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Original Article

Association of ATG7 gene polymorphisms with microscopic polyangiitis in Chinese individuals

Liepeng Chu[1], Huan Zhong[1], Yan Zhu[1,2], Lizhen Li[1], Jingsi Wei[1], Li Huang[1], Chao Xue[1]

[1]*Department of Nephrology, The Second Affiliated Hospital of Guangxi Medical University, Nanning 530000, Guangxi, China;* [2]*The First Affiliated Hospital of University of South China, Hengyang 421000, Hunan, China*

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Abstract: Microscopic polyangiitis (MPA) is a type of antineutrophil cytoplasmic antibody (ANCA)-related vasculitis. Autophagy-related gene 7 (ATG7) protects against complicated disorder states in model organisms, but the way ATG7 dysfunction contributes to MPA remains elusive. This investigation assessed the impacts of ATG7 single-nucleotide polymorphisms (SNPs) on microscopic polyangiitis (MPA) in China. A total of 211 controls and 214 MPA patients were recruited and analyzed. Polymerase chain reaction (PCR) and high-throughput sequencing were adopted to detect the ATG7 SNPs (*rs75492008*, *rs2594966*, *rs6442260* and *rs8154*), and stratification analysis, different genetic models and differences in allele and genotype frequencies were evaluated. Haplotype evaluation was performed after linkage disequilibrium (LD) analyses, and interactions between alleles were assessed. Generalized multifactor dimensionality reduction (GMDR) was adopted to analyze SNP-SNP interactions among the four ATG7 SNPs and phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) and unc-nc-like autophagy activating kinase 1 (ULK1) SNPs previously studied by our team. Relationships between ATG7 polymorphisms, disease activity biomarkers and therapeutic effects in MPA were analyzed. Sex stratification analysis of the *rs2594966* GG genotype with codominant and recessive models showed OR=3.42, 95% CI [1.19-9.80], P=0.041 and OR=3.31, 95% CI [1.23-8.90], P=0.012, respectively. Haplotype G-G-C-T was related to an increased MPA risk (OR=1.5, 95% CI [0.999-2.266], P=0.029). Permutation testing of GMDR suggested that ATG7 *rs6442260* and *rs8154*, PIK3CA *rs1607237*, and ULK1 *rs4964879* might interact with each other in MPA development (P<0.05). Among 214 MPA patients, 79 available complete follow-up clinical datasets were gathered from September 2009 to October 2020, showing that *rs75492008* and *rs4964879* affect the correlation between C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) in MPA activity. Patients with *rs8154* TT and *rs1607237* CC genotypes had better clinical treatment effects (P<0.05). Gene polymorphisms may be related to MPA in China, exhibiting correlation with MPA activity indicators, treatment and prognosis.

Keywords: ATG7 gene, microscopic polyangiitis (MPA), polymorphisms

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-related vasculitis (AAV) is characterized by inflammation of medium and small vessels. AAV is an autoimmune disease comprised of three clinical subtypes: eosinophilic GPA (EGPA), microscopic polyangiitis (MPA) and granulomatosis with polyangiitis (GPA). MPA belongs to a group of systemic vasculitis diseases featuring necrotizing inflammation of medium to small-sized blood vessels [1]. MPA constitutes the main clinical subtype in Chinese and Japanese patients, and most patients with MPA are positive for MPO-ANCAs [2, 3].

Although the precise mechanism of AAV remains unclear, a strong genetic relationship is known. Indeed, genome-wide association investigations (GWASs) show that HLA-DP (rs3117242) variants facilitate the pathogenesis of MPO-ANCA-related vasculitis [4-7].

Autophagy constitutes a complex and highly modulated course, and dozens of autophagy-associated genes (ATGs) are involved in molecular mechanisms. Autophagy-related protein 7 (ATG7) participates in two ubiquitin-like protein coupling systems, namely, the ATG12-ATG5 and ATG8 conjugation systems. In these systems, ATG7 functions as an E1-like ligase, promotes

extension of the separation membranes and holds the key to autophagosome formation [8]. Research on autophagy has been increasing, and investigations have shown that autophagy is related to susceptibility to various autoimmune system diseases [9-12]. Nevertheless, the role of ATG7 mutation in AAV remains to be confirmed. As in China, MPA constitutes the most typical clinical subtype of AAV, and our research team prioritized whether ATG7 gene polymorphisms played a role in MPA sensitivity. In this research, in the functional region of the ATG7 gene (*rs75492008*, *rs2594966*, *rs6442260*, and *rs8154*), SNP loci featuring a minor allele frequency (MAF) ≥5% were selected, and their association with susceptibility to MPA was investigated between MPA patients and healthy people in China. We used generalized multifactor dimensionality reduction (GMDR) to analyze SNP-SNP interactions among the four ATG7 SNPs as well as SNPs of the PIK3CA and ULK1 genes researched by our team previously. We also analyzed ATG7 polymorphisms in MPO disease activity and therapeutic effects.

Materials and methods

Ethics statement

This investigation was permitted by the Ethics Committee of the Second Affiliated Hospital of Guangxi Medical University (No. 2018 KY-0100). The investigation was committed to the principles of the Declaration of Helsinki.

