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RESEARCH ARTICLE

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Genome-wide association study of 7661 Chinese Han individuals and fine-mapping major histocompatibility complex identifies HLA-DRB1 as associated with IgA vasculitis

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Abstract

Background: Immunoglobulin-A vasculitis (IgAV) is an immune-related systemic vasculitis with an unclear etiology. Genetic predisposition is now considered to be closely associated with the development of the disease, and it is essential to reveal the relationship between them. To explore the role of heredity in the disease, we performed a genome-wide association study (GWAS) of 496 IgAV cases and 7165 controls using an Illumina Infinium Global Screening Array chip.

Methods: In the first stage of analysis, a significant correlation between the major histocompatibility complex (MHC) and IgAV was observed. Subsequently, human leukocyte antigen (HLA) analysis was conducted using a new large-scale Han-MHC reference panel. Fine mapping of IgAV risk in the MHC region indicated that two amino acid positions, 120 and 11, of HLA-DRB1 and three potential HLA alleles (HLA-DRB1∗04, HLA-DRB1∗16, and HLA-DRB1∗16:02) were significantly associated.

Results: Further stepwise conditional analysis demonstrated that 3 amino acid positions (120, 26, 96) of HLA-DRB1 and 6 HLA-DRB1 alleles (HLA-DRB1*04, HLA-DRB1*16, HLA-DRB1*01, HLA-DRB1*12:02, HLA-DRB1*10, and HLA-DRB1*15:02) were independent signals. Among them, the most significant signal was HLA-DRB1 amino acid Ser120 (OR = 1.59, $p = 3.19 \times 10^{-8}$); no independent signal in the MHC region except for HLA-DRB1 was found.

Conclusions: Our study confirms that the pathogenesis of IgAV has a genetic component and that HLA-DRB1 is strongly associated with susceptibility to IgAV.

KEYWORDS
Henoch-Schönlein purpura, HLA-DRB1, Immunoglobulin-A vasculitis, susceptibility

1 | INTRODUCTION

Immunoglobulin-A (IgA) vasculitis (IgAV; formerly called Henoch-Schönlein purpura) is an autoimmune vasculitis characterized by extensive deposition of IgA1-dominant immune complexes in capillaries, arterioles, and venules, with a reported incidence of 3–27/100.000 in children and 0.8–5.1/100.000 in adults.[1–3] IgAV is the most common subtype of vasculitis in children aged 2–10 years.

the median age of onset is 4 years, and it is also present during adulthood.[4] IgAV is clinically characterized by nonthrombocytopenic purpura, which can be combined with joint swelling and pain, joint cavity effusion, gastrointestinal symptoms (abdominal pain and blood in the stool), and kidney involvement (hematuria and proteinuria); a few cases may involve the central nervous system and testis.[5,6] Although most patients have a good prognosis, with self-limiting disease, kidney involvement is often considered to result in a relatively poor prognosis.[7]

The etiology and pathogenesis of IgAV have not yet been fully elucidated. Pathogenic microbial infections, drugs, vaccination, insect bites, and foods are generally considered to trigger onset of IgAV. Compared with the Caucasian population, Asians have a higher incidence rate,[8] a significantly higher risk of affected first-degree relatives of patients[4] and the phenomenon of family clusters,[9] suggesting that IgAV has a strong genetic predisposition. Overall, understanding genetic abnormalities of IgAV will be crucial for understanding the development of disease. A series of studies to date have reported that genetic abnormalities contribute to the development of IgAV and that the genetic component of IgAV is complex, perhaps as a consequence of gene–gene interactions.[10] A series of human leukocyte antigen (HLA) gene alleles located in the major histocompatibility complex (MHC) region are currently considered to be closely related to susceptibility to IgAV through a few candidate gene studies.[11] Genome-wide association study (GWAS) research explores single-nucleotide polymorphisms (SNPs) at the upstream genome-wide level, and its value has been confirmed in a series of primary vasculitis diseases, such as aortic arteritis, Behçet disease, and ANCA-related vasculitis.[12] In addition to HLA alleles, amino acid site anomaly signals can also be found by further imputation of the HLA region. The first GWAS of IgAV in 2017 from Spain reporting that the HLA-II region between *HLA-DQA1* and *HLA-DQB1* in those of Caucasian ancestry is closely related to disease susceptibility.[13] But GWAS with IgAV in the East Asian population, especially in the Chinese Han population, are still lacking.

