caucasians and replicated in Asian patients, including novel loci identified in Asian populations. Details of these SNPs are shown in Table 1. The SNPs rs27037, rs27980 and rs27582 were reported to be in strong linkage disequilibrium (LD), as well as the SNPs rs30187 and rs27434 and the SNPs rs2549782 and rs2248374. Based on conflicting results in several studies [9,10,24–26], all sites were selected for the current study (Fig. 1).

The iPLEX Gold Genotyping Assay technique was used for genotyping analysis on a Sequenom MassArray System (Sequenom, San Diego, CA, USA). MassArray Assay Design software (Sequenom, San Diego, CA, USA) was applied for primer design. The study was performed strictly following the standard procedures of the manufacturer (Agena Bioscience, San Diego, CA, USA).

Statistical analysis

The $\chi^2$ test was applied for Hardy–Weinberg equilibrium (HWE) analysis in all tested SNPs. Allele and genotype frequency were calculated with the Sequenom MassArray System platform. SPSS (SPSS Inc., Chicago, IL, USA) version 19.0 was used to calculate 95% confidence intervals (CI) and odds ratios (ORs). The Bonferroni correction was employed to correct multiple comparisons. A corrected P-value (Pc) of less than 0.05 was considered to be statistically significant.

Meta-analysis

A literature search was performed using the PubMed database for studies on the associations of polymorphisms in ERAP1/ERAP2 or RUNX3 with AS susceptibility. Ankylosing spondylitis (AS), acute anterior uveitis (AAU), RUNX3, ERAP1 (ARTS1), ERAP2 and polymorphisms were searched in the PubMed database (up to May 2017). The following inclusion criteria were used: (i) case–control studies on AS susceptibility examining the role of ERAP1/ERAP2 or RUNX3 polymorphisms (ii) containing data of minor allele frequencies (MAF) and 95% CI and OR (iii) showing numbers of cases and controls. Exclusion criteria were: (i) duplicate data in references, (ii) replication studies and (iii) twins and family studies. The author, year of publication, ethnicity, numbers of controls and cases and MAF of polymorphisms in the ERAP1/ERAP2 gene or RUNX3 gene were collected from each study. RevMan version 5.3 (http://ims.cochrane.org/revman/download) software was applied for the meta-analysis. Both the fixed- and random-effect models were used to account for pooled ORs ($F$ statistics). A list of the included studies is shown in the Supporting information, Table S1.
Table 1. Seventeen candidate single nucleotide polymorphisms (SNPs) in endoplasmic reticulum-associated aminopeptidase (ERAP1/ERAP2) and runt-related transcription factor 3 (RUNX3) as reported in Europeans and Asians

| Chr | Gene   | SNP      | Dis | Ethnic groups           | Reference                  |
|-----|--------|----------|-----|-------------------------|----------------------------|
| 1   | RUNX3  | rs9438876| AS  | Chinese                 | Liu et al. [28]            |
| 1   | RUNX3  | rs1395621| AS  | Chinese                 | Liu et al. [28]            |
| 1   | RUNX3  | rs7551188| AS  | Chinese                 | Liu et al. [28]            |
| 1   | RUNX3  | rs4648889| AS  | European                | Vecellio et al. [29]       |
| 1   | RUNX3-MIR4425 | rs11249215 | AS | Chinese/European/Korean | Evans et al. [8], Cho et al. [24], Liu et al. [28] |
| 1   | RUNX3-MIR4425 | rs6600247 | AS | European/East Asian     | Cortes et al. [10]         |
| 5   | CAST   | rs27980  | AS  | Chinese/Taiwanese       | Wang et al. [30], Zhang et al. [31] |
| 5   | CAST   | rs27582  | AS  | Chinese                 | Zhang et al. [31]          |
| 5   | ERAP1  | rs1065407| AS  | Chinese                 | Zhang et al. [31]          |
| 5   | ERAP1  | rs27044  | AS  | Chinese/Caucasian/Korean| Choi B et al. [26], Zhang et al. [31], Burton et al. [32] |
| 5   | ERAP1  | rs2032890| AS  | European                | Robinson et al. [33]       |
| 5   | ERAP1  | rs30187  | AS  | European/East Asians/Korean| Cortes et al. [10], Choi et al. [26] |
| 5   | ERAP1  | rs27434  | AS  | Chinese/Korean/European  | Reveille et al. [9], Bang et al. [34], Li et al. [35] |
| 5   | ERAP2  | rs2549782| AS  | Caucasian               | Haroon et al. [36]         |
| 5   | ERAP2  | rs2248374| AS  | European                | Robinson et al. [33]       |
| 5   | LNPEP  | rs10044354| AS | European               | Cortes et al. [10]         |
| 5   | ERAP1  | rs27037  | AS  | Chinese/Taiwanese/Koreans/European| Reveille et al. [9], Bang et al. [34], Wang et al. [30], Zhang et al. [31] |