Study population

From September 2009 to October 2020, 214 eligible MPA patients were recruited from the Department of Nephrology, the Second Affiliated Hospital of Guangxi Medical University. The inclusion criteria were as follows: (a) diagnosis of MPA at the Department of Nephrology, the Second Affiliated Hospital of Guangxi Medical University, the diagnosis of MPA was based on the 2012 Chapel Hill International Conference on Vasculitis Nomenclature [13], (b) adults equal to or older than 18 years old, and (c) born in China with no familial relation to others in the study. Patients with malignant tumors, chronic disorders, other autoimmune disorders or secondary vasculitis were excluded. A total of 211 healthy controls were matched with the MPA group concerning to age and sex.

DNA extraction

Blood (5 ml) was collected from all participants, and total genomic DNA was extracted using a Tiangen blood DNA extraction kit. DNA quality was evaluated using a Nanodrop 2000 spectrophotometer. Samples with an A260/A280 proportion of 1.7-1.9 were analyzed and the separated DNA was kept at -80°C.

SNP selection

rs75492008, *rs2594966*, *rs6442260* and *rs8154* of the ATG7 gene were selected from the Chinese genotype data of 1000 Genomes (http://grch37.ensembl.org/). The choice standards were as follows: (1) MAF ≥0.05, (2) HWE P>0.5, (3) located in the functional area.

SNP genotyping

ATG7 was detected by PCR and high-throughput sequencing by Sangon Biotech. PCR amplification conditions were set up using a two-step method. Paired-end sequencing was performed on the library using the HiSeq XTen sequencer of Illumina (California, American), and the data were analyzed with Samtools 0.1.18 software. The accuracy of genotyping was verified by sequencing approximately 10% of the random samples via the Sangon Biotechnology Company of China, and the duplication rate of all SNP genotyping reached 100%.

GMDR analysis

GMDR 0.7 software was used to evaluate multiple effects among susceptible SNPs of the autophagy gene family. The test level was α=0.05, and P<0.05 was considered statistically significant. We selected different genetic loci from the same batch of experimental samples for analysis, including the ATG7 gene (*rs75492008*, *rs2594966*, *rs6442260*, and *rs8154*), PIK3CA gene (*rs9838117* and *rs16-07237*) and ULK1 gene (*rs7138581*, *rs730-0908*, *rs4964879*, *rs12303764*, *rs10902469*, and *rs9481*).

Clinical and laboratory data

We retrospectively collected all clinical and laboratory information after screening the patients' medical records. The following data were collected: age, sex, blood pressure, serum

Table 1. Baseline characteristics of the study participants

Parameter	MPA group (n=214)	Control group (n=211)	*p*
Sex (Male/Female)	82/132	83/128	0.829
Age (years)	55.0±14.5	51.2±12.6	0.009
<60	118 (55.1%)	160 (75.8%)	
≥60	96 (44.9%)	51 (24.2%)	
Ethnicity (Han/Zhuang)	135/79	155/56	0.022
SBP (mmHg)	135.75±23.26	125.23±14.58	<0.001
DBP (mmHg)	79.53±12.95	76.67±10.61	0.017
SCR (μmol/L)	388.09±385.59	69.34±15.64	<0.001
Uric acid (μmol/L)	410.76±160.12	329.70±98.73	<0.001
24-h UPR (g/24 h)	1579.58±1938.55	21.23±21.67	<0.001

creatinine, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), uric acid, and 24 h urinary protein quantitation.

Treatment effects

We analyzed treatment effects from September 2009 to October 2020. Through this follow-up of 11 years, we evaluated whether the treatment effect was related to gene polymorphisms.

Statistical analysis

Genotypic and allelic frequencies in the MPA group and control were evaluated via the Fisher's exact test or the chi-squared test. Employing the chi-squared test, we assessed Hardy-Weinberg equilibrium (HWE) for each SNP in the control participants. Genetic models and stratification discussions with 95% confidence intervals (CIs) and odds ratios (ORs) were analyzed to examine the link between MPA risk and genetic variation adopting online SNP stats software (https://www.snpstats.net/start.htm), with adjustment for sex and age. Haplotype blocks and LD determined by D' were evaluated using online software (SHEsis) (http://analysis.biox.cn/myAnalysis.php) [14, 15]. SPSS Statistics version 23.0 (IBM, Armonk, New York, USA) was applied for data analyses, and P<0.05 was considered statistically significant.

Results

Baseline features of the study participants

The fundamental clinical information of the healthy controls and MPA patients is shown in **Table 1**. The MPA group was comprised of 79 Zhuang nationality and 135 Han nationality individuals. The age range at the time of the disease was 18-82 years, with an average age of 55.0±14.5 years. A total of 132 patients were female and 118 were <60 years. The control group was composed of 155 Han Chinese individuals and 128 females (**Table 1**).

Association of MPA susceptibility with gene polymorphisms

The genotype dispersion of the four ATG7 gene SNPs in the MPA was in HWE (all P>0.05). The selected ATG7 gene SNPs *rs75492008*, *rs2594966*, *rs6442260*, and *rs8154* had an MAF of >5%. Based on single-SNP analysis, the alleles and genotypes of ATG7 did not display any great diversity between MPA patients and controls. (P>0.05) (**Table 2**).

