

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-1 β and tumour necrosis factor- α). 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

Gene/region	SNP	Allele	Western		Asian		This study			
			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
<i>NOS2</i> ²	rs2297518	A	1.13	6.30E-07	NA	NA	0.176/0.1387	1.33	1.83E-04	8.60E-03
<i>SULT1A1</i> ²	rs75301646	A	1.11	1.40E-07	1.16	0.012	0.292/0.2472	1.26	2.55E-04	8.60E-03
<i>UBE2E3</i> 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
<i>ERAP1</i> ^{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.

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