Genetic association of non-MHC region with ankylosing spondylitis in a Chinese population

Ankylosing spondylitis (AS) is a chronic inflammatory disease mainly affecting the sacroiliac joints and spine.[1] In order to examine overall genetic susceptibility of AS, several genome-wide association studies (GWASs) were performed in Caucasian populations, and a number of genetic polymorphisms in non-major histocompatibility complex (MHC) regions were found, such as *ERAP1*, *IL23R* as well as many others.[2–6] However, only a small portion of the single nucleotide polymorphisms (SNPs) were validated in East Asian (EA) populations.[2] In recent years, multiple studies indicated significant genetic differences between different European and EA populations.[2 7] To resolve this further, we have examined all reported AS-associated SNPs in non-MHC regions in Chinese patients with AS. A total of 1289 patients with AS and 1536 controls were included in this analysis, which have not been included in prior analyses.[2 7] The sociodemographic and clinical characteristics of the cohort are presented in online supplementary table S1.

In this study, the candidate SNPs were mainly selected from six GWAS studies published from 2007 to 2016.[2–6 8] Overall, 69 SNPs in non-MHC regions were included, 32 of which had not been validated in EA population before. The results of six loci (rs6759298, rs2297518, rs75301646, rs12615545, rs5837881 and rs27044) were consistent with prior reports in the Caucasian populations (table 1). Among them, the SNP rs2297518 in the *NOS2* gene (OR/p_value (false discovery rate (FDR))=1.328/8.6E-03) and rs5837881 in an intergenic region (OR/p_value (FDR)=0.80/0.04) were not validated previously in EA populations, whereas four other SNPs were consistent with previous reports in Asian cohorts.[2 7] Nine loci that had been previously associated with AS in relatively small cohorts of Chinese population were not validated in the present results (online supplementary table S2). Twenty-four loci that previously showed an association with AS but with p value not at genome-wide significance ($>10^{-8}$) in both Caucasian and EA populations were not validated in our results (online supplementary table S3). Thirty-one loci previously reported implicated only in Caucasians did not show an association with AS in this study (online supplementary table S4).

In this study, all six SNPs showed significant differentiation between AS and controls in both Caucasians and our samples. Among them, rs6759298 located in a 'gene desert' at chromosome 2p15 showed highest significance in both Caucasian and Chinese data (OR/p value=1.31/3.60E-41, 1.22/3.81E-04, respectively). The Genotype-Tissue Expression dataset showed that rs6759298 has expression quantitative trait loci (eQTL) with *B3GNT2* (online supplementary figure 1A). In addition, the database of Mouse Genome Informatics (MGI) showed that mice with *B3gnt2*-knocked-out showed increased levels of inflammatory cytokines such as interleukin (IL)-6, IL-1β and tumour necrosis factor-alpha). An association with another SNP (rs2297518) located in *NOS2*, which has never been reported in Chinese data of patients with AS, was found. It is a mutation that causes deleterious alterations in the coding region of *NOS2*. Rs75301646 is an intron variant on *SULT1A2*, which has eQTL with *SULT1A1* expression (online supplementary figure 1B). MGI showed *Sult1c1*-knockout- mice have abnormal immune responses and increased sacral vertebrae number. Rs12615545 is near *UBE2E3* and has eQTL with *UBE2E3* (ubiquitin enzyme)

Table 1 Genetic associations with AS and controls in the Chinese population

			Western		Asian		This study			
Gene/region	SNP	Allele	OR	P values	OR	P values	MAF	OR	P values	P_adj
2p15[2]	**rs6759298**	G	1.31	3.60E-41	1.28	1.60E-06	0.4418/0.3917	1.22	3.81E-04	8.60E-03
NOS2[2]	**rs2297518**	A	1.13	6.30E-07	NA	NA	0.176/0.1387	1.33	1.83E-04	8.60E-03
SULTIA1[2]	**rs75301646**	A	1.11	1.40E-07	1.16	0.012	0.292/0.2472	1.26	2.55E-04	8.60E-03
UBE2E3[2]	**rs12615545**	T	0.90	2.30E-07	0.83	8.50E-04	0.2959/0.3379	0.82	6.66E-04	0.01
Chr2 Indel[6]	**rs5837881**	T	0.88	1.26E-13	NA	NA	0.149/0.18	0.80	3.14E-03	0.04
ERAP1[3 10]	**rs27044**	C	0.71	1.00E-06	1.30	9.37E-07	0.4694/0.5046	0.86	4.70E-03	0.05

AS, ankylosing spondylitis; MAF, minor allele frequency.

in oesophageal mucosa, suggesting it might be related to inflammation status (online supplementary figure S1C).

Other loci were not validated in our samples such as *IL12B*, *ANTXR2* and *FCGR2A*.[9] We postulated that some different SNPs located in the same genes or genes in the same pathway can substitute for those loci in the Chinese population as has been observed with *IL23R*.[2] Therefore, it is essential to find associated SNPs in different cohorts by sequencing or different chip analyses to extend our knowledge to the pathogenesis of AS.

In conclusion, we genotyped 69 previously reported non-MHC AS-associated SNPs in a different Chinese cohort and found that six loci showed significant differences between patients with AS and controls in both Caucasian and EA populations. Usually, several SNPs may have similar effect to the same gene. Therefore, in the future, we can perform functional study of genes controlled by several SNPs in the mouse.

Jing Liu,[1,2] Weilin Pu,[1,2] Yuan Li,[1,2] Yanyun Ma,[2,3] Qi Zhu,[4] Wei Wan,[5] Chengde Yang,[6] Xiaofeng Wang,[1,2] Xingdong Chen,[1,7] Xiaodong Zhou,[8] John D Reveille,[8] Li Jin,[1,2] Hejian Zou,[9,10] Jiucun Wang[1,11]

[1]State Key Laboratory of Genetic Engineering, Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Fudan University, Shanghai, China
[2]Human Phenome Institute, Fudan University, Shanghai, China
[3]Ministry of Education Key Laboratory of Contemporary Anthropology, Department of Anthropology and Human Genetics, School of Life Sciences, Fudan University, Shanghai, China
[4]Institute of Arthritis Research, Shanghai Academy of Chinese Medical Sciences, Guanghua Integrative Medicine Hospital, Shanghai, China
[5]Division of Rheumatology and Immunology, Changhai Hospital, Shanghai, China
[6]Division of Rheumatology, Ruijin Hosptial, Shanghai Jiaotong University Schools of Medicine, Shanghai, China
[7]Fudan-Taizhou Institute of Health Sciences, Taizhou, China
[8]Division of Rheumatology and Clinical Immunogenetics, The University of Texas-McGovern Medical School, Houston, TX, United States
[9]Division of Rheumatology, Huashan Hospital, Fudan University, Shanghai, China
[10]Institute of Rheumatology, Immunology and Allergy, Fudan University, Shanghai, China
[11]Division of Dermatology, Huashan Hospital, Fudan University, Shanghai, China

Correspondence to Professor Jiucun Wang, State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Division of Dermatology, Huashan Hospital, Shanghai 200438, China; jcwang@fudan.edu.cn and Hejian Zou, Division of Rheumatology, Huashan Hospital, Fudan University, Shanghai, China; hjzou@fudan.edu.cn

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Contributors JW and HZ designed the project and wrote the manuscript. JL and WP collected data, performed genotyping and analysis and wrote the manuscript. YL and YM managed all samples. QZ, WW and CY provided the ankylosing spondylitis samples. XW and XC provided the control samples. XZ, JDR and LJ revised the manuscript and provided valuable suggestions.

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