

## RESEARCH ARTICLE

# Associations between *ATG16L1* gene polymorphism and antineutrophil cytoplasmic antibody-associated vasculitis in the Chinese Guangxi population: A case-control study

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## Abstract

**Background:** Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is an autoimmune disease often accompanied by rapidly progressive renal failure, and the genetic background is still unknown. Our study was performed to test whether autophagy-related 16 like 1 (*ATG16L1*) rs4663402 and rs4663396 single nucleotide polymorphisms (SNPs) were associated with AAV in the Chinese Guangxi population.

**Methods:** One hundred seventy seven unrelated AAV patients and 216 healthy controls were included in this case-control study. Multiplex polymerase chain reaction combined with high-throughput sequencing was used for typing, and SNPStats and SHEsis were used for association analysis, pairwise linkage disequilibrium, and haplotype analysis.

**Results:** rs4663402 and rs4663396 were in Hardy-Weinberg equilibrium in AAV and control groups. The frequencies of rs4663402 AA, AT, and TT genotypes were 82.5%, 16.9%, and 0.6%, respectively, in patients with AAV, and 83.5%, 16.2%, and 0.5%, respectively, in controls. The frequencies of rs4663396 CC, CT, and TT genotypes were 63.8%, 33.9%, and 2.3%, respectively, in patients with AAV, and 69.2%, 26.6%, and 4.2%, respectively, in controls. Haplotype analysis revealed two SNPs in a single haplotype block ( $D' = 1.0$ ). Our logistic regression adjusted for sex and age showed no association between rs4663402 and rs4663396 and the risk for AAV in genetic models ( $p > 0.05$ ). However, *ATG16L1* rs4663396 CC and CT + TT genotypes exhibited statistically significant differences in the incidence of arthralgia ( $p = 0.03$ ).

**Conclusions:** Our results indicated that *ATG16L1* rs4663402 and rs4663396 polymorphisms were not associated with AAV in the Chinese Guangxi population. *ATG16L1* rs4663396 CT + TT genotype may be associated with arthralgia.

## KEYWORDS

antineutrophil cytoplasmic antibody-associated vasculitis, *ATG16L1*, autophagy, single nucleotide polymorphism

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## 1 | INTRODUCTION

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a rare autoimmune disease characterized by systemic vasculitis.<sup>1</sup> The number of cases is gradually increasing each year. According to a recent study of a large insurance claims data set in the United States, the incidence of granulomatous polyangiitis is approximately 30.5 per million person-years among adults aged 18–65 years.<sup>2</sup> The clinical manifestations of AAV involve a variety of tissue and organ damage, especially in the kidney, which often presents as rapidly progressive renal failure due to crescentic glomerulonephritis.<sup>3</sup> The pathogenesis of AAV is still unclear but is mainly due to a complex interaction between genetic susceptibility and environmental factors.<sup>4</sup> The genetic background of AAV affects not only the clinical symptoms but also the specificity of ANCA, the classification of the disease, the prognosis, and the tendency to relapse.<sup>5,6</sup> Therefore, the identification of genes that may be involved in the pathogenesis and progression of AAV is important for the development of new prevention and treatment strategies.

Autophagy is an important and highly conserved regulatory mechanism for maintaining intracellular homeostasis and plays an integral role in pathogen clearance and cell differentiation.<sup>7,8</sup> Studies have shown that autophagy is involved in the pathology of several immune nephropathies (lupus nephritis,<sup>9</sup> IgA nephropathy,<sup>10</sup> membranous nephropathy,<sup>11</sup> diabetic nephropathy,<sup>12</sup> and ANCA-associated glomerulonephritis<sup>13</sup>) and transplanted kidneys. Systemic lupus erythematosus, multiple sclerosis, Crohn's disease, and autoimmune and inflammatory diseases such as rheumatoid arthritis are also closely associated with abnormal autophagy.<sup>12,14</sup>

Autophagy-related 16 like 1 (*ATG16L1*) is a key molecule necessary for the ATG12-ATG5-ATG16L1 complex to extend autophagosomes during autophagy.<sup>7,15</sup> This complex mediates the growth and closure of phagosomes and the formation of autophagosomes by anchoring microtubule-associated protein 1 light chain 3 to the target membrane by linking it to phosphatidylethanolamine.<sup>16</sup> Defective ATG12-ATG5-ATG16L1 complex formation affects the prolongation of autophagic vesicles and autophagy is inhibited, leading to impaired neutrophil extracellular traps formation.<sup>17</sup>