The purpose of this study was to perform a GWAS of the Chinese Han population with IgAV and to use the complete 5-Mb MHC local database constructed by the Institute of Dermatology of Anhui Medical University covering 10,689 cases of HLA gene variants in a healthy population[14] for fine mapping of the MHC to identify IgAV-related susceptible gene variant sites.

2 | MATERIAL AND METHODS

2.1 | Study population

The initial stage of the GWAS analysis included 514 cases of the Chinese Han population diagnosed with IgAV mainly from the First Affiliated Hospital of Anhui Medical University in central China and 7186 healthy controls from the Chinese Han population. At least two doctors diagnosed and classified IgAV according to guidelines for the diagnosis and classification of childhood vasculitis developed by the European Society of Children's Rheumatology.[15] EDTA-anticoagulated blood samples were collected for DNA extraction for genotyping. All subjects signed informed consent before participating in the trial. Clinical information was collected from the patients or their guardians through comprehensive clinical examinations, and questionnaires were used to gather other demographic information from the patient and control populations. None of the controls had IgAV or other autoimmune diseases and no family history of IgAV or other autoimmune diseases (including first-, second-, and third-degree relatives). All trial protocols were carried out after being authorized and approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University in accordance with the principles of the Declaration of Helsinki.

2.2 | GWAS genotyping and quality control

Genomic DNA was extracted from the EDTA-anticoagulated blood samples according to standard procedures and adjusted to a concentration of 50 ng/μl for genotyping. We used the Illumina Infinium Global Screening Array BeadChip, which includes 700,078 markers; a cohort including 7698 samples (512 patients and 7186 controls) was genotyped using the GSA array. Genotyping was conducted at the Key Laboratory of Dermatology of Anhui Medical University and Shanghai Jingneng Biology. Genotype calling and the clustering of study sample genotypes were performed using Illumina's GenTrain (version 1.0) clustering algorithm in Genome Studio (version 2011.1). Finally, 500 patients and 7186 controls were subjected to subsequent genotyping. Smartpca software33 was used to process principal component analysis (PCA) outliers.[16] PLINK v.1.071 software was applied for quality control for filtering the original data. Furthermore, we excluded SNPs with a call rate <98%, minimum allele frequency (MAF) <1%, and Hardy–Weinberg equilibrium (HWE) p value $<1 \times 10^{-4}$; SNPs and copy number variant (CNV) probes on the X chromosome, Y chromosome, and mitochondrial chromosome were removed from further analysis. After quality control, genotype data for 33,347 autosomal variants in 496 patients and 7165 controls were included for further analysis.

2.3 | Imputation of HLA alleles, amino acid sites, and SNPs in the MHC region

The 5814 SNP genotypes contained in the MHC region (chromosome 6:29–34 Mb) of the GSA chip were extracted for analysis. HLA allele region genotype filling was performed with SNP2HLA package v1.03 and Beagle software.[17] The reference dataset relied on a newly established MHC reference library based on deep sequencing of the MHC region in the Chinese Han population, containing 29,948 variants in 10,689 cases of a healthy Han population in China.[14] This MHC reference library contains 8 HLA genes (HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQA1, HLA-DQB1, HLA-DPA1, and HLA-DPB1) and amino acid polymorphism SNPs. PLINK version 1.07 software was

employed to refer to previous genotype filling data, and low-quality genotype filling ($r^2 < 0.3$), minimum allele frequency (MAF) <0.01 and significantly different from Hardy–Weinberg law ($p < 1 \times 10^{-4}$) mutations were removed. The imputation predicted r^2 with true genotypes used as imputation quality, and the imputed markers which had r^2 value <0.3 was filtered. Ultimately, 22,966 variants remained for further analysis. Among the above variants, there were 634 amino acid variants for HLA class I and II antigens, 165 = classic HLA alleles (63 two alleles and 102 four alleles), and 22,167 SNP insertions or missing.

2.4 | Statistical analysis

All HLA variants were recorded as binary tags, similar to those recorded elsewhere. To evaluate how the variation affects the risk of IgAV, we used the logistic regression model by PLINK software (v.1.9) to assume that each allele exerts an additive effect on the scale. The statistical results are not presented using genome control correction because according to λgc (genome expansion coefficient) results reported earlier, there is negligible evidence of population stratification, which is consistent with our previous research results.[18] To identify distinctive independent HLA allelic variants and corresponding amino acid changes, a stepwise condition analysis was performed to evaluate the independent signal with PLINK software (v.1.9). Because the statistical analysis is done at the genetic level, the analysis was completed until no significant variants appeared in the results ($p > 0.05$). The R language (4.0.3) was used to visualize the results.