CAST = calpastatin; MIR = microRNA.

Results

Case–control study

Clinical features of AS+AAU− and AS+AAU+ patients are shown in Table 2. The proportion of male patients in the AS+AAU− and AS+AAU+ groups are similar (68–69%). The average age of AS patients also having experienced AAU is older than that of the AS patients without AAU. The ratio of HLA-B27 was 97.17% in AS+AAU− and 82.92% in AS+ AAU+ patients. Seventeen SNPs (eleven located in or near the ERAP1/ERAP2 gene, six in the RUNX3 gene) were genotyped in 1766 AS patients with or
without AAU (AS\(^+\)AAU\(^-\) 882, AS\(^+\)AAU\(^+\) 884) and 1727 healthy controls. All the tested polymorphisms in ERAP1/ERAP2 genes and RUNX3 gene are shown in the Supporting information, Table S2. Genotype frequencies of all SNPs were in line with the HWE (Fisher’s P-value > 0.05).

Our results showed that some SNPs (Supporting information, Table S2) (rs10044354, rs1065407, rs27434, rs27582 and rs27037) showed no association of polymorphisms with risk of AS\(^+\)AAU\(^-\) after correction for multiple comparisons. This was also the case for SNPs rs27434, rs30187 and rs27037 polymorphisms with risk of AS\(^+\)AAU\(^+\). A case–control study performed on HLA-B27-positive patients and non-typed healthy controls also showed similar results (data not shown). Haplotype analysis was performed on the ERAP1 gene using Haploview version 4.2 software. None of the haplotypes showed an association with AS with or without AAU in our Chinese Han population (Supporting information, Table S3).

**Meta-analysis**

A literature search in the PubMed database resulted in the identification of 22 studies; 12 studies met the study inclusion criteria, including four studies from European populations and eight studies from Asian populations (two from Korean, six from Chinese populations), including the present study [9,25,26,30,31,34,35,37–40]. Ten studies were excluded (seven studies due to lack of data and three studies having duplicate data). A literature search in the PubMed database showed that there were no reports concerning the association of ERAP1/ERAP2 or RUNX3 gene polymorphisms with AS susceptibility in Japanese individuals. At the same time, we found that ERAP1/ERAP2 or RUNX3 gene polymorphisms were a risk factor for AS in Korean and Taiwanese patients, but this was not obvious in Chinese mainland patients. Therefore, Chinese Han were divided into two subgroups in this meta-analysis (Chinese, including data from the Chinese mainland and China Taiwan Island; Chinese1, including data only from the Chinese mainland). The meta-analysis results of the association of ERAP1/ERAP2 polymorphisms with risk of AS is shown in Table 3. Insufficient reports were available for a meta-analysis of the RUNX3 gene.