The *ATG16L1* gene, located at the long arm of chromosome 2 (2q37.1), is 44,028bp long and contains 19 exons. Interestingly, single nucleotide polymorphisms (SNPs) in the introns region of *ATG16L1* (rs4663402, rs4663396) might be associated with genetic susceptibility to head and neck squamous cell carcinoma,<sup>18</sup> hepatocellular carcinoma,<sup>19</sup> ankylosing spondylitis,<sup>20</sup> and inflammatory bowel disease.<sup>21</sup> However, we have not found a study on the relationship between *ATG16L1* and AAV.

In this study, we investigated the relationship between *ATG16L1* SNPs (rs4663402, rs4663396) polymorphisms and AAV to provide clues and evidence for the pathogenesis, early prevention, and treatment of AAV.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

We included 177 patients with AAV (AAV group) who were treated and hospitalized in the Second Affiliated Hospital of Guangxi Medical University between January 2005 and January 2019. They all met the diagnostic criteria of the 2012 Chapel Hill International Vasculitis Naming Conference. The exclusion criteria were secondary vasculitis caused by infection, drugs, malignant tumors, or other autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, Crohn's disease, etc.). In the same period, we selected 216 healthy people without AAV from the physical examination center of the same hospital, as the normal control group (control group). All the participants were Han or Zhuang Chinese in Guangxi, China. They are unrelated, and the genetic background of the participants is highly consistent. This case-control study was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Guangxi Medical University (No. KY-0100 of 2018). All participants provided written informed consent.

### 2.2 | Biochemical analyses

Hemoglobin (Hb), white blood cell (WBC), neutrophils (NEUT), platelets (PLT), blood urea nitrogen (BUN), serum creatinine (SCr), uric acid (UA), total cholesterol (TC), triglyceride (TG), albumen (ALB), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), anti-hemolytic streptococcus "O" test (ASO), complement (C3 and C4), double-stranded DNA (dsDNA), antinuclear antibodies (ANA), extractable nuclear antigen (ENA), perinuclear antineutrophil cytoplasmic antibody (pANCA), and cytoplasmic antineutrophil cytoplasmic antibody (cANCA) were determined by standard biochemical and immunological methods.

### 2.3 | DNA extraction and purity determination

Following the steps of the DP319 Kit (Tiangen, China), DNA samples were purified from 2 to 3 ml of anticoagulated venous blood. To determine the concentration, 1.5  $\mu$ l of extracted DNA solution was carefully dropped into the ultraviolet spectrophotometer. When the OD260/280 ratio was 1.8–2.0, the DNA purity was regarded as standard, and these samples were stored at  $-80^{\circ}\text{C}$ .

### 2.4 | PCR amplification

Amplification of the target SNP site sequence and preparation of the Illumina-compatible sequencing library were completed by two-step polymerase chain reaction (PCR).

The first PCR reaction system contained 25  $\mu$ l of the mixture: template DNA (10 ng/ $\mu$ l) 2  $\mu$ l, primer-F (10  $\mu$ M) 1  $\mu$ l, primer-R (10  $\mu$ M) 1  $\mu$ l, enzyme-free water 6  $\mu$ l, and 2 $\times$  PCR Ready Mix 15  $\mu$ l (KAPA HiFi

Ready Mix). The following reaction procedure was performed on the PCR instrument (BIO-RAD, T100TM): pre-denaturation at 98°C for 3 min and then denaturation at 98°C for 30s. The mixture was annealed at 50°C for 30s, extended at 72°C for 30s, and eight cycles were performed. This was followed by denaturation at 98°C for 30s, annealing at 66°C for 30s, and extension at 72°C for 30s; 25 cycles were performed. Finally, it was extended at 72°C for 5 min, and the PCR products were stored at 4°C.

After completion of the PCR reaction, the PCR product was detected by 1% agarose gel electrophoresis to determine the correct size of the product, and AMPureXP magnetic beads were used to purify and recover the PCR product.