3 | RESULTS

3.1 | General situation of the research subjects and SNPs

This study included statistical analysis of the average age, the male to female ratio, and the sample size of the Chinese Han population. The results are shown in Table 1.

3.2 | Statistical analysis of genome-wide SNP typing data

PCA was used to assess typing data for 496 IgAV patients and 7165 controls. No abnormal deviation was found among the samples, and the case and control samples were basically matched. The PCA chart is shown in Figure 1.

After strict quality control, Plink1.07 software was used to count 377,302 SNPs genome-wide in 496 patients with IgAV and 7165 normal controls. SNPs in the HLA region were strongly related to IgAV (Figure 2A). This finding suggested that the HLA gene located in the MHC region may be closely related to susceptibility to IgAV. By analyzing 377,302 whole-gene SNPs, corrected genome expansion coefficient (λgc) of 1.39778 (1.37931 after excluding the MHC region) was calculated; the QQ plot is shown in Figure 2B. Among them, we detected 112 SNPs in the HLA region (Chr. 6: 29–34 Mb), with a P_GWAS value $<10 \times 10^{-5}$. The correlation between 17SNPs and IgAV reached the significance level of the entire genome ($p < 5 \times 10^{-8}$), and 18 SNPs (rs9275407, rs9275440, rs9275332, rs9275393, rs9275428, rs9275371, rs9275439, rs9275390, rs9275377, rs9272346, rs9275464, rs660895, rs521539, rs9275365, rs17427599, rs9269110, rs9275327, and rs6903608) are located in the MHC region (Table 2).

3.3 | HLA genotype filling and quality control results

We used SNP2HLA software to fill in the HLA genotypes for 496 IgAV patients and 7165 controls in the MHC region using the improved MHC region reference dataset above and obtained corresponding data for 29,948 loci in the HLA region. Quality control was performed again, and 22,966 sites were obtained, including 165 HLA alleles, 634 HLA gene corresponding amino acid positions and 22,167 SNP sites. After Bonferroni correction, the correlation analysis result $p < 2.21 \times 10^{-6}$ was defined as statistically significant.

3.4 | Association analysis results of classic HLA alleles and amino acid site SNPs

We performed logistic single-point association analysis on the data for 496 IgAV patients and 7165 controls after the previous quality control step. After imputation of the newly constructed MHC region reference data, the association analysis showed multiple allele and amino acid variations in HLA-DRB1 to be associated with susceptibility to IgAV. We conducted a single-point association analysis of 165 classic HLA alleles and found that 3 HLA-DRB1 alleles were infinite near the significance level of this study ($p < 2.21 \times 10^{-6}$), with HLA-DRB1*04 being the most significant site ($p = 3.17 \times 10^{-6}$). The frequency of HLA-DRB1*04 was 16.1% among the IgAV patients and 11.23% among the controls. The OR value calculated by the logistic regression model was 1.517 (95% CI: 1.207–1.809), and the specific results are shown in Table 3. After analyzing the corresponding positions of 634 variants in HLA

TABLE 1 Summary of the samples used for GWAS

Cases			Control			
Sample size	male/female	Mean age	Sample size	male/female	Mean age	Total
496	283/213	16.58 (±14.16)	7165	3537/3628	33.46 (±15.38)	7661