Stratification analyses on the basis of gender, age, and ethnicity

The outcomes showed that sex obviously affected the relativity between MPA and ATG7 SNPs. According to codominant and recessive models, the ATG7 *rs2594966* GG genotype (OR=3.42, 95% CI [1.19-9.80], P=0.041; OR=3.31, 95% CI [1.23-8.90], P=0.012) was related to a higher MPA incidence in males. Stratification analyses of age and ethnicity did not reveal an association with MPA (**Tables 3**-**5**).

Linkage disequilibrium analysis

Figure 1 exhibits the pairwise LD pattern concerning the ATG7 SNPs analyzed in the current investigation. r^2 and D' were computed to clarify the degree of LD in pairwise combinations of the four SNPs. Strong LD between two SNPs (*rs2594966*, *rs6442260*) was observed (D' >0.8) (**Figure 1**). Based on the LD plot, ATG7 *rs75492008*, *rs2594966*, *rs6442260*, and *rs8154* loci form seven haplotypes. Haplotype G-G-C-T was related to an increased risk of MPA (OR=1.5, 95% CI [0.999-2.266], P=0.029), whereas other haplotypes did not present a relativity (**Table 6**).

Table 2. Association analysis for ATG7 gene SNPs in cases and healthy controls

SNP ID	Alleles	Genetype n (%)			Allele n (%)		OR (95% CI)
rs75492008	C>T	CC	CT	TT	C	T	
Control		142 (67.9%)	59 (28.2%)	8 (3.8%)	343 (82.1%)	75 (17.9%)	1.099 (0.770-1.571)
Case		151 (70.6%)	55 (25.7%)	8 (3.7%)	357 (83.4%)	71 (16.6%)	
p			0.836		0.602		
rs8154	T>C						
Control		7 (3.4%)	46 (22%)	156 (74.6%)	60 (14.4%)	358 (85.6%)	0.035 (0.634-1.380)
Case		5 (2.3%)	48 (22.4%)	161 (75.2%)	58 (13.6%)	370 (86.4%)	
p			0.820		0.736		
rs6442260	G>A	GG	GA	AA	G	A	
Control		123 (58.6%)	75 (35.7%)	12 (5.7%)	321 (76.4%)	99 (23.6%)	0.999 (0.727-1.371)
Case		124 (57.9%)	79 (36.9%)	11 (5.1%)	327 (76.4%)	101 (23.6%)	
p			0.945		0.993		
rs2594966	G>A						
Control		22 (10.5%)	104 (49.5%)	84 (40%)	148 (35.2%)	272 (64.8%)	1.119 (0.846-1.481)
Case		36 (16.8%)	90 (42.1%)	88 (41.1%)	162 (37.8%)	266 (62.2%)	
p			0.108		0.430		

GMDR analysis

We used twelve loci (ATG7 (*rs75492008*, *rs2594966*, *rs6442260* and *rs8154*), PIK3CA (*rs9838117* and *rs1607237*) and ULK1 (*rs-9481*, *rs7138581*, *rs7300908*, *rs4964879*, *rs12303764* and *rs10902469*)) to analyze SNP-SNP interactions. We developed a significant four-locus model including ATG7 *rs64-42260* and *rs8154*, PIK3CA *rs1607237*, and ULK1 *rs4964879* (P=0.0001), implying a potential SNP-SNP interaction among *rs64-42260*, *rs1607237*, and *rs4964879*. The cross-verification consistency of the four-locus mode reached 3/10, and the testing accuracy stood at 0.6735 (1000 permutation tests P>0.05; **Table 7**).

Correlation between gene polymorphism and the biomarkers of disease activity CRP/ESR in MPA patients

Among 214 patients, complete follow-up datasets were available for 79 of them. The outcomes showed that *rs75492008* impacted the relationship between ESR and disease activity in MPA patients (P<0.05). Additionally, *rs4964879* affected the relationship between disorder activity and CRP (P<0.05) (**Table 8**).

Correlation between Atg7 gene polymorphism and treatment effect in MPA patients

Complete follow-up datasets were obtained for 79 of the 214 patients. The outcomes showed that patients featuring the *rs8154* TT genotype and the *rs1607237* CC genotype had better clinical treatment effects (P<0.05) (**Table 9**).

Discussion

Our work showed that ATG7 *rs2594966* may be associated with MPA. The ATG7 loci *rs75492008*, *rs2594966*, *rs6442260*, and *rs8154* that form haplotype G-G-C-T were discovered to be related to an increased MPA risk in Chinese individuals. Overall, ATG7, PIK3CA and ULK1 may influence the development of MPA through internal connections. *rs754920-08* was found to affect the relativity between disorder activity and ESR in MPA cases. Additionally, *rs4964879* influenced the relativity between disorder activity and CRP. Cases with *rs8154* TT and *rs1607237* CC genotypes had better clinical treatment effects.

