Gene polymorphisms in *ULK1* and *PIK3CA* are associated with the risk of microscopic polyangiitis in the Guangxi Zhuang Autonomous Region in China

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Abstract

Background. Microscopic polyangiitis (MPA) is a systemic autoimmune disease characterized by inflammation of small- and medium-sized blood vessels. Autophagy-related protein polymorphisms are involved in autoimmune disease. The aim of this study was to evaluate the effects of single-nucleotide polymorphisms (SNPs) in the *ULK1* and *PIK3CA* genes on the risk of MPA.

Method: A total of 208 patients with MPA and 211 controls in the Guangxi Zhuang Autonomous Region were recruited and analyzed. The SNPs selected were detected by polymerase chain reaction and high-throughput sequencing. The differences in allele and genotype frequency, various genetic models, and stratification analyses were evaluated, haplotype evaluation was performed after linkage disequilibrium analysis, and the interaction between gene alleles was analyzed.

Result: A statistically significant difference was detected in the genotypic distribution of two SNPs between the two groups: *ULK1* rs4964879 ($p = 0.019$) and *PIK3CA* rs1607237 ($p = 0.002$). The results of the genetic models revealed that *ULK1* rs4964879 and rs9481 are statistically significantly associated with an increased risk of MPA, whereas *PIK3CA* rs1607237 is associated with a reduced risk. The association between SNPs and MPA risk is affected by age, sex, and ethnicity. The *ULK1* haplotype (G-T-A-C-G-A) and *PIK3CA* haplotype (T-G) are associated with a reduced risk of MPA, while the *PIK3CA* haplotype (C-G) is associated with an increased risk.

Conclusion: In this study, polymorphisms in the autophagy-related genes *ULK1* and *PIK3CA* and their association with MPA were examined. The results showed that the polymorphisms in *ULK1* (rs4964879 and rs9481) and *PIK3CA* (rs1607237) were significantly associated with MPA risk in the Guangxi population. However, the molecular mechanisms are still unclear; basic science research and studies with larger samples are needed to confirm our conclusions and explore the underlying mechanisms.

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a group of autoimmune diseases characterized by the inflammation of small- and medium-sized blood vessels. AAV is divided into granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic GPA (EGPA). Each clinical phenotype is associated with the presence of circulating ANCAs, mainly proteinase-3 (PR3) and myeloperoxidase (MPO) (Ramponi et al. 2021). AAV is more common in males than females, and its incidence rate increases with age, especially in the range of 60–70 years (Geetha & Jefferson 2020). Additionally, a notable geographic preponderance of MPA rather than GPA and EGPA is observed in China (Chang et al. 2019).

The precise mechanism of AAV remains unestablished, but a genome-wide association study (GWAS) identified the genetic component in the development of this autoimmune disease. A GWAS conducted in a North American cohort demonstrated that GPA is significantly associated with single-nucleotide polymorphisms (SNPs) in the HLA region encoding major histocompatibility complex (MHC) Class II (Xie et al. 2013). A European study showed that the genetic association with AAV is antigen specificity. PR3-ANCA is associated with HLA-DP, which encodes SERPINA1 and PRTN3, while MPO-ANCA is associated with HLA-DQ (Lyons et al. 2012). Consistent with previous studies, a new large GWAS revealed that MHC and non-MHC gene variates are related to GPA/MPA susceptibility, and changing the expression of genes and proteins is associated with the immune response (Merkel et al. 2017).

80   Autophagy is an essential metabolic process in eukaryotic cells, and autophagy-related proteins are
81   involved in various pathologies, including disorders of immune regulation, inflammation, and cancer (Wu
82   & Adamopoulos 2017). The PI3K/AKT/mTOR/ULK1 signaling pathway is one of the main regulators of
83   autophagy. Uncoordinated 51-like kinase 1 (ULK1) is a serine/threonine kinase that plays a key role in
84   the formation of the ULK1 complex. The human ULK1 complex can induce the initiation of the
85   autophagy pathway and maturation of autophagosomes (Lin & Hurley 2016). PIK3CA encodes the p110α
86   catalytic subunit of phosphatidylinositol 3-kinase (PI3K), which can inactivate mTOR through the
87   PIK3CA/AKT signaling pathway and lead to autophagy (Qu et al. 2016). PIK3CA and ULK1 are the core
88   components of this signaling pathway, and their mutations may alter the autophagy response and cause a
89   change in the incidence of disease risk (Morgan et al. 2012; Qu et al. 2016; Zhang & Zhou 2019; Zhang et
90   al. 2017).