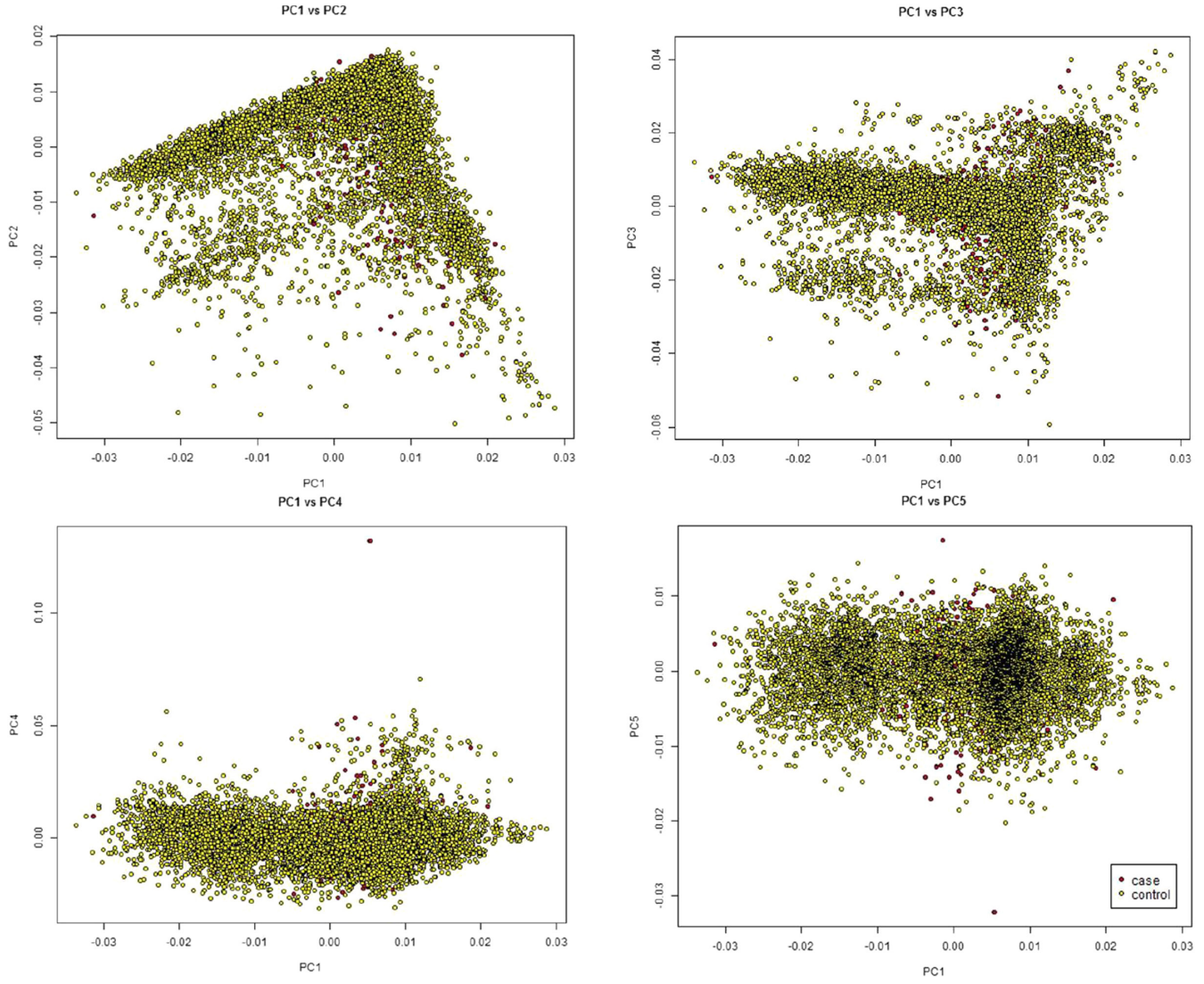

FIGURE 1 Principal component analysis (PCA) of samples in the central Chinese Han population

genes, we found that 3 amino acid positions reached a significant difference ($p < 2.21 \times 10^{-6}$); the results are also shown in Table 3. The most significantly related site were amino acid 120 of HLA-DRB1: serine (Ser), aspartic acid (Asp) and position 11: valine (Val). The former is a risk type change (OR = 1.593, $p = 3.19 \times 10^{-8}$). All of the findings suggest that the HLA-DRB1 gene is related to susceptibility to IgAV. In a comparison of 22,167 SNPs between the cases and controls, 143 reached the level of association significance ($p < 2.21 \times 10^{-6}$), and the specific results are shown in Table 3.

3.5 | Stepwise regression analysis in the MHC region

The most relevant gene in the MHC region is HLA-DRB1 (Figure 3A). Since HLA genes and the corresponding amino acids are the most basic units for function, we conducted stepwise regression analysis for HLA alleles and corresponding amino acid positions and found 6 independent HLA-DRB1 allele signals: HLA-DRB1*04, HLA-DRB1*16, HLA-DRB1*01, HLA-DRB1*1202, HLA-DRB1*10, and HLA-DRB1*1502 (Table 4). We also detected 3 independent signals of HLA-DRB1 amino acid positions: HLA-DRB1 amino acid Ser120 (OR = 1.593, $p = 3.19 \times 10^{-8}$), HLA-DRB1 amino acid Leu26, and HLA-DRB1 amino acid Glu96 (Table 5). After controlling for HLA-DRB1 alleles and amino acids, we did not discover any other significant independent signal in the MHC region (Figure 3B,C). The statistical significance in both association and stepwise regression analyses of the change at amino acid 120 suggests that HLA-DRB1 amino acid 120 is a marker of susceptibility to IgAV. After selecting independent signal sites in HLA genes, the signal changes in the entire stepwise regression are shown in Figure 3.