In addition to their role in autophagy, ATGs are strongly implicated in AAV [16]. It has been reported that neutrophils treated with ANCAs exhibit a higher extent of autophagy and release more NETs [17]. Anti-LAMP-2 antibody-treated human neutrophils exhibit a decreased apoptosis rate and strong autophagy activity [18]. The effects are decreased by autophagy suppressors but not by apoptosis suppressors. Autophagy constitutes a basic eukaryotic intracellular biological process necessary for activating adaptive and innate immune responses, comprised of the modulation of cytokine generation, maintenance of lymphocyte homeosta-

Table 3. Distribution of ATG7 in population of different sex and its association with the risk of MPA

SNP ID	Model	Genetype	male OR (95% CI)	*p* value	female OR (95% CI)	*p* value
rs75492008	Codominant	CC	1	0.75	1	0.98
		CT	0.75 (0.35-1.58)		0.95 (0.55-1.61)	
		TT	0.93 (0.28-3.06)		0.95 (0.13-6.86)	
	Dominant	CC	1	0.49	1	0.83
		CT+TT	0.79 (0.40-1.55)		0.95 (0.56-1.60)	
	Recessive	CC+CT	1	NA	1	0.97
		TT	1.00 (0.31-3.24)		0.96 (0.13-6.93)	
	Overdominant	CC+TT	1	0.45	1	0.84
		CT	0.75 (0.36-1.58)		0.95 (0.56-1.61)	
rs8154	Codominant	TT	1	0.85	1	0.69
		CT	1.24 (0.59-2.58)		0.89 (0.49-1.61)	
		CC	1.05 (0.14-7.69)		0.55 (0.13-2.38)	
	Dominant	CC	1	0.59	1	0.54
		CT+TT	1.22 (0.60-2.47)		0.84 (0.48-1.47)	
	Recessive	TT+CT	1	NA	1	0.44
		CC	1.00 (0.14-7.27)		0.57 (0.13-2.42)	
	Overdominant	TT+CC	1	0.57	1	0.75
		CT	1.23 (0.59-2.57)		0.91 (0.51-1.64)	
rs6442260	Codominant	GG	1	0.95	1	0.95
		GA	1.11 (0.58-2.12)		1.00 (0.60-1.68)	
		AA	1.04 (0.25-4.41)		0.84 (0.29-2.44)	
	Dominant	GG	1	0.75	1	0.93
		GA+AA	1.11 (0.59-2.06)		0.98 (0.60-1.60)	
	Recessive	GG+GA	1	1	1	0.74
		AA	1.00 (0.24-4.14)		0.84 (0.30-2.39)	
	Overdominant	GG+AA	1	0.75	1	0.94
		GA	1.11 (0.59-2.10)		1.02 (0.61-1.69)	
rs2594966	Codominant	AA	1	0.041*	1	0.4
		GA	1.06 (0.54-2.06)		0.71 (0.42-1.20)	
		GG	3.42 (1.19-9.80)		0.99 (0.46-2.12)	
	Dominant	AA	1	0.34	1	0.29
		GA+GG	1.36 (0.73-2.55)		0.77 (0.47-1.26)	
	Recessive	AA+GA	1	0.012*	1	0.65
		GG	3.31 (1.23-8.90)		1.18 (0.58-2.40)	
	Overdominant	AA+GG	1	0.43	1	0.18
		GA	0.78 (0.42-1.45)		0.71 (0.44-1.17)	

*P<0.05.

sis, and cardiometabolic, phagocytosis and self-antigen presentation [9, 19]. More evidence implies that autophagy is involved in vertebrate growth by regulating different cellular courses, comprised of programmed cell death, differentiation, survival and proliferation [20]. Currently, autophagy is boosted by over 40 ATG proteins, most of which participate in forming autophagosomes [10]. Of them, ATG7 has long been regarded as a crucial molecule for autophagy. Mutations in autophagy-associated genes are associated with many human disorders, revealing new potential therapeutic targets in the autophagy pathway. Studies have also shown that ATG7 variants are related to some autoimmune diseases [21-27]. Overall, the association of ATG7 with various disorders implies its pleiotropic impacts on numerous

Table 4. Distribution of ATG7 in population of different ages and its association with the risk of MPA