As shown in Table 3, the meta-analysis showed a strong association of rs30187, rs27434 and rs27044 in ERAP1 with AS susceptibility in European descendants (\(P = 0.00001, \text{OR} = 1.29 (1.17–1.42), \hat{I}^2 = 0\)%; \(P = 0.00001, \text{OR} = 1.35 (1.25–1.45), \hat{I}^2 = 0\)%; \(P = 0.00001, \text{OR} = 1.32 (1.20–1.46), \hat{I}^2 = 18\)%) (Supporting information, Figs S1–S3). However, the association of these SNPs with AS in Asian populations was controversial. Meta-analysis confirmed that there was no association of rs30187, rs27037 or rs27980 in ERAP1 with AS in Chinese Han (\(P = 0.11, \text{OR} = 1.05 (0.99–1.12), \hat{I}^2 = 36\)%; \(P = 0.11, \text{OR} = 1.09 (0.98–1.20), \hat{I}^2 = 0\)%; \(P = 0.07, \text{OR} = 0.92 (0.83–1.01), \hat{I}^2 = 0\)%) (Supporting information, Figs S1, S4 and S6), and also showed no association of rs27434 and rs27582 in ERAP1 with AS in Chinese Han (Supporting information, Figs S2 and S5), but with a high \(\hat{I}^2\) value (\(\hat{I}^2 = 84\)%; \(\hat{I}^2 = 77\)%) and rs27044 with a boundary \(\text{P-value} (P = 0.05)\) and a high \(\hat{I}^2\) value (\(\hat{I}^2 = 88\)%) (Supporting information, Fig. S3) was observed in Asian populations. When taking ERAP1: rs30187 as an example, forest plots showed a high \(\hat{I}^2\) value (\(\hat{I}^2 = 78\)%) and a lower \(\text{P-value} (P < 0.0005)\) when all studies from Europe and Asia were pooled. Stratified analysis on ethnicity showed a forest plot with \(\hat{I}^2 = 0\)% and \(P < 0.00001\) in Europe and \(\hat{I}^2 = 36\)% and \(P = 0.11\) in mainland China. However, heterogeneity was obviously increased when Korea and Taiwan were added (Supporting information, Fig. S1).

Heterogeneity was found among all the studies. No obvious heterogeneity was found in studies on the association of rs30187, rs27434 and rs27044 with AS susceptibility in European populations (\(\hat{I}^2 = 0\)%; \(\hat{I}^2 = 0\)%; \(\hat{I}^2 = 18\)%) (Supporting information, Figs S1–S3). However, heterogeneity was obvious in the studies from Asian populations (\(\hat{I}^2 = 0\sim89\)%) (Supporting information, Figs S1–S6), even in Chinese populations (\(\hat{I}^2 = 0\sim84\)%) (Supporting information, Figs S1–S6). The funnel plot of the loci showed no significant asymmetry. The meta-analysis was thus considered not to have a significant publication bias in each group of included studies. Forest plots of the meta-analysis of those loci are shown as Supporting information, Figs S1–S6.

**Discussion**

In the present study, known AS-associated SNPs were assayed in a large Chinese Han population to evaluate the
association of polymorphisms in ERAP1/ERAP2 and RUNX3 genes with AS in Chinese Han. Our study demonstrated a trend of several polymorphisms for AS or AS with AAU in Chinese Han, including one SNP in ERAP2 and five in ERAP1. However, all these polymorphisms lost statistical significance after correction for multiple comparisons. Furthermore, a meta-analysis of the existing literature supported our findings that there was no association of polymorphisms in these genes with AS in Chinese Han. Subgroup analysis indicated that geographical factors might be the important causes of heterogeneity. South Koreans, Chinese Han and Taiwanese belong to the East Asian population. However, the studies from the peninsula and inland China are quite different, suggesting that environmental factors may play a role.

Aminopeptidases encoded by ERAP1/ERAP2 have two major functions presumed to be related to inflammatory and immune responses. One function is that of a ‘molecular ruler’, trimming peptides along with ERAP2 prior to or after loading them onto MHC class I molecules. SNPs in ERAP1/ERAP2 may affect the peptide repertoire, possibly resulting in an aberrant CD8+ T cell response [20,23]. ERAP1 gene polymorphisms have been confirmed to be associated with AS in Caucasians [8–10]. However, our meta-analysis of all data reported on the association of polymorphisms in these genes with AS indicated that population and geographical differences might be one of the causes of heterogeneity. Even in Asian populations, the association of ERAP1 gene polymorphisms with AS differs depending on the studied populations [25,30,34]. Results of studies from Korea and Taiwan were similar, but different from data from mainland China. Our study showed no association of ERAP1 gene polymorphisms with AS susceptibility, which was in agreement with an earlier GWAS by Lin et al. [25]. Results from our meta-analysis also showed that data from Taiwan and Korea were similar. Little is known about the association of ERAP1 gene polymorphisms with AS in Japanese. This may be due to the fact that HLA-B27 is rare in the Japanese population and functional ERAP abnormalities are probably seen only in relation with HLA-B27. Another function of aminopeptidases encoded by ERAP1/ERAP2 is presumed to be related to inflammatory and immune responses in regulating inflammation via the shedding of membrane-bound cytokine receptors, such as tumour necrosis factor receptor 1 (TNF-R1) [41], interleukin (IL)-1RII [42] and IL-6Rα [43]. Whether this activity is involved in AS pathogenesis seems unlikely, as earlier studies showed that there were no differences in the levels of IL-6R, IL-1R2 and TNF-R1 in cell culture supernatants or serum when comparing AS patients with controls [8,36].