Then, using the first step PCR product as the template, the second step PCR reaction was performed to obtain the sequencing library with molecular tags. The reaction system was acted as follows: template DNA (10 ng/μl) 2 μl, Universal P7 primer (including molecular tag, 10 μM) 1 μl, Universal P5 primer (10 μM) 1 μl, enzyme-free water 6 μl, and 2× PCR Ready Mix 15 μl (total volume 25 μl). The second PCR reaction program was as follows: pre-denaturation at 98°C for 5 min; denatured at 94°C for 30s; annealed at 55°C for 20s; extended at 72°C for 30s; and performed five cycle procedures. It was extended at 72°C for 5 min, and the PCR products were stored at 4°C. Finally, the PCR products were purified and recovered using AMPure XP magnetic beads. Equal amounts of PCR products were mixed and sequenced using a HiSeq XTen sequencer (Illumina, San Diego, CA).

## 2.5 | Data quality control and genotyping analysis

First, any partial sequences containing sequencing splice sequences were removed using cutadapt (v1.2.1) software. Second, the remaining sequences were controlled with PRINSEQ-lite (v0.20.3) software, and bases with quality thresholds below 20 were removed in the order of sequences 3' to 5', and the remaining sequences were considered qualified. Then, the qualified sequences were compared with the reference genome by using BWA (v0.7.13-r1126) software

with default parameters. Based on the comparison, the genotype results of the target loci were calculated using SAMtools software (v0.1.18). Finally, the mutant loci were genetically annotated with ANNOVAR software.

## 2.6 | Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics Version 25.0 for Windows. SNP frequencies in control and AAV participants were tested for departure from Hardy-Weinberg equilibrium. Continuous variables were compared by either unpaired Student's *t* test or Mann-Whitney *U* test. Chi-squared (Pearson's  $\chi^2$ ) test or Fisher's exact test was used to compare discrete variables. The relationship between ATG16L1 rs4663402 and rs4663396 polymorphism and AAV was assessed by logistic regression adjusted for sex and age. Five models (codominant, dominant, recessive, overdominant, and log-additive) were used to evaluate associations of SNPs with AAV risk by using SNPStats online software (<https://www.snpstats.net/start.htm>). Pairwise linkage disequilibrium (LD) values, haplotype structure, and haplotype frequencies were determined using SHEsis online software (<http://analysis.bio-x.cn/myanalysis.php>). Haplotypes with a frequency < 0.03 were not considered for the analysis. Odds ratios (ORs), 95% confidence intervals (CIs), and *p*-values were calculated. Bonferroni correction method was used for the test level of multiple comparisons among multiple groups. Values of *p* < 0.05 were considered statistically significant, and all statistical tests were two-sided.

## 3 | RESULTS

### 3.1 | Characteristics of the participants

Demographic and clinical characteristics of patients with and without AAV are shown in Table 1. Of the AAV patients, 68 were male and 109 female, and their mean age was 53.90 ± 1.13 years. For

TABLE 1 Characteristics of patients with and without AAV

Characteristics	Control (n = 216)	AAV (n = 177)	<i>p</i>
Ethnic (Zhuang, %)	58 (26.9)	62 (35.0)	0.099
Male (%)	84 (38.9)	68 (38.4)	1.000
Age (years)	51.35 ± 0.86	53.90 ± 1.13	0.074
SBP [≥140 vs. <140 (mmHg), %]	32 (14.8)	56 (38.9)	<0.001
DPB [≥90 vs. <90 (mmHg), %]	28 (13.0)	30 (20.8)	0.057
SCr (μmol/L)	69 (17–111)	261.5 (35–2682)	<0.001
UA (mol/L)	327 (101–701)	403.5 (88–821)	<0.001
TC	5.38 (2.54–9.17)	4.22 (1.54–8.90)	<0.001
TG	1.01 (0.07–86.67)	1.26 (0.37–6.25)	0.065

Note: Data are presented as mean ± SD, proportions/percentages, or median (IQR) values. *P* < 0.05 was considered statistically significant.

Abbreviations: IQR, Interquartile range; SCr, serum creatinine; TC, total cholesterol; TG, triglyceride; UA, uric acid.

the healthy controls, 84 male and 132 female, the mean age was  $51.35 \pm 0.86$  years. There were no statistically significant differences in age or sex between the AAV and control groups ( $p > 0.05$ ), which were comparable. High systolic blood pressure was significantly more frequent in patients with AAV ( $p < 0.001$ ). In addition, serum creatinine, UA, and TC levels were significantly higher in patients with AAV ( $p < 0.001$ ).