SNP ID	Model	Genetype	Age <60 years OR (95% CI)	*P* value	Age ≥60 years OR (95% CI)	*P* value
rs75492008	Codominant	C/C	1	0.66	1	0.61
		C/T	0.78 (0.46-1.34)		1.45 (0.61-3.44)	
		T/T	1.01 (0.26-3.89)		0.73 (0.15-3.42)	
	Dominant	C/C	1	0.41	1	0.55
		C/T+TT	0.81 (0.48-1.34)		1.27 (0.58-2.79)	
	Recessive	C/C+CT	1	0.9	1	0.61
		T/T	1.09 (0.29-4.14)		0.67 (0.14-3.10)	
	Overdominant	CC+TT	1	0.37	1	0.36
		C/T	0.78 (0.46-1.33)		1.48 (0.63-3.49)	
rs8154	Codominant	TT	1	0.58	1	0.9
		CT	0.99 (0.56-1.76)		1.11 (0.47-2.59)	
		CC	0.44 (0.09-2.24)		1.58 (0.16-15.75)	
	Dominant	CC	1	0.75	1	0.73
		CT+TT	0.92 (0.53-1.58)		1.15 (0.51-2.60)	
	Recessive	TT+CT	1	0.3	1	0.7
		CC	0.44 (0.09-2.23)		1.55 (0.16-15.29)	
	Overdominant	TT+CC	1	0.94	1	0.84
		CT	1.02 (0.58-1.80)		1.09 (0.47-2.55)	
rs6442260	Codominant	GG	1	0.8	1	0.72
		GA	0.87 (0.52-1.45)		1.31 (0.64-2.68)	
		AA	0.76 (0.27-2.20)		1.47 (0.27-8.06)	
	Dominant	GG	1	0.53	1	0.43
		GA+AA	0.86 (0.53-1.39)		1.32 (0.66-2.65)	
	Recessive	GG+GA	1	0.68	1	0.74
		AA	0.80 (0.28-2.28)		1.32 (0.25-7.05)	
	Overdominant	GG+AA	1	0.66	1	0.51
		GA	0.89 (0.54-1.47)		1.27 (0.63-2.57)	
rs2594966	Codominant	A/A	1	0.36	1	0.47
		G/A	0.79 (0.48-1.32)		0.94 (0.45-1.97)	
		G/G	1.34 (0.62-2.91)		1.75 (0.61-5.06)	
	Dominant	A/A	1	0.62	1	0.78
		GA+GG	0.88 (0.54-1.43)		1.11 (0.55-2.21)	
	Recessive	AA+GA	1	0.26	1	0.22
		GG	1.51 (0.73-3.14)		1.81 (0.67-4.87)	
	Overdominant	AA+GG	1	0.22	1	0.53
		GA	0.74 (0.46-1.20)		0.80 (0.40-1.60)	

physiological courses and the potentially shared pathological mechanism of MPA disorders.

To date, no investigations have exhibited relativity between MPA and ATG7 gene variants. Although this study focused on such associations, we detected no statistically significant difference in ATG7 allele frequency or genotype (*rs75492008*, *rs2594966*, *rs6442260*, and *rs8154*) between MPA and healthy control groups in China. Moreover, age and ethnicity stratification analyses exhibited no obvious differences in allele frequencies or genotype between the groups. The probable causes are listed below. In general, the action mechanism of transcription factors is relatively complex, and nucleotides surrounding a mutation site and mutation types differ. Furthermore, if the mutation greatly alters the DNA structure, the

Table 5. Distribution of ATG7 in population of different ethnicity and its association with the risk of MPA

SNP ID	Model	Gene type	Ethnicity = Han OR (95% CI)	*P* value	Ethnicity = Zhuang OR (95% CI)	*P* value
rs75492008	Codominant	CC	1	0.78	1	0.78
		CT	1.20 (0.71-2.03)		1.20 (0.71-2.03)	
		TT	1.20 (0.37-3.84)		1.20 (0.37-3.84)	
	Dominant	CC	1	0.48	1	0.48
		CT+TT	1.20 (0.72-1.98)		1.20 (0.72-1.98)	
	Recessive	CC+CT	1	0.82	1	0.82
		TT	1.14 (0.36-3.62)		1.14 (0.36-3.62)	
	Overdominant	CC+TT	1	0.52	1	0.52
		CT	1.19 (0.70-2.00)		1.19 (0.70-2.00)	
rs8154	Codominant	TT	1	0.43	1	0.43
		CT	0.75 (0.42-1.33)		0.75 (0.42-1.33)	
		CC	0.52 (0.13-2.15)		0.52 (0.13-2.15)	
	Dominant	CC	1	0.23	1	0.23
		CT+TT	0.71 (0.41-1.24)		0.71 (0.41-1.24)	
	Recessive	TT+CT	1	0.4	1	0.4
		CC	0.56 (0.14-2.27)		0.56 (0.14-2.27)	
	Overdominant	TT+CC	1	0.36	1	0.36
		CT	0.77 (0.43-1.36)		0.77 (0.43-1.36)	
rs6442260	Codominant	GG	1	0.61	1	0.61
		GA	1.17 (0.71-1.91)		1.17 (0.71-1.91)	
		AA	1.59 (0.57-4.47)		1.59 (0.57-4.47)	
	Dominant	GG	1	0.41	1	0.41
		GA+AA	1.22 (0.76-1.95)		1.22 (0.76-1.95)	
	Recessive	GG+GA	1	0.43	1	0.43
		AA	1.50 (0.54-4.14)		1.50 (0.54-4.14)	
	Overdominant	GG+AA	1	0.64	1	0.64
		GA	1.12 (0.69-1.82)		1.12 (0.69-1.82)	
rs2594966	Codominant	AA	1	0.23	1	0.23
		GA	0.89 (0.54-1.47)		0.89 (0.54-1.47)	
		GG	1.67 (0.80-3.47)		1.67 (0.80-3.47)	
	Dominant	AA	1	0.9	1	0.9
		GA+GG	1.03 (0.64-1.65)		1.03 (0.64-1.65)	
	Recessive	AA+GA	1	0.099	1	0.09
		GG	1.77 (0.89-3.51)		1.77 (0.89-3.51)	
	Overdominant	AA+GG	1	0.31	1	0.31
		GA	0.79 (0.49-1.25)		0.79 (0.49-1.25)	

effects of nucleotides can be stronger. Accurate binding site anticipation is not easy, and transcriptional modulation may occur through many various mechanisms [21]. For example, *rs75492008* is located in the 5'UTR, and the resulting transcription product is not translated, with translational regulation occurring mainly through secondary structure; *rs2594966* and *rs6442260* are located within the intronic region, with reduced effects on gene expression, and *rs8154* is located within the synonymous CSNP area and features a low impact on gene expression.