### Table 3. Meta-analysis of association between ERAP1 polymorphisms and AS

| SNP     | Ethnicity | Ref. | No. | OR  | 95% CI      | P-value | Model | I²  | P-value |
|---------|-----------|------|-----|-----|-------------|---------|-------|-----|---------|
| rs30187 | Overall   | [25,26,30,38–40] | 7   | 1.1 | 1.04–1.17 | 0.0005  | R     | 78  | 0.0002 |
|         | European  | [38–40]    | 3   | 1.29| 1.17–1.42 | 0.00001 | F     | 0   | 0.99   |
|         | Asian     | [25,26,30]  | 4   | 1.17| 1.01–1.35 | 0.03    | R     | 84  | 0.0004 |
|         | Chinese   | [25,30]     | 3   | 1.09| 1.03–1.15 | 0.004   | F     | 67  | 0.05   |
|         | Chinese1  | [25]        | 2   | 1.05| 0.99–1.12 | 0.11    | R     | 36  | 0.21   |
| rs27434 | Overall   | [9,25,31,34,35,37,38] | 8   | 1.19| 1.04–1.36 | 0.01    | R     | 88  | 0.00001|
|         | European  | [9,38]      | 2   | 1.35| 1.25–1.45 | 0.00001 | F     | 0   | 0.44   |
|         | Asian     | [25,31,34,35,37] | 6   | 1.14| 0.97–1.35 | 0.12    | R     | 89  | 0.0001 |
|         | Chinese   | [5,31,35,37] | 5   | 1.08| 0.92–1.28 | 0.35    | R     | 84  | 0.0001 |
| rs27044 | Overall   | [26,30,38–40] | 6   | 1.25| 1.18–1.33 | 0.00001 | F     | 77  | 0.0006 |
|         | European  | [38–40]     | 3   | 1.32| 1.20–1.46 | 0.00001 | F     | 18  | 0.29   |
|         | Asian     | [26,30]     | 3   | 1.26| 1.00–1.59 | 0.05    | R     | 88  | 0.0002 |
| rs27037 | Overall   | [9,30,31,34] | 5   | 1.2 | 1.14–1.27 | 0.00001 | F     | 44  | 0.13   |
|         | European  | [9]         | 1   | n.a.| n.a.     | n.a.    | n.a.  | n.a. | n.a.   |
|         | Asian     | [30,31,34]  | 4   | 1.2 | 1.12–1.29 | 0.00001 | F     | 58  | 0.07   |
|         | Chinese   | [30,31]     | 3   | 1.16| 1.02–1.32 | 0.02    | R     | 59  | 0.09   |
|         | Chinese1  | [31]        | 2   | 1.09| 0.98–1.20 | 0.11    | F     | 0   | 0.99   |
| rs27582 | Overall   | [31,35,38]  | 4   | 0.96| 0.90–1.03 | 0.28    | F     | 74  | 0.009  |
|         | Chinese   | [31,35]     | 3   | 0.95| 0.79–1.14 | 0.61    | R     | 77  | 0.01   |
| rs27980 | Overall   | [30,31,38]  | 4   | 1   | 0.93–1.06 | 0.88    | F     | 62  | 0.05   |
|         | Chinese   | [30,31]     | 3   | 0.99| 0.85–1.16 | 0.91    | R     | 75  | 0.02   |
|         | Chinese1  | [31]        | 2   | 0.92| 0.83–1.01 | 0.07    | F     | 0   | 0.91   |

Chinese = people from China mainland and Taiwan Island (Taiwanese); Chinese1 = people from China mainland; R = random-effects model; F = fixed-effects model; OR = odds ratio; CI = confidence interval; n.a. = not available; No. = number of included studies (include the present study); AS = ankylosing spondylitis; ERAP = endoplasmic reticulum-associated aminopeptidase; OR = odds ratio; CI = confidence interval.
In this study, we found no association between ERAP1 gene polymorphisms and AS susceptibility in Chinese Han, which might be due to the following three reasons. First, the vast territory of mainland China has different climate zones as well as a variety of different natural environments, all of which might play a role. Secondly, we only collected patients from two affiliated hospitals, which might not be sufficient to represent the huge Chinese population and widespread distribution over the country. Thirdly, ERAP1 gene polymorphisms and AS susceptibility may vary ethnically, and the SNPs in ERAP1 found to be associated with AS in European Caucasians might not be the susceptibility loci for Chinese Han.