### 3.2 | SNP analysis

The frequency distributions of ATG16L allele, genotype, and models associated with the risk of AAV are shown in Table 2. All investigated polymorphisms were in Hardy-Weinberg equilibrium in cases and controls, which suggests the participants were population-representative. In this study, the 391 samples were successfully genotyped, except for 2 cases of rs4663396 in the control group. There was no significant difference between allele frequency, genotype frequency, or differing genetic models in rs4663402 and rs4663396 of the ATG16L1 gene with and without AAV. A logistic regression was used to evaluate whether rs4663402 and rs4663396

polymorphisms were independently associated with AAV after adjusting for sex and age. The results indicated no relationship between rs4663402 and rs4663396 polymorphisms and AAV in our cohort of patients (Table 2).

### 3.3 | Haplotype analysis and pairwise LD analysis

The pairwise LD analysis of the ATG16L1 gene polymorphism indicated a linkage imbalance in rs4663396 and rs4663402 ( $D' = 1.00$ ,  $r^2 = 0.418$ ), and the two SNPs were in the same haplotype block, preferring overall heredity (Figure 1). Four haplotypes were constructed from the two ATG16L1 SNPs (Table 3). Our results show no significant difference between the various haplotypes and the risk of AAV ( $p > 0.05$ ).

### 3.4 | Genotypes and clinical phenotypes

We also determined whether different genotypes of the two genetic polymorphisms (rs4663402 and rs4663396) were associated with

TABLE 2 Frequency distributions of ATG16L allele and genotype and their associations with the risk of developing AAV<sup>a</sup>

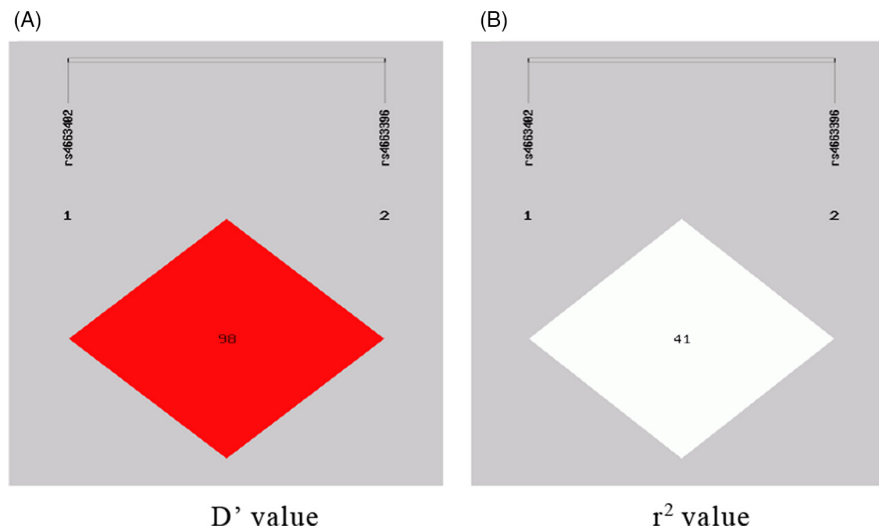
SNP	Model	Genotype	Control (n = 216)	AAV (n = 177)	OR (95% CI)	p	
rs4663402	Codominant	A/A	180 (83.5%)	146 (82.5%)	1	0.70	
		A/T	35 (16.2%)	30 (16.9%)	1.05 (0.61–1.80)		
		T/T	1 (0.5%)	1 (0.6%)	1.39 (0.09–22.56)		
	Dominant	A/A	180 (83.3%)	146 (82.5%)	1	0.43	
		A/T-T/T	36 (16.7%)	31 (17.5%)	1.06 (0.62–1.80)		
	Recessive	A/A-A/T	215 (99.5%)	176 (99.4%)	1	0.90	
		T/T	1 (0.5%)	1 (0.6%)	1.38 (0.09–22.35)		
	Overdominant	A/A-T/T	181 (83.8%)	147 (83%)	1	0.41	
		A/T	35 (16.2%)	30 (16.9%)	1.05 (0.61–1.79)		
	Log-additive	-	-	-	-	1.07 (0.64–1.77)	0.47
Allele		A	395 (91.4%)	322 (91.0%)	0.943 (0.574–1.547)		
rs4663396	Codominant	C/C	148 (69.2%)	113 (63.8%)	1	0.48	
		C/T	57 (26.6%)	60 (33.9%)	1.40 (0.90–2.18)		
		T/T	9 (4.2%)	4 (2.3%)	0.62 (0.18–2.06)		
	Dominant	C/C	148 (69.2%)	113 (63.8%)	1	0.52	
		C/T-T/T	66 (30.8%)	64 (36.2%)	1.30 (0.85–1.98)		
	Recessive	C/C-C/T	205 (95.8%)	173 (97.7%)	1	0.41	
		T/T	9 (4.2%)	4 (2.3%)	0.55 (0.17–1.83)		
	Overdominant	C/C-T/T	157 (73.4%)	117 (66.1%)	1	0.32	
		C/T	57 (26.6%)	60 (33.9%)	1.43 (0.93–2.22)		
	Log-additive	-	-	-	-	1.14 (0.79–1.65)	0.78
		Allele	C	353 (82.5%)	286 (80.8%)	0.894 (0.621–1.285)	
			T	75 (17.5%)	68 (19.2%)		0.58