Sex stratification analysis revealed that the ATG7 *rs2594966* GG genotype was related to a

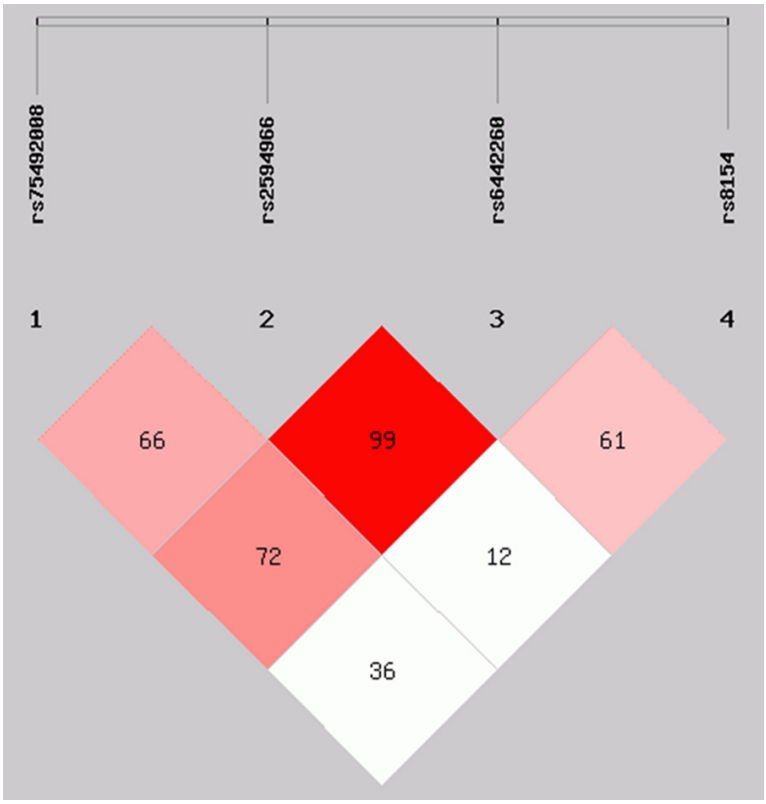

Figure 1. Graphical representation of SNP locations and LD structure. LD plots containing 4 SNPs in ATG7. D' and R^2 value heatmap of linkage disequilibrium in ATG7 gene SNPs. Linkage disequilibrium (LD) testing and haplotype analysis were carried out using SHEsis online software (http://analysis.biox.cn/myAna lysis.php; Shi and He).

higher MPA incidence in males based on codominant and recessive models. Our discoveries resemble those of a previous investigation, exhibiting that ATG7 could feature a significant impact in serious cerebral palsy (CP) types, with possible sex-related differences [26]. The findings indicated haplotype G-G-C-T to be related to an increased MPA risk (OR= 1.5, 95% CI [0.999-2.266], P=0.029). The exact biological mechanisms underpinning the relationship between MPA risk and ATG7 polymorphism remain elusive, and further investigations featuring larger specimen sizes are required to clarify these mechanisms.

To study multigene diseases [28], we employed GMDR software to analyze the relationship between ATG7 gene SNPs and other autophagy-related genes and AAV incidence. The results showed an interaction for ATG7 *rs6442260* and *rs8154*, PIK3CA *rs1607237* and ULK1 *rs4964879*, suggesting that mutations in these genes can interact and raise susceptibility risk to MPA. In the present study, ATG7 (*rs6442260*, G>A) was located in the LD of chromosome region 3p25.3, PIK3CA (*rs1607237*, C>T) was located in the LD of chromosome region 3q26.32, ULK1 (rs4964879, A>G) was located in the LD of chromosome region 12q24.33. Our previous investigation discovered that the PIK3CA gene and ULK1 gene may be obviously related to MPA in the Guangxi population [16]. SNPs *rs6442260*, *rs1607237* and *rs4964879* mentioned in this study are all located in introns. Intronic mutations may influence gene regulation by aberrantly splicing or disrupting DNA-protein interactions. The SNP *rs6442260* is located in the ATG7 gene, encoding an ubiquitin-activating E1-like enzyme key to autophagy [29]. The sequential activities of the E1-like enzyme ATG7 are required in a process in which that the ATG12 system mediates the activation, transfer, and covalent conjugation of ATG12 to ATG5, controls autophagosome formation, and modulates p53/TP53 activity to control the cell cycle and survival during metabolic stress. Atg5 and Atg7 are target genes of YTHDF2, and according to recent research, YTHDF2 controls the mRNA stability of ULK1 in an m6A-dependent way [30]. ATG7 may modulate p53 and control the mRNA stability of ULK1 in an m6A-dependent way, enhancing the susceptibility to MPA. The SNP *rs1607237* is located in the PIK3CA gene, which encodes for the α-isoform of the catalytic subunit (p110a) of class IA PI3K kinase. The PI3K signaling pathway exerts an effect on many cellular processes, such as proliferation, angiogenesis, survival, and metabolism [31]. Mutations in the PIK3CA gene may positively regulate the mTOR/ULK1 pathway through the PIK/AKT signaling pathway, and activation of AKT may reduce the autophagy response, ultimately enhancing the susceptibility to MPA. The SNP