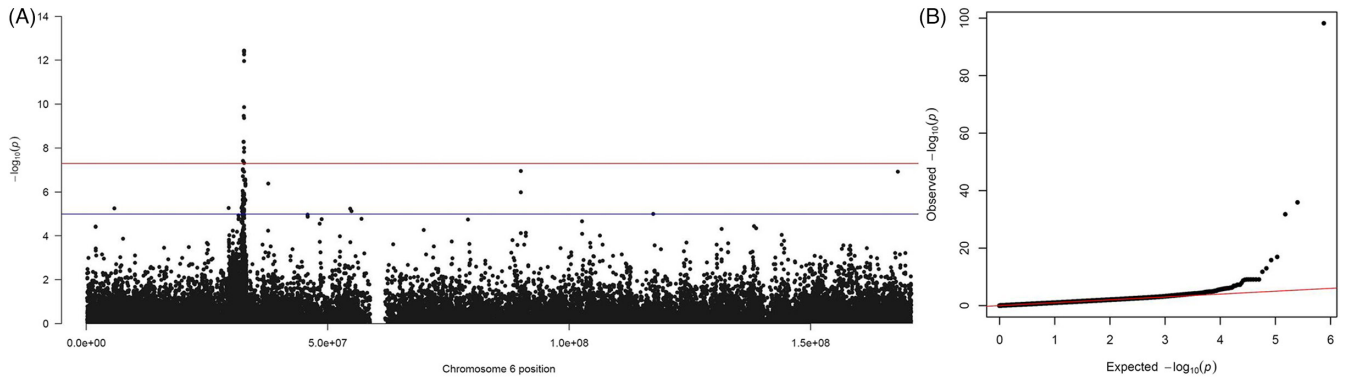

FIGURE 2 Summary of genome-wide association results for 496 cases and 7165 controls. (A) The genome-wide *p* values ($-\log_{10} p$) of logistic regression analysis adjusted for sex (19,899 SNPs) plotted against the position on chromosome 6. (B) Quantile–quantile plots of the observed *p* values versus the expected values from the *p* value of association. The plot in black is based on the entire set of 377,302 SNPs

TABLE 2 Association evidence for 18 SNPs at MHC loci in GWAS

CHR[a]	SNP	Position[b]	Allele[c]	MAF[d]		OR (95% CI)[e]	*p* value
				Case	Control		
6	rs9275407	32,670,037	T/G	0.3303	0.2289	1.66 (1.45–1.91)	3.63×10^{-13}
6	rs9275440	32,671,596	T/C	0.3303	0.229	1.66 (1.45–1.91)	3.65×10^{-13}
6	rs9275332	32,666,943	A/G	0.3303	0.229	1.66 (1.45–1.91)	3.8×10^{-13}
6	rs9275393	32,669,439	A/G	0.3303	0.229	1.66 (1.45–1.91)	3.8×10^{-13}
6	rs9275428	32,670,978	G/A	0.3303	0.2291	1.66 (1.45–1.91)	3.87×10^{-13}
6	rs9275371	32,668,296	C/T	0.3303	0.2292	1.66 (1.45–1.91)	4.15×10^{-13}
6	rs9275439	32,671,521	C/T	0.3296	0.2291	1.65 (1.44–1.90)	5.43×10^{-13}
6	rs9275390	32,669,156	C/T	0.3289	0.2295	1.65 (1.43–1.89)	1.08×10^{-13}
6	rs9275377	32,668,667	G/A	0.2319	0.1546	1.65 (1.41–1.93)	1.34×10^{-10}
6	rs9272346	32,604,372	G/A	0.2436	0.3538	0.59 (0.50–0.70)	3.38×10^{-10}
6	rs9275464	32,672,082	G/A	0.2327	0.1567	1.63 (1.40–1.91)	4.28×10^{-10}
6	rs660895	32,577,380	G/A	0.2834	0.2051	1.53 (1.33–1.77)	5.13×10^{-9}
6	rs521539	32,581,973	T/A	0.2828	0.2047	1.53 (1.33–1.77)	5.23×10^{-9}
6	rs9275365	32,668,125	A/G	0.2581	0.1843	1.54 (1.33–1.79)	9.69×10^{-9}
6	rs17427599	32,667,364	T/C	0.2692	0.1948	1.52 (1.32–1.76)	1.46×10^{-8}
6	rs9269110	32,443,269	A/C	0.3222	0.4109	0.68 (0.60–0.78)	3.87×10^{-8}
6	rs9275327	32,666,802	T/C	0.2581	0.1874	1.51 (1.30–1.75)	4.7×10^{-8}