91   Numerous studies have revealed that autoimmune diseases may share susceptibility genes. *ULK1* has
92   been shown to be associated with ankylosing spondylitis (Zhang et al. 2017) and Crohn's disease
93   (Morgan et al. 2012). The cooccurrence of systemic lupus erythematosus (SLE) and AAV reported in
94   cases suggests that these two diseases may have shared genetic factors, especially in MPO-ANCA-
95   positive AAV (Hervier et al. 2012). Emerging evidence shows that autophagy-related gene
96   polymorphisms, such as mTOR (Saravani et al. 2020) and ATG5 (López et al. 2013), participate in SLE.
97   However, as an autoimmune disease, the role of autophagy-related gene mutations in AAV has not yet
98   been reported. Considering that MPA is the most common clinical subtype of AAV in China, we focused
99   on whether gene polymorphisms of *ULK1* and *PIK3CA* play a role in susceptibility to MPA. In the
100   present study, SNP loci with a minor allele frequency (MAF) ≥5% in the functional region of the *ULK1*
101   and *PIK3CA* genes were selected, and the association between these two gene polymorphisms and
102   susceptibility to MPA was explored in a group of patients with MPA and a healthy control group from the
103   Guangxi Zhuang Autonomous Region in China.

104

Materials & Methods

106   **Study population**
107   A total of 208 eligible patients with MPA were recruited from September 2009 to April 2020 in the
108   Department of Nephrology of the Second Affiliated Hospital of Guangxi Medical University (formerly
109   Western Hospital of the First Affiliated Hospital of Guangxi Medical University). The inclusion criteria
110   were as follows: (i) all cases were classified and evaluated as MPA according to the 2012 Revised
111   International Chapel Hill Consensus Conference Nomenclature of Vasculitis (Jennette et al. 2013), (ii)
112   age ≥18 years, and (iii) all patients were born in the Guangxi Zhuang Autonomous Region and had no
113   blood relationship. Patients with secondary vasculitis, other autoimmune diseases, chronic disease and
114   malignant tumors were excluded. A total of 211 healthy volunteers matching the MPA group with respect
115   to age and sex were enrolled as the control group.
116   The basic clinical information of the patients with MPA and the healthy controls is presented in
117   Table 1. The age range at presentation was 18–82 years, with a mean age of 54.6 ± 14.9 years, of which
118   114 cases were < 60 years, and 62.5% were female. The MPA group had 131 Han and 75 Zhuang
119   nationality populations. The mean BVAS at diagnosis was 16.8 ± 4.43. In this study, 36 biopsy specimens
120   (40.4%) were classified as focal, 9 (10.1%) as crescentic, 20 (22.5%) as mixed, and 24 (27%) as sclerotic.
121   Tubulointerstitial injury was graded as follows: 37 (41.6%) had a score of 1, 41 (46.1%) had a score of 2,
122   and 11 (12.4%) had a score of 3. The control group (mean age 51.2 ± 12.6 years) consisted of 128 females
123   and 155 Han nationality populations. This study was approved by the Ethics Committee of the Second

124   Affiliated Hospital of Guangxi Medical University (No. 2018 KY-0100) and followed the principles of
125   the Helsinki Declaration. Written informed consent was obtained from all participants.
126

DNA isolation

128   Blood (5 ml) was collected from the ulnar vein of each participant. Total genomic DNA was
129   extracted from peripheral blood samples using a blood DNA extraction kit (Tiangen, Beijing, China)
130   according to the manufacturer's instructions, and the quality was checked by a Nanodrop 2000
131   spectrophotometer (Thermo Scientific). Samples with an A260/A280 ratio of 1.7–1.9 were included in the
132   study, and the isolated DNA was stored at −80 °C for further studies.
133

Tag SNP selection

135   Six SNPs of the *ULK1* gene (rs10902469, rs12303764, rs4964879, rs7300908, rs7138581 and
136   rs9481) and two SNPs of the *PIK3CA* gene (rs1607237 and rs9838117) were selected from genotype data
137   of Chinese people in the 1000 Genomes (http://grch37.ensembl.org/). The selection criteria included the
138   following: 1) sites located in the functional region, 2) previously reported associations with autoimmune
139   or inflammatory diseases, 3) select tag SNPs as determined using HaploReg, and 4) MAF ≥0.05.
140