Table 6. Correlation between the haplotype of ATG7 gene SNPs and MPA susceptibility

Haplotype	Case (n=214)	Control (n=211)	OR (95% CI)	*p*
GACT	145.83 (0.341)	162.74 (0.389)	0.776 (0.584-1.031)	0.079
AGCT	75.44 (0.176)	72.31 (0.173)	0.994 (0.696-1.420)	0.973
GATT	60.83 (0.142)	64.89 (0.155)	0.876 (0.599-1.282)	0.495
GGCT	65.85 (0.154)	44.09 (0.105)	1.505 (0.999-2.266)	0.029*
GACC	28.37 (0.066)	20.33 (0.049)	1.355 (0.754-2.438)	0.308
GGCC	20.58 (0.048)	21.57 (0.052)	0.906 (0.487-1.684)	0.753
AACT	17.41 (0.041)	9.25 (0.022)	1.83 (0.814-4.112)	0.138

*P<0.05.

Table 7. GMDR analysis for the best interaction combination model

Data set	χ^2	*p*	OR (95% CI)
Training set	23.4606	0.0001*	4.4074 (2.3837, 8.1495)
Validation set	0.5781	0.4471	1.8949 (0.3209, 11.1896)
The whole data set	24.0491	0.0001*	4.0563 (2.2913, 7.1810)

*P<0.05.

rs4964879 is located in the ULK1 gene, which is a core serine/threonine protein kinase that participates in initiating autophagy. ULK1 and autophagy-specific Class III PI3K complexes are greatly controlled. The MTOR complex 1 (mTORC1) is one of the main regulators of macroautophagy, powerfully suppressing autophagosome generation by catalysing the inactivating phosphorylation of ATG13 and ULK1 [32]. A study showed that renal tubular autophagy in diabetic kidney disease was impaired by the p53/microRNA-214/ULK1 axis [33]. ULK1 may act though the mTOR/ULK1/PI3K and p53/microRNA-214/ULK1 pathways, eventually increasing susceptibility to MPA. Overall, we infer that ATG7, PIK3CA and ULK1 may influence MPA development through the PI3K/AKT/mTOR/ULK1signaling pathway and the p53/microRNA-214/ULK1 signalling pathway through internal connections. Nevertheless, we did not conduct further research to determine how these SNPs interact or how their interactions increase susceptibility to AAV, and future experimental investigations are needed.

ESR is a biomarker that refers to the sedimentation rate of red blood cells under certain conditions. ESR is significantly higher in acute inflammation, and the active stage of rheumatic disease and is used for assessing disease activity for systemic inflammation and diseases such as rheumatoid arthritis, polymyalgia rheumatica, and giant cell arteritis [34]. An enhanced ESR is an independent prognostic element for mortality [35]. CRP is synthesized and degraded in hepatocytes, and serum CRP levels increase dramatically within 24-72 hours during inflammation [36]. CRP features a short half-life and is typically regarded as the lab marker of selection for acute inflammatory disorders. Clinically, we judge the activity of vasculitis by detecting CRP and ESR. Although they are not as specific and sensitive as ANCAs, they are still of great value in assessing the disease activity of vasculitis and in predicting recurrence. Our study showed that *rs75492008* affects the association between CRP and ESR in MPA patients; however, there was no difference in CRP. *rs75492008* with the TT genotype may lead to more severe disease activity in vasculitis. Additionally, *rs4964879* affects the association between CRP and ESR in MPA patients; however, there was no difference in ESR. *Rs4964879* with the GA genotype may also be related to worse disorder activity in vasculitis. It can be inferred that gene polymorphisms may affect the disease activity of MPA, which may be of great significance in judging the severity of vasculitis and in predicting recurrence. Atg7-deficient endothelial cells augmented endothelial to mesenchymal transition [37]. Atg7 defect caused apoptosis induced by mitochondrial damage in mature T lymphocytes resulting in lymphopenia [38]. Another study revealed that T-cell-specific Atg7 deletion impaired IL-2 and IFN-y production and stimulated proliferation instead of inducing apoptosis [39]. ATG7 may act through autophagy, leading to autoimmune and inflammatory reactions. Together, MPA and ATG7 may cause an elevated ESR indirectly through the expression of E-selectin by endothelial cells, which causes neutrophils to adhere to endothelial cells. It