RUNX3 is also involved in the pathogenesis of immune-related diseases, such as psoriasis [44,45], asthma [46], coeliac disease [47], Crohn's disease [48], AS [8] and ulcerative colitis [49,50]. Differentiation and function of CD8+ T cells, NK cells and dendritic cells are controlled partially by RUNX3 [14,15,51]. RUNX3 gene polymorphism, however, did not increase the risk of AS susceptibility in Chinese Han.

The MHC gene HLA-B27 has been shown to be associated strongly with ankylosing spondylitis susceptibility, and confers 20–30% of the genetic risk [1]. The combined risk of ERAP1/ERAP2 and HLA-B27 in AS is up to 70% in European populations [52,53], and these two factors are now presumed to be the main risk alleles for AS. As mentioned above, combining earlier data from Lin et al. [25] with our results showed that there is no significant association of ERAP1/ERAP2 polymorphisms and the risk of AS in Chinese Han. The reason for this discrepancy is unclear, and may be due to as yet unknown environmental factors.

Our study has several limitations. First, samples included in the study came from only two affiliated hospitals. In view of the large population of Han Chinese in mainland China, a selection bias for AS and AS with AAU may be present. Thus, multi-centre studies are needed in future studies. Secondly, geographical factors were not taken into account for sample collection. Environmental differences are known to exist between northern and southern, inland, coastal and peninsular areas of China. Thirdly, the meta-analysis confirmed our result and also indicated that geographical factors may be the main reason of high heterogeneity, but the high value of $I^2$ indicates that part of the data should be subject of further analysis.

In conclusion, the present study shows that genetic polymorphisms of ERAP1/ERAP2 and RUNX3 genes may not be involved in AS susceptibility in Chinese Han.

Acknowledgements

This work was supported by National Key R&D Program of China (grant number 2016YFC0904000), Natural Science Major International (Regional) Joint Research Project (grant number 81320108009), National Natural Science Foundation Project (grant number 31370893), National Key Clinical Specialties Construction Program of China, Chongqing Science & Technology Platform and Base Construction Program (grant number cstc2014pt-sy10002). Thanks to all donors enrolled in the present study. The authors thank the patients and controls for their participation in this study.

Author contributions

P. Y., W. S., L. D. and J. D. designed and performed the experiments and wrote the draft. S. L., Q. C. and G. Y. prepared the samples, materials and reagents. P. Y. and A. K. finished the final manuscript.

Disclosure

All authors have declared no conflicts of interest.

References

1. Braun J, Sieper J. Ankylosing spondylitis. Lancet 2007; 369: 1379–90.
2. Ng SC, Liao Z, Yu DT, Chan ES, Zhao L, Gu J. Epidemiology of spondyloarthritides in the People’s Republic of China: review of the literature and commentary. Semin Arthritis Rheum 2007; 37:39–47.
3. Braun J, Bollow M, Remlinger G et al. Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. Arthritis Rheum 1998; 41:58–67.
4. Stolwijk C, Essers I, van Tubergen A et al. The epidemiology of extra-articular manifestations in ankylosing spondylitis: a population-based matched cohort study. Ann Rheum Dis 2015; 74:1373–8.
5. Robinson PC, Leo PJ, Poinot JJ et al. The genetic associations of acute anterior uveitis and their overlap with the genetics of ankylosing spondylitis. Genes Immun 2016; 17:46–51.
6. Yang P, Wan W, Du L et al. Clinical features of HLA-B27-positive acute anterior uveitis with or without ankylosing spondylitis in a Chinese cohort. Br J Ophthalmol 2018; 102:215–9.
7. Tsui FW, Haroon N, Reveille JD et al. Association of an ERAP1 ERAP2 haplotype with familial ankylosing spondylitis. Ann Rheum Dis 2010; 69:733–6.
8. Evans DM, Spencer CC, Poinot JJ et al. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. Nat Genet 2011; 43:761–7.
9. Reveille JD, Sims AM, Danoy P et al. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. Nat Genet 2010; 42:123–7.
10. Cortes A, Hadler J, Poinot JP et al. Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. Nat Genet 2013; 45:730–8.
11. Saveau L, Carroll O, Lindo V et al. Concerted peptide trimming by human ERAP1 and ERAP2 aminopeptidase complexes in the endoplasmic reticulum. Nat Immunol 2005; 6:897–9.
12. Biond J, Rodriguez S, Nicolau ML, al Saati T, Gairin JE. In vivo major histocompatibility complex class I (MHC-I) expression on
MHCI\textsubscript{low} tumor cells is regulated by gammadelta T and NK cells during the early steps of tumor growth. Cancer Immun 2009; 9:10.