[a]CHR: chromosome.
[b]Positions are based on human genome version 19 (hg19).
[c]Minor/major alleles.
[d]MAF: minor allele frequency.
[e]OR (95% CI):Odds ratio (95% Confidence interval).

4 | DISCUSSION

IgAV is a primary autoimmune small vasculitis disease, and genetic components play a key role in the development of disease. HLA, as the main molecule involved in the presentation of endogenous and exogenous antigens in the human body, has been confirmed to be strongly associated with systemic lupus erythematosus, rheumatoid arthritis, psoriasis, and some other autoimmune diseases[19] and closely related to some primary vasculitis, such as antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and giant cell vasculitis (GCA). Furthermore, recent GWASs have shown that *HLA–DPB1* is associated with the pathogenesis of AAV and *HLA–DRB1* with GCA.[20–22]

The correlation between the HLA gene and IgAV has been identified by a series of studies based on candidate gene methods. Among HLA class I molecules, HLA-B*4102, HLA-B*7, HLA-B*15,

TABLE 3 Association of HLA alleles and amino acid changes with IgAV susceptibility

HLA variant	A1/A2	Frequency of A1		OR(95% CI)	*p* value
		Cases	Controls		
HLA allele					
HLA-DRB1*04	P/A	0.161	0.1123	1.52 (1.27–1.81)	3.17×10^{-6}
HLA-DRB1*16	P/A	0.014	0.0441	0.31 (0.18–0.52)	4.6×10^{-6}
HLA-DRB1*1602	P/A	0.014	0.0441	0.31 (0.18–0.52)	4.6×10^{-6}
Amino acid polymorphism					
HLA-DRB1 amino acid Asn120	P/A	0.187	0.1261	1.59 (1.35–1.88)	3.19×10^{-8}
HLA-DRB1 amino acid Ser120	A/P	0.187	0.1261	1.59 (1.35–1.88)	3.19×10^{-8}
HLA-DRB1 amino acid Val11	P/A	0.187	0.1262	1.59 (1.35–1.88)	3.33×10^{-8}

Abbreviations: 95% CI, 95% confidence interval for the odds ratio; A1, effective allele; A2, alternative allele, the letter "A" stands for "Absent," the letter "P" stands for "Present"; OR, estimated odds ratio.

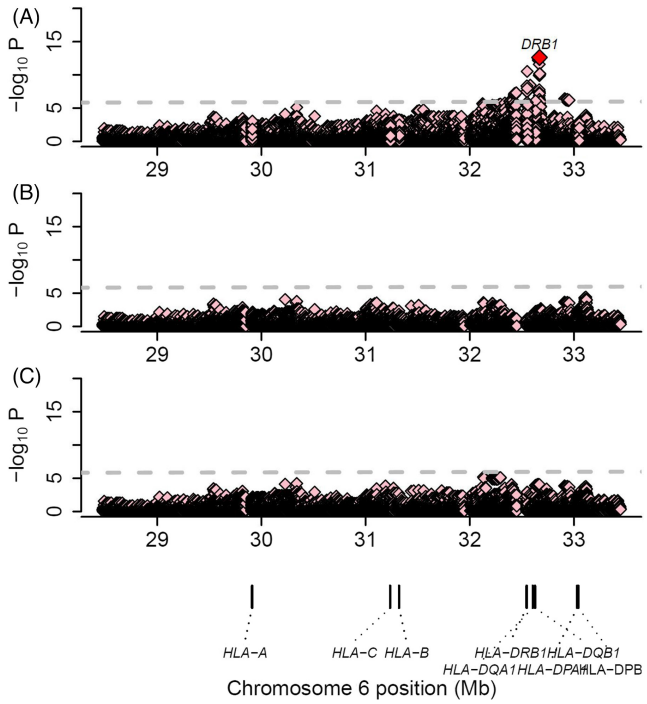

FIGURE 3 Stepwise conditional analysis of genotyped and imputed HLA classical alleles and AAs and with the IgAV GWA cohort. The horizontal axis of each panel represents the -log10 (*p*) value of the site, and the vertical axis represents the corresponding physical location of the hg19 version of the human genome. The gray dashed line represents the significance level of this study. (A) Association results before conditional analysis, marked as HLA-DRB1. (B) Conditional analyses controlling for HLA-DRB1 alleles. (C) Conditional analysis by further controlling for HLA-DRB1 amino acids