Table 8. Relationship between gene polymorphisms and CRP and ESR indicators

SNP ID	Gene type	CRP	χ	*p*	ESR	χ	*p*
rs75492008*	CC	43.89±62.00	1.151	0.319	70.50±42.95	3.702	0.027
	CT	36.96±46.83			86.46±45.86		
	TT	70.94±41.43			104.38±22.80		
rs8154	CC	43.5±59.82	0.052	0.95	97.5±0.71	0.415	0.661
	CT	40.91±55.18			72.23±44.62		
	TT	44.46±59.05			77.20±43.85		
rs6442260	GG	45.40±63.32	1.294	0.277	76.82±43.13	0.632	0.533
	GA	36.79±43.26			77.66±45.56		
	AA	72.42±77.36			58.14±38.58		
rs2594966	GG	41.49±56.58	0.474	0.623	76.24±42.48	0.009	0.991
	GA	49.00±67.41			75.71±43.71		
	AA	39.15±47.71			76.75±44.87		
rs1607237	CC	47.67±61.33	0.865	0.423	79.04±42.74	0.279	0.757
	CT	34.33±50.87			77.39±42.74		
	TT	35.37±42.69			70.61±42.33		
rs4964879#	GG	20.03±25.96	2.947	0.046	78.40±49.69	2.197	0.115
	GA	52.00±57.50			81.68±42.74		
	AA	42.69±67.25			65.19±40.28		

Abbreviations: CRP: C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate. *P<0.05, #P<0.05.

Table 9. Relationship between gene polymorphisms and disease treatment outcomes

SNP ID/Outcomes	Gene type	Inefficiency	Efficient	F	p
rs75492008	CC	3/77	49/77	1.5955	0.4503
	CT	3/77	19/77		
	TT	0	3/77		
rs8154*	CC	0	1/77	65.07	0.00001
	CT	0	16/77		
	TT	6/77	54/77		
rs6442260	GG	3/77	42/77	0.9158	0.6326
	GA	3/77	24/77		
	AA	0	5/77		
rs2594966	GG	2/77	11/77	2.3601	0.3073
	GA	1/77	33/77		
	AA	3/77	27/77		
rs1607237#	CC	1/77	43/77	16.42	0.0003
	CT	1/77	21/77		
	TT	4/77	6/77		
rs4964879	GG	1/77	15/77	1.274	0.5289
	GA	2/77	36/77		
	AA	3/77	20/77		

*P<0.05, #P<0.05.

may increase the chemotaxis of neutrophils and T cells, and promote a systemic inflammatory response. The MPO-ANCA-positive patients showed significantly higher serum levels of C-reactive protein than the MPO-ANCA-negative patients [40]. ANCA-related vasculitis leading to ischemic effects and inflammation is important in myeloperoxidase (MPO)-ANCA-positive patients. CRP can prime neutrophils and strengthen the respiratory burst induced by ANCAs and might be pathogenic in AAV [41]. CRP plays prothrombotic roles by dissociating from pentameric CRP (pCRP) into changed or monomeric CRP (mCRP). ANCA can induce the production of extra-neutrophilic traps (NETs). The NETs-dependent formation of mCRP induced by pCRP and ANCA can activate platelets and then contribute to mCRP generation on activated platelets. The newly produced mCRP can further strengthen platelet activation, the process of thrombosis, and the inflammatory response [42]. ULK1 (*rs4964879*) variation may initiate autophagy, and NETs influences CRP and then affect the disease activity of MPA.

With regard to MPA treatment, induction therapy emphasizes the application of low-dose hormones. Rituximab (RTX) and cyclophosphamide are the core drugs for the remedy of serious active GPA/MPA. The phase III clinical study results of a C5a receptor inhibitor (avacopan) showed that the treatment effect of avacopan is not inferior to that of hormones at 26 weeks, that the continuous remission rate was better than that of hormones at 52 weeks, and that there were fewer side effects [43]. Avacopan has good application prospects in the treatment of AAV. Discoveries related to translating immunopathogenesis into clinical practice as targeted therapies are increasing, and our team explored whether MPA treatment is related to genetic polymorphisms. This result shows that MPA patients with the rs8154 TT genotype and rs1607237 CC genotype had better clinical treatment effects, although the molecular mechanisms are still not clear.

This study has several limitations. First, the participant quantity was very small resulting from low MPA incidence, particularly with regard to subpopulations after stratified analyses, which may have provided insufficient evidence for definitive conclusions. Second, some subsequent data, such as the curative impact of mortality, relapse rate, renal survival rate, glucocorticoids and immunosuppressants, were lacking. Third, we did not explore molecular mechanisms for verifying the relationship between the gene polymorphisms identified in the investigation and MPA.

Conclusions

There are 214 MPA patients and 211 controls in this study and among the 214 MPA patients, 79 available complete follow-up clinical datasets were gathered from September 2009 to October 2020. The present investigation indicates that gene polymorphisms in the ATG7, PIK3CA and ULK1 genes may be associated with MPA in China, with possible relationships to activity indicators, treatment and prognosis.

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Disclosure of conflict of interest

None.

Address correspondence to: Li Huang and Chao Xue, Department of Nephrology, The Second Affiliated Hospital of Guangxi Medical University, No. 166 Da Xue Dong Lu Road, Nanning 530000, Guangxi, China. E-mail: 619414407@qq.com (LH); xuechao@stu.gxmu.edu.cn (CX)

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