13 Martín-Esteban A, Sanz-Bravo A, Guasp P, Barnea E, Admon A, López de Castro JA. Separate effects of the ankylosing spondylitis associated ERAP1 and ERAP2 aminopeptidases determine the influence of their combined phenotype on the HLA-B*27 peptideome. J Autoimmun 2017; 79:28–38.

14 Fainaru O, Woelf E, Lotem J et al Runx3 regulates mouse TGF-beta-mediated dendritic cell function and its absence results in airway inflammation, EMBO J 2004; 23:969–79.

15 Levanon D, Negreanu V, Lotem J et al. Transcription factor Runx3 regulates interleukin-15-dependent natural killer cell activation. Mol Cell Biol 2014; 34:1138–69.

16 Cruz-Guilloty F, Pipkin ME, Djuretic IM et al. Runx3 and T-box proteins cooperate to establish the transcriptional program of effector CTLs. J Exp Med 2009; 206:51–9.

17 Taniguchi I, Osato M, Egawa T et al. Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development. Cell 2002; 111:621–33.

18 Atagunduz P, Appel H, Kuon W et al. HLA-B27-restricted CD8+ T cell response to cartilage-derived self peptides in ankylosing spondylitis. Arthritis Rheum 2005; 52:892–901.

19 Zhang L, Jarvis LB, Baek HJ, Gaston JS. Regulatory IL4+CD8+ T cells in patients with ankylosing spondylitis and healthy control. Ann Rheum Dis 2009; 68:1345–51.

20 Kuhne M, Erben U, Schulze-Tanzil G et al. ARTS1 polymorphisms are associated with ankylosing spondylitis in Han Chinese. Tissue Antigens 2014; 83:324–9.

21 Nagarajan NA, Shastri N. Immune surveillance for ERAAP and RUNX3, TBKBP1, PPARGC1B polymorphisms the influence of their combined phenotype on the HLA-B*27 association ERAP1 and ERAP2 aminopeptidases determine the transcriptional program of effector CTLs. J Exp Med 2009; 206:51–9.

22 York IA, Brehm MA, Zendzian S, Towne CF, Towne CF, Rock KL. Endoplasmic reticulum aminopeptidase 1 (ERAP1) trims MHC class I-presented peptides in vivo and plays an important role in immunodominance. Proc Natl Acad Sci USA 2006; 103:9202–7.

23 Rastall DP, Aldhamen YA, Seregine SS, Godbehere S, Amalfitano A. ERAP1 functions override the intrinsic selection of specific antigens as immunodominant peptides, thereby altering the potency of antigen-specific cytolytic and effector memory T-cell responses. Int Immunol 2014; 26:685–95.

24 Cho SM, Jung SH, Chung YJ. A variant in RUNX3 is associated with the risk of ankylosing spondylitis in Koreans. Genomics Inform 2017; 15:65–8.

25 Lin Z, Bei JX, Shen M et al. A genome-wide association study in Han Chinese identifies new susceptibility loci for ankylosing spondylitis. Nat Genet 2011; 44:73–7.

26 Choi CB, Kim TH, Jun JB et al. ARTS1 polymorphisms are associated with ankylosing spondylitis in Koreans. Ann Rheum Dis 2010; 69:582–4.

27 van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984; 27:361–8.

28 Liu J, Lian Z, Xiao Y, Shi LL, Chai W, Wang Y. Analysis of clinical indexes and RUNX3, TBKBP1, PPARGC1B polymorphisms in Chinese Han patients with ankylosing spondylitis. Genet Test Mol Biomarkers 2015; 19:37–43.

