Both *HLA* class I and II regions identified as genome-wide significant susceptibility loci for adult-onset Still's disease in Chinese individuals

Adult-onset Still's disease (AOSD) is a rare autoinflammatory condition with unclear aetiology and highly heterogeneous manifestations, such as spiking fever, skin rash and arthralgia (or arthritis).[1 2] AOSD is usually the result of both genetic and environmental factors. However, only a few genetic loci have been associated with AOSD, and none of them have reached the threshold for genome-wide significance (GWS) of $p<5\times10^{-8}$.[2 3] For example, *HLA-DRB1* was strongly associated with AOSD in previous studies (the p value for the most significant association was 8.60×10^{-6}).[3] *HLA-DRB1* also influences the risk of systemic juvenile idiopathic arthritis (JIA),[4] which presents similarly to AOSD but differs in age of onset. In addition, functional variations in periodic fever syndrome genes have been identified in some AOSD patients (Bonferroni corrected p values ranged from 2.34×10^{-3} to 2.40×10^{-4}).[5]

In this study, for the first time, we conducted a genome-wide association study (GWAS) to systematically screen genetic factors influencing susceptibility to AOSD (online supplementary text). Principal component analysis was adopted to evaluate population stratification between cases and controls and to detect outliers for removal (online supplementary figure S1). After quality control and imputation, a total of 3 547 931 variants in 264 AOSD cases and 2420 controls (discovery: 247 cases vs 2163 controls; replication: 17 cases vs 257 controls; online supplementary text and table S1) from China were analysed. The genomic inflation, λ_{GC}, was 1.018, indicating the presence of population stratification to a minimal degree (online supplementary figure S2). A total of 1281 variants, from the *HLA* class I and II regions, exceeded GWS in this analysis (online supplementary figure S3).

The most significant association was with rs9268791 (OR=2.363, $p=4.10\times10^{-19}$; table 1 and figure 1A), located in the intergenic region between *HLA-DRA* and *HLA-DRB5* (the *HLA* class II region). For the *HLA* class I region, the top GWS signal was observed for rs3094178 (OR=2.139, $p=1.97\times10^{-8}$; table 1 and figure 1B), which is located 19 kb downstream of *HLA-G*. Outside the *HLA* region, a suggestive association ($p<10^{-5}$) was observed at *VEGFC* (rs514410, OR=2.211, $p=1.81\times10^{-6}$; online supplementary table S2, figure S4 and text).

We explored regulatory annotations for the GWS SNPs and variants in strong linkage disequilibrium (LD) with them ($r^2>0.8$) using HaploReg[6] and RegulomeDB,[7] and 94 SNPs had a RegulomeDB score from 1a–1f to 2a–2c, suggesting that they are located in regulatory regions with maximum evidence (online supplementary table S3). Of them, the top SNPs in each region were rs3115628 ($p=2.92\times10^{-8}$; *HLA* class I) and rs9268832 ($p=3.41\times10^{-18}$; *HLA* class II). In addition, the expression quantitative trait loci analyses suggested that rs9268832 was significantly associated with the expression of several genes (including *HLA-DRB1*, *HLA-DRB6*, *HLA-DQA2* and *HLA-DQB1*; $p<5\times10^{-8}$) and that rs3115628 was significantly correlated with *ZFP57* and four *HLA* pseudogenes ($p<5\times10^{-8}$) in different types of tissues (online supplementary table S4).[8] Further studies are required to clarify the mechanisms underlying these associations.

Previous GWAS of JIA on primarily European ancestry samples has also identified three GWS SNPs in the *HLA* region.[9 10] However, none of them overlapped with the AOSD-associated markers identified in this study. Pair-wise LD analysis showed that these SNPs are in low LD ($r^2<0.1$, online supplementary table S5). This finding suggests genetic heterogeneity in these diseases and across different populations.