HLA-B*40, HLA-B*52 HLA -B*35, and HLA-B*49 alleles have been found to be closely related to susceptibility to IgAV in Caucasian, Turkish, and Asian populations, respectively,[23–25] whereas HLA-A*1, HLA-A*2, HLA-A*3, HLA-A*11, and HLA-A*26 alleles are considered to be closely related in Turkish and Asian populations.[24,25] Previous research on HLA class II molecules has mainly focused on HLA-DRB1. HLA-DRB1*01, HLA-DRB1*0103, HLA-DRB1*03, HLA-DRB1*07, and HLA-DRB1*11 are closely related to susceptibility to IgAV.[26–28] In 2017, the first GWAS for IgAV (285 patients vs. 1006 controls) in Caucasians reported the potential relevance of HLA-DRB1 amino acid positions 11 and 13 ($p = 1.88 \times 10^{-5}$, $p = 6.67 \times 10^{-5}$, respectively).[13] In our study, we also observed a strong correlation signal in the MHC region, and subsequent fine-mapping analysis showed amino acid positions 120 and 11 of HLA-DRB1 and three potential HLA-DRB1 alleles (HLA-DRB1*04, HLA-DRB1*16, HLA-DRB1*1602) to be independently associated with IgAV. Our findings are largely consistent with a study from Spain and precisely localize HLA-DRB1 amino acid abnormalities in IgAV. Given that the changes in amino acids of HLA-DRB1 may significantly affect function, such alterations might participate in the occurrence of IgAV. Unlike some previous conclusions obtained through candidate gene studies, all of the abnormal signals in the HLA region in our research are in HLA-DRB1, with no other HLA genes found to have abnormally independent signals. This may be closely linked to population specificity and test deviations due to limitations of previous methods. Given the smaller sample size, we need to further expand the sample capacity to confirm the specificity of the HLA-DRB1 gene in susceptibility to disease. Moreover, how genetic abnormalities affect the function of immune cells and trigger disease needs further exploration. Some studies have shown that amino acid site variation in the antigen-binding region of the HLA gene may change the presenting peptide repertoire and cause a significant risk of autoimmune diseases.[29,30] This suggests that dysfunction of the antigen-presenting cells that express HLA molecules may be an important initiating factor in the pathogenesis of IgAV.

HLA-DRB1 is an HLA II molecule that is mainly distributed on the surface of antigen-presenting cells responsible for immune system regulation. HLA-DRB1 is involved in the recognition of exogenous antigen peptides and in charge of presenting exogenous antigen peptides to CD4+ Th cells, participating in T-cell activation signal transduction. A study from Spain showed that 40% of IgAV patients had a history of pro-respiratory infection before disease onset,[31] suggesting that antigen presentation dysfunction