Note: The SNPStats online software and SHEsis online software were used to analyze both SNPs in alleles, genotypes and models.  $p$ -value was computed using Pearson's  $\chi^2$  test or Fisher's exact test, adjusted for sex and age.  $p < 0.05$  was considered statistically significant.

Abbreviations: CI, confidence interval; OR, odd ratio.

<sup>a</sup>The number of cases included in the analysis after deleting the cases with missing data.

**FIGURE 1**  $D'$  and  $R^2$  value heat map of linkage disequilibrium in SNPs of *ATG16L1* gene. The SHEsis online software was used to analyze the Pair-wise linkage disequilibrium plot for *ATG16L1* SNPs. (A)  $D' = 0.98$ ; (B)  $R^2 = 0.41$ .



**TABLE 3** Relationship between *ATG16L1* haplotype and the risk of AAV<sup>a</sup>

Haplotype	Control	AAV	OR (95% CI)	$p^a$	$p^b$
AC	351.9 (82.2%)	286 (80.8%)	0.896 (0.623–1.289)	0.555	>0.05
AT	39.1 (9.1%)	36 (10.2%)	1.123 (0.697–1.808)	0.633	>0.05
TT	35.9 (8.4%)	32 (9.0%)	1.082 (0.657–1.782)	0.756	>0.05
TC	1.1 (0.03%)	0.0 (0.0%)	-	-	-

Note: We used the SNPStats online software to analyze both SNPs in haplotype.  $p^a$  values were assessed by Logistic regression analysis, adjusted for sex and age;  $p^b$ : Bonferroni correction.  $p < 0.05$  was considered statistically significant.

Abbreviations: AAV, ANCA-associated vasculitis; CI, confidence interval; OR, odds ratio.

<sup>a</sup>The number of cases included in the analysis after deleting the cases with missing data.

clinical phenotypes. However, the results showed that, except for a difference in the incidence of arthralgia between the rs4663396 CC and CT+TT genotypes being statistically significant ( $p = 0.03$ ), no association was found in comparing the clinical symptoms and laboratory markers with different genotypes by using Pearson's  $\chi^2$  or Fisher's exact test (Table 4).

## 4 | DISCUSSION

Current research shows that autophagy dysfunction is one of the reasons for the occurrence and development of AAV.<sup>12</sup> *ATG16L1* gene is involved in the whole process of autophagy, and a change in the gene sequence might affect the function of the protein, and consequently influence the pathogenesis of AAV. This assumption was the basis of our study, a case-control in Guangxi to observe an association between two SNPs of *ATG16L1* and AAV. However, the results showed that the allele frequencies, genotype frequencies, haplotypes, and genetic models of rs4663402 and rs4663396 were not significantly associated with genetic susceptibility to AAV. Interestingly, when considering clinical variables, there was a statistically significant difference between the rs4663396 CC genotype and the CT+TT genotype, where the latter had a higher incidence of arthralgia.