29 Vecchietti M, Roberts AR, Cohen CJ et al. The genetic association of RUNX3 with ankylosing spondylitis can be explained by allelo-specific effects on IRF4 recruitment that alter gene expression. Ann Rheum Dis 2016; 75:1534–40.

30 Wang CM, Ho HH, Chang SW et al. ERAP1 genetic variations associated with HLA-B27 interaction and disease severity of syndesmophytes formation in Taiwanese ankylosing spondylitis. Arthritis Res Ther 2012; 14:R125.

31 Zhang Z, Dai D, Yu K et al. Association of HLA-B27 and ERAP1 with ankylosing spondylitis susceptibility in Beijing Han Chinese. Tissue Antigens 2014; 83:324–9.

32 Burton PR, Clayton DG, Cardon LR et al. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmune variants. Nat Genet 2007; 39:1329–37.

33 Robinson PC, Claushuis TA, Cortes A et al. Genetic dissection of acute anterior uveitis reveals similarities and differences in associations observed with ankylosing spondylitis. Arthritis Rheumatol 2015; 67:140–51.

34 Bang SY, Kim TH, Lee B et al. Genetic studies of ankylosing spondylitis in Koreans confirm associations with ERAP1 and 2p15 reported in white patients. J Rheumatol 2011; 38:322–4.

35 Li C, Lin Z, Xie Y et al. ERAP1 is associated with ankylosing spondylitis in Han Chinese. J Rheumatol 2011; 38:317–21.

36 Haroon N, Tsui FW, Chiu B, Tsui HW, Imman RD. Serum cytokine receptors in ankylosing spondylitis: relationship to inflammatory markers and endoplasmic reticulum aminopeptidase polymorphisms. J Rheumatol 2010; 37:1907–10.

37 Wang J, Li H, Wang J, Gao X. Association between ERAP1 gene polymorphisms and ankylosing spondylitis susceptibility in Han population. Int J Clin Exp Pathol 2015; 8:11641–6.

38 Harvey D, Pointon JJ, Evans DM et al. Investigating the genetic association between ERAP1 and ankylosing spondylitis. Hum Mol Genet 2009; 18:4204–12.

39 Pimentel-Santos FM, Ligeiro D, Matos M et al. Association of IL23R and ERAP1 genes with ankylosing spondylitis in a Portuguese population. Clin Exp Rheumatol 2009; 27:800–6.

40 Szczypiorska M, Sanchez A, Bartolome N et al. ERAP1 polymorphisms and haplotypes are associated with ankylosing spondylitis susceptibility and functional severity in a Spanish population. Rheumatology (Oxford) 2011; 50:1969–75.

41 Cui X, Hawari F, Alsaaey S et al. Identification of ARTS-1 as a novel TNFR1-binding protein that promotes TNFR1 ectodomain shedding. J Clin Invest 2002; 110:515–26.

42 Cui X, Rouhani FN, Hawari F, Levine SJ. Shedding of the type II IL-1 decoy receptor requires a multifunctional aminopeptidase, aminopeptidase regulator of TNF receptor type 1 shedding. J Immunol 2003; 171:6814–9.

43 Cui X, Rouhani FN, Hawari F, Levine SJ. An aminopeptidase, ARTS-1, is required for interleukin-6 receptor shedding. J Biol Chem 2003; 278:28677–85.

44 Tsoi LC, Spain SL, Knight J et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. Nat Genet 2012; 44:1341–8.

45 Apel M, Ube S, Bowes J et al. Variants in RUNX3 contribute to susceptibility to psoriatic arthritis, exhibiting further common ground with ankylosing spondylitis. Arthritis Rheum 2013; 65:1224–31.

46 Laprise C. The Sagueneay-Lac-Saint-Jean asthma familial collection: the genetics of asthma in a young founder population. Genes Immun 2014; 15:247–55.

47 Trynka G, Wijmenga C, van Heel DA. A genetic perspective on coeliac disease. Trends Mol Med 2010; 16:537–50.
Franken A, McGovern DP, Barrett JC et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn’s disease susceptibility loci. Nat Genet 2010; 42:1118–25.