Table 1 Summary statistics for the genome-wide significant association of single nucleotide polymorphisms with adult-onset Still's disease

SNP	Nearby gene(s)	Chr	Position (hg19)	Risk/non-risk allele	Stage*	RAF in cases	RAF in controls	OR	SE	P values	P_het
rs9268791	*HLA-DRA|HLA-DRB5*	6	32 421 073	T/C	Meta	–	–	2.363	0.096	4.10E−19	0.686
					Discovery	0.468	0.273	2.389	0.100	2.85E−18	
					Replication	0.382	0.224	2.054	0.360	4.54E−02	
rs3094178	*HLA-G*	6	29 818 000	C/G	Meta	–	–	2.139	0.135	1.97E−08	0.806
					Discovery	0.875	0.769	2.116	0.143	1.48E−07	
					Replication	0.767	0.568	2.366	0.432	4.62E−02	

*The discovery data set comprises 247 cases and 2163 controls from northern and central China. The replication data set comprises 17 cases and 257 controls from southern China.
Chr, chromosome;P_het, p value for heterogeneity test across different cohorts using Cochran's Q test; RAF, frequency of risk allele;SNP, single nucleotide polymorphism.

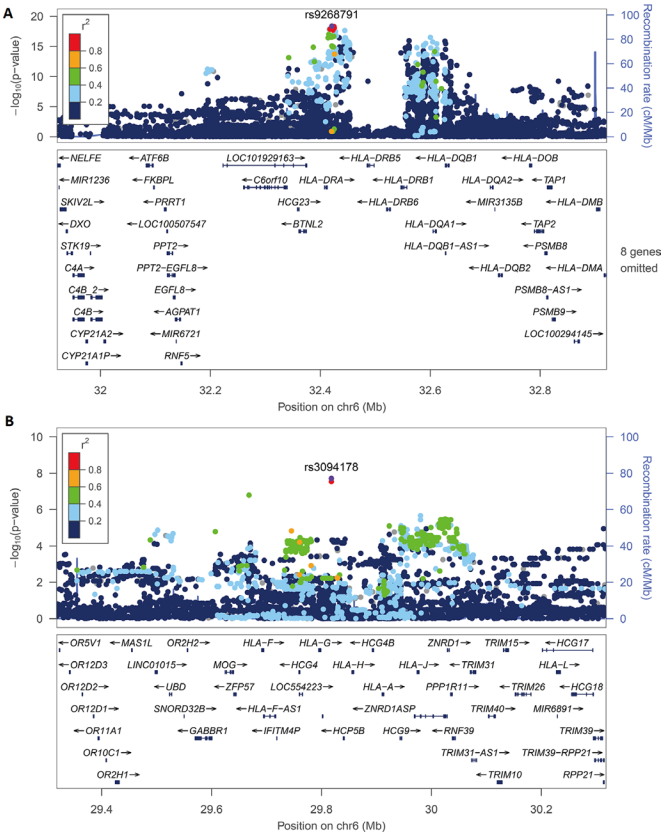

Figure 1 Regional association plots of the adult-onset Still's disease-associated *HLA* class I and II regions. (A) *HLA* class II region and (B) *HLA* class I region. Purple circles represent the most significantly associated single nucleotide polymorphisms (SNPs) (marker SNPs) in each region in the meta-analysis of discovery and replication. $-\log^{10}$p values (Y-axis) of the SNPs (within the regions spanning 500 kb on either side of the marker SNP) are presented according to the chromosomal positions of the SNPs (X-axis, hg19). SNPs are coloured according to their linkage disequilibrium (LD) with the marker SNP. The LD values were established based on the 1000 Genomes Asian (ASI) Data (March 2012). Estimated recombination rates with samples from the 1000 Genomes Project March 2012 release are shown as blue lines, and the genomic locations of genes within the regions of interest annotated from the University of California, Santa Cruz (UCSC) genome browser are shown as arrows.

In summary, we identified the first GWS risk loci and indicated an important role for both *HLA* class I and II regions in the genetic architecture of AOSD using a genome-wide scan in a Chinese sample. Further replication studies of other populations are necessary to confirm our findings and to investigate the possible ancestral differences.

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