Several inflammatory diseases have been associated with *ATG16L1* gene polymorphism.<sup>22–25</sup> *ATG16L1* SNPs (rs2241880 and

rs6754677) are significantly associated with the risk of Crohn's disease in Malaysia, and people with the homozygous AA genotype have a higher risk of developing Crohn's disease.<sup>26</sup> In addition, *ATG16L1* rs2241880 polymorphism is associated with rheumatoid arthritis in China.<sup>27</sup> Pugazhendhi<sup>21</sup> confirmed that the allele and genotype frequencies of the *ATG16L1* mutant rs4663402 are associated with Crohn's disease and ulcerative colitis in India. Similarly, Glas et al.<sup>28</sup> indicated that a protective association between rs4663396 and Crohn's disease has also been confirmed in Germany. In addition, one study found that among the three haplotypes constructed by rs4663396 and rs6758317, the TT haplotype was associated with an increased risk of ankylosing spondylitis in women, but there was no association after Bonferroni correction, and its genotype was not correlated with clinical characteristics such as CRP and ESR.<sup>20</sup> Comparable to the results of the current study, there was no statistical significance for rs4663402 and rs4663396 T alleles in a study by Shen and Li.<sup>19,20</sup> In the light of the partially shared genetic background and etiology between AAV and the autoimmune diseases, the *ATG16L1* rs4663402 and rs4663396 polymorphisms were also included in our current study. However, we did not find significant evidence for the associations of *ATG16L1* rs4663402 and rs4663396 polymorphisms with either AAV or different serotypes of AAV.

We indicate that *ATG16L1* rs4663402 and rs4663396 polymorphisms are not associated with the autoimmune disease AAV, which is not consistent with reported results for some immune diseases,

TABLE 4 Association between clinical phenotypes and genotypes of rs4663402 and rs4663396 in patients with AAV<sup>a</sup>

Clinical phenotypes	rs4663402 genotype			rs4663396 genotype		
	AA	AT + TT	p	CC	CT + TT	p
Hb (g/L)	79.8 (63.1–99.0)	81.6 (59.0–105.3)	0.75	77.4 (63.0–97.2)	81.1 (63.0–106.4)	0.75
WBC (10 <sup>9</sup> /L)	8.22 (6.10–10.70)	7.49 (5.94–11.57)	0.94	8.06 (6.16–10.52)	8.68 (5.82–11.30)	0.49
NEUT (10 <sup>9</sup> /L)	5.63 (4.14–8.44)	5.58 (4.17–8.34)	0.89	5.39 (4.13–8.34)	6.28 (4.14–8.49)	0.41
PLT (10 <sup>12</sup> /L)	278.0 (202.0–359.4)	253.5 (210.3–384.0)	0.96	273.0 (201.5–350.0)	276.6 (211.7–406.5)	0.25
BUN (mmol/L)	14.61 (5.90–24.36)	13.19 (4.98–23.23)	0.50	15.44 (6.08–24.52)	13.05 (4.90–23.18)	0.39
SCr (μmol/L)	291 (118–506)	237 (64–441)	0.36	292 (119–524)	225 (81–452)	0.24
UA (mol/L)	409.78 ± 153.89	389.14 ± 188.78	0.56	418.55 ± 152.92	384.90 ± 170.08	0.22
TC (mmol/L)	4.49 (3.70–4.89)	4.49 (3.88–5.05)	0.76	4.49 (3.65–4.88)	4.46 (3.83–5.01)	0.77
TG (mmol/L)	1.33 (0.95–1.50)	1.50 (1.04–1.86)	0.15	1.37 (0.95–1.50)	1.42 (0.98–1.75)	0.37
ALB (g/L)	30.29 ± 6.23	29.13 ± 8.19	0.43	29.99 ± 6.39	30.11 ± 6.65	0.77
CRP (mg/L)	28.84 (7.10–61.28)	16.63 (10.50–51.59)	0.91	26.00 (7.55–57.54)	24.06 (9.46–77.05)	0.66
ESR (mm/h)	79 (54–104)	79 (39–96)	0.34	79 (59–100)	79 (49–99)	0.52
RF (U/ml)	5.9 (0–200.1)	6.7 (0.5–141.8)	0.82	5.6 (0–156.0)	8.4 (0.5–200.1)	0.33
ASO (U/ml)	44 (0.8–299)	37 (15–114)	0.29	43 (0.8–299)	51 (4–162)	0.85
C3 (mg/L)	0.92 (0.27–1.53)	1.01 (0.11–1.64)	0.43	0.91 (0.27–1.53)	0.97 (0.11–1.64)	0.58
C4 (mg/L)	0.28 (0.11–2.25)	0.28 (0.04–0.49)	0.66	0.29 (0.11–2.25)	0.28 (0.04–0.53)	0.37
dsDNA (%)	4 (2.3)	2 (1.1)	0.28	1 (0.6)	5 (2.8)	0.06
ANA (%)	31 (17.5)	7 (4.0)	0.79	22 (12.4)	16 (9.0)	0.69
ENA (%)	14 (7.9)	4 (2.3)	0.66	10 (5.6)	8 (4.5)	0.82
pANCA (%)	95 (53.7)	17 (9.6)	0.55	72 (40.7)	40 (22.6)	0.88
cANCA (%)	15 (8.5)	2 (1.1)	0.78	10 (5.6)	7 (4.0)	0.88
Fever (%)	40 (22.6)	6 (3.4)	0.52	31 (17.5)	15 (8.5)	0.82
Edema (%)	55 (31.1)	9 (5.1)	0.56	43 (24.3)	21 (11.9)	0.78
Rash (%)	7 (4.0)	2 (1.1)	0.59	5 (2.8)	4 (2.3)	0.87
Cough (%)	51 (28.8)	7 (4.0)	0.40	41 (23.2)	17 (9.6)	0.39
Hemoptysis (%)	9 (5.1)	3 (1.7)	0.42	8 (4.5)	4 (2.3)	1.00
Myalgia (%)	4 (2.3)	1 (0.6)	0.55	2 (1.1)	3 (1.7)	0.55
Arthralgia (%)	15 (8.5)	6 (3.4)	0.16	8 (4.5)	13 (7.3)	0.03