Guo C, Yao F, Wu K, Yang L, Zhang X, Ding J. Chromatin immunoprecipitation and association study revealed a possible role of Runt-related transcription factor 3 in the ulcerative colitis of Chinese population. Clin Immunol 2010; 135:483–9.

Weersma RK, Zhou L, Nolte IM et al. Runt-related transcription factor 3 is associated with ulcerative colitis and shows epistasis with solute carrier family 22, members 4 and 5. Inflamm Bowel Dis 2008; 14:1615–22.

Dicken J, Mildner A, Leshkowitz D et al. Transcriptional reprogramming of CD11b+Esam(hi) dendritic cell identity and function by loss of Runx3. PLOS ONE 2013; 8:e77490.

Brown MA. Breakthroughs in genetic studies of ankylosing spondylitis. Rheumatology (Oxford) 2008; 47:132–7.

Seregin SS, Rastall DP, Evnouchidou I et al. Endoplasmic reticulum aminopeptidase-1 alleles associated with increased risk of ankylosing spondylitis reduce HLA-B27 mediated presentation of multiple antigens. Autoimmunity 2013; 46:497–508.

Supporting information
Additional Supporting information may be found in the online version of this article at the publisher’s web-site:

Fig. S1. Forest plots of odds ratios (OR) with 95% confidence intervals (CI) for rs30187 in endoplasmic reticulum-associated aminopeptidase (ERAP1) and risk of ankylosing spondylitis (AS) of all studies [(a) studies from both Europe and Asia, (b) studies from Europe, (c) studies from Asia (Chinese mainland, China Taiwan Island, Korea), (d) studies from Chinese mainland and China Taiwan Island and (e) studies by Lin and Su WC]. As shown in (e), data from our study and genome-wide association study (GWAS) of Lin, $I^2 = 36\%$ and $P = 0.11$.

Fig. S2. Forest plots for endoplasmic reticulum-associated aminopeptidase (ERAP1): rs27037 and susceptibility of ankylosing spondylitis (AS) of all studies [(a) studies from both Europe and Asia, (b) studies from Asia (Chinese mainland, China Taiwan Island, Korea), (c) studies from Chinese mainland and China Taiwan Island, (d) studies by Zhang and Su from Chinese mainland]. As shown in (d), data from our study and Zhang, $I^2 = 0\%$ and $P = 0.11$.

Fig. S3. Forest plots for endoplasmic reticulum-associated aminopeptidase (ERAP1): rs27434 and susceptibility of ankylosing spondylitis (AS) of all studies [(a) studies from both Europe and Asia, (b) studies from Europe, (c) studies from Asia (Chinese mainland, China Taiwan Island, Korea), (d) studies from Chinese mainland, (e) studies by Zhang, I² 50% and P 5 0.05].

Fig. S4. Forest plots for endoplasmic reticulum-associated aminopeptidase (ERAP1): rs27582 and susceptibility of ankylosing spondylitis (AS) of all studies [(a) studies from both Europe and Asia, (b) studies from Europe, (c) studies from Asia (Chinese mainland, China Taiwan Island, Korea), (d) studies by Zhang and Su from Chinese mainland)]. As shown in (d), data from Chinese mainland, $I^2 = 84\%$ and $P = 0.13$.

Fig. S5. Forest plots for endoplasmic reticulum-associated aminopeptidase (ERAP1): rs27980 and susceptibility of ankylosing spondylitis (AS) of all studies [(a) studies from both Europe and Asia, (b) studies from Chinese mainland]. As shown in (b), data from Chinese mainland, $I^2 = 77\%$ and $P = 0.61$.

Fig. S6. Forest plots for endoplasmic reticulum-associated aminopeptidase (ERAP1): rs27980 and susceptibility of ankylosing spondylitis (AS) of all studies [(a) studies from both Europe and Asia, (b) studies from Chinese mainland and China Taiwan Island, (c) studies by Zhang and Su from Chinese mainland)]. As shown in (c), data from Chinese mainland, $I^2 = 0\%$ and $P = 0.07$.

Table S1. Included and excluded studies in the meta-analysis
Table S2. Allele and genotype frequencies of single nucleotide polymorphisms (SNPs) in Chinese Han patients with ankylosing spondylitis (AS) and AS with AAU
Table S3. Haplotype analysis of 11 SNP sites of ERAP1 in Chinese Han ankylosing spondylitis (AS) patients with or without acute anterior uveitis (AAU)