TABLE 4 Summary results of stepwise conditional analysis of HLA alleles for IgAV

HLA allele	*HLA-DRB1*04*	*HLA-DRB1*16*	*HLA-DRB1*01*	*HLA-DRB1*12:02*	*HLA-DRB1*10*	*HLA-DRB1*15:02*	*HLA-DRB1*1404*
Nominal association							
p	3.17×10^{-6}	4.6×10^{-6}	2.67×10^{-4}	8.22×10^{-3}	1.97×10^{-3}	5.45×10^{-2}	4.33×10^{-2}
OR (95% CI)	1.517 (1.272–1.809)	0.308 (0.181–0.525)	1.892 (1.335–2.682)	1.343 (1.078–1.673)	1.901 (1.257–2.876)	1.376 (0.993–1.908)	0.508 (0.138–1.009)
Condition on HLA-DRB1*04							
p	NA	4.68×10^{-5}	1.23×10^{-4}	1.23×10^{-4}	8.7×10^{-4}	2.23×10^{-2}	6.7×10^{-2}
OR (95% CI)	NA	0.331 (0.195–0.564)	1.978 (1.397–2.802)	1.434 (1.146–1.793)	2.034 (1.339–2.089)	1.468 (1.056–2.04)	0.393 (0.145–1.067)
Condition on HLA-DRB1*04&HLA-DRB1*16							
p	NA	NA	2.49×10^{-4}	4.42×10^{-3}	1.8×10^{-3}	3.8×10^{-2}	5.4×10^{-2}
OR (95% CI)	NA	NA	1.918 (1.354–2.718)	1.385 (1.107–1.732)	1.947 (1.282–2.959)	1.417 (1.02–1.97)	0.376 (0.139–1.019)
Condition on HLA-DRB1*04&HLA-DRB1*16&HLA-DRB1*01							
p	NA	NA	NA	1.61×10^{-3}	1.71×10^{-3}	2.72×10^{-2}	5.76×10^{-2}
OR (95% CI)	NA	NA	NA	1.437 (1.147–1.8)	1.955 (1.286–2.972)	1.45 (1.043–2.017)	0.38 (0.14–1.031)
Condition on HLA-DRB1*04&HLA-DRB1*16&HLA-DRB1*01&HLA-DRB1*1202							
p	NA	NA	NA	NA	9.18×10^{-4}	1.36×10^{-2}	6.7×10^{-2}
OR (95% CI)	NA	NA	NA	NA	2.035 (1.337–3.097)	1.518 (1.09–2.115)	0.393 (0.145–1.068)
Condition on HLA-DRB1*04&HLA-DRB1*16&HLA-DRB1*01&HLA-DRB1*1202&HLA-DRB1*10							
p	NA	NA	NA	NA	NA	9.31×10^{-3}	7.16×10^{-2}
OR (95% CI)	NA	NA	NA	NA	NA	1.554 (1.115–2.166)	0.399 (0.147–1.084)
Condition onHLA-DRB1*04&HLA-DRB1*16&HLA-DRB1*01&HLA-DRB1*12:02&HLA-DRB1*10&HLA-DRB1*15:02							
p	NA	NA	NA	NA	NA	NA	7.67×10^{-2}
OR (95% CI)	NA	NA	NA	NA	NA	NA	0.406 (0.149–1.102)

TABLE 5 Summary results of stepwise conditional analysis of HLA amino acid changes for IgAV

Amino acid polymorphism	Nominal association		condition on HLA-DRB1 position 120		condition on HLA-DRB1 position 120&HLA-DRB1 position 26		condition on HLA-DRB1 position 120&HLA-DRB1 position 26&HLA-DRB1 position 96	
	p	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)
HLA-DRB1 amino acid Ser120	3.19×10^{-8}	1.593 (1.349–1.882)	NA	NA	NA	NA	NA	NA
HLA-DRB1 amino acid Leu26	3×10^{-5}	1.413 (1.2–1.664)	1.77×10^{-5}	1.436 (1.217–1.695)	NA	NA	NA	NA
HLA-DRB1 amino acid Glu96	2.67×10^{-4}	1.892 (1.335–2.682)	1.06×10^{-4}	1.992 (1.406–2.822)	2.6×10^{-2}	1.559 (1.067–2.277)	NA	NA
HLA-DRB1 amino acid Gln74	0.795	1.029 (0.827–1.281)	0.316	1.117 (0.89–1.388)	9.6×10^{-2}	1.205 (0.967–1.503)	9.6×10^{-2}	1.206 (0.967–1.503)

caused by extraneous antigen stimulation plays an important role in the onset of IgAV. Amino acids at positions 120 and 11 are located in the peptide-binding grooves of HLA-DRB1 molecules, suggesting latent abnormal antigen presentation and protein stability of the corresponding MHC molecules.[32,33] Such a change in the HLA-DRB1 gene encoding the antigen peptide-binding domain may be an important component of IgAV pathogenesis. Whether the abnormal independent signals of HLA-DRB1 amino acid sites found in our study have a marked effect on gene function and lead to immune disorders and further increase susceptibility to disease deserve further experimental confirmation.

In summary, this is the first study to utilize GWAS and MHC region genotype filling to identify multiple phenotypes of HLA-DRB1 alleles and amino acid position changes at the genomic level in the Chinese Han population with IgAV. Our findings suggest that HLA-DRB1 has a strong association with susceptibility to IgAV. Nonetheless, further research is needed to elucidate how the HLA-DRB1 allele and amino acid site changes found in this study result in abnormal activation of antigen-presenting cells and Th cells after antigen stimulation and ultimately lead to the occurrence of IgAV.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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