Note: Data are presented as mean ± SD, proportions/percentages, or median (IQR) values.  $p < 0.05$  was considered statistically significant.

Abbreviations: ALB, albumen; ANA, antinuclear antibodies; ASO, anti-hemolytic streptococcus "O" test; BUN, blood urea nitrogen; C3 C4, complement; cANCA, cytoplasmic antineutrophil cytoplasmic antibody; CRP, C-reactive protein; dsDNA, double-stranded DNA; ENA, extractable nuclear antigen; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; IQR, interquartile range; NEUT, neutrophils; pANCA, perinuclear antineutrophil cytoplasmic antibody; PLT, platelets; RF, rheumatoid factor; SCr, serum creatinine; TC, total cholesterol; TG, triglyceride; UA, uric acid; WBC, white blood cell.

<sup>a</sup>The number of cases included in the analysis after deleting the cases with missing data.

but is consistent with others. For example, we found that rs4663402 and rs4663396 TT genotypes were rarely found in healthy people and AAV patients in this study, which is consistent with an Indian study.<sup>21</sup> Therefore, we assume that differences in the frequency of A/T and C/T alleles between diverse and geographically distant populations might, at least partially, explain the discrepant results and the lack of association between ATG16L1 SNPs (rs4663402 and rs4663396) and AAV in our study.

Several potential limitations of the present study should be acknowledged. First, the participants including AAV patients and controls were recruited from only one hospital; thus, selection bias was unavoidable. Second, the sample size of our study was not large enough to detect associations with clinical symptoms and laboratory markers. Unfortunately, we did not detect serum

ATG16L1 levels in the case and control groups. These ATG16L1 SNPs may be rare in Guangxi. Therefore, further studies with a relatively larger sample size should be conducted to validate these associations.

## 5 | CONCLUSIONS

Taken together, we did not find an association between autophagy-related genes ATG16L1 rs4663402 and rs4663396 polymorphisms and AAV in Chinese Guangxi populations. This implies that ATG16L1 rs4663402 and rs4663396 may not be a marker for AAV in Chinese Guangxi populations. However, ATG16L1 rs4663396 CT+TT genotype may be associated with arthralgia. Further



larger-scale studies of different ethnic populations are warranted to validate our results.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

All the data in this study can be obtained from the corresponding author.

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