SNP genotyping assay

142   SNPs of the *ULK1* and *PIK3CA* genes were detected by polymerase chain reaction (PCR) and high-
143   throughput sequencing (Sangon Biotech, Shanghai, China). The PCR amplification conditions were
144   settled by the two-step method. HiSeq XTen sequencers (Illumina, San Diego, CA, USA) were used to
145   perform paired-end sequencing of the library, and the data were analyzed using Samtools 0.1.18 software.
146   Approximately 10% of the randomly selected samples were sequenced by Sangon Biotechnology
147   Company (Shanghai, China) to verify the accuracy of genotyping, and the reproducibility rate of all SNP
148   genotyping was 100%.
149

Statistical analysis

151   The genotypic and allelic frequencies in the MPA group and the control group were evaluated by the
152   chi-square test or Fisher's exact test. Hardy–Weinberg equilibrium (HWE) in the control participants was
153   tested using the chi-square test for each SNP. Genetic models and stratification analyses with odds ratios
154   (ORs) and 95% confidence intervals (CIs) were analyzed to estimate the relationship between genetic
155   variation and the risk of MPA through online SNPstats software (https://www.snpstats.net/start.htm)
156   adjusted by age and sex. Pairwise linkage disequilibrium (LD) and haplotype blocks as measured by D'
157   were evaluated by online software (SHEsis) ((Shi & He 2005). The interactions between SNPs of the
158   *ULK1* gene and *PIK3CA* gene were evaluated using generalized multifactor dimensionality reduction
159   (GMDR). SPSS Statistics version 23.0 (IBM, Armonk, NY, USA) was used to analyze the data, and
160   *p*<0.05 was considered statistically significant.
161

Results

Association of gene polymorphisms with MPA susceptibility

164   The genotyping results for quality control ranged from 97.18% to 99.76%. Detailed information on
165   all SNPs is provided in Table 2 (SNP IDs, locations and allele frequencies). In the selected SNPs, all
166   ANPs had a MAF of >5%, and the genotype distribution in the control group was in HWE (*p* >0.05).

167 According to the single-SNP analyses, the allele frequencies of *PIK3CA* rs1607237 (C>T) were
168 significantly different between the MPA group and the control group ($p = 0.011$).
169   The association between the SNPs and the risk of MPA was identified by genetic models
170 (codominant, dominant, recessive, and overdominant) and genotype frequencies (Table 3). The results
171 adjusted by age and sex showed that rs4964879 in the *ULK1* gene significantly increased the risk of MPA
172 with the GA genotype in the codominant model (GA versus AA, OR = 1.76, 95% CI: 1.15–2.70, and $p =$
173 0.03), the dominant model (GA/GG versus AA, OR = 1.60, 95% CI: 1.07–2.40, and $p = 0.022$) and
174 overdominant model (GA versus AA/GG, OR = 1.68, 95% CI: 1.13–2.49, and $p = 0.0096$). The risk of
175 MPA in the *ULK1* gene rs9481 was 1.77 times that in healthy controls in the recessive model (GG versus
176 AA/AG, 95% CI: 1.06–2.94, and $p = 0.027$). The mutations of rs1607237 in the *PIK3CA* gene had a
177 lower incidence of MPA with the CT genotype in the codominant model (CT versus CC, OR = 0.47, 95%
178 CI: 0.30–0.73, and $p = 0.0039$), the dominant model (CT/TT versus CC, OR = 0.55, 95% CI: 0.37–0.82,
179 and $p = 0.0031$), and the overdominant model (CT versus CC/TT, OR = 0.49, 95% CI: 0.32–0.76, and $p =$
180 0.0013). No significant difference was observed for the other gene loci between the cases and controls (p
181 > 0.05).
182

Linkage disequilibrium analysis
184   Figure 1 shows the pattern of pairwise LD with respect to the analyzed SNPs of two genes in the
185 current study. The LD plot indicated that the *ULK1* rs10902469, rs12303764, rs4964879, rs7300908,
186 rs7138581, and rs9481 loci formed six haplotypes (Table 4). Haplotype G-T-A-C-G-A was the most
187 commonly observed haplotype in the cases (49.2%) and in the healthy controls (56.1%) and was
188 associated with a reduced risk of MPA (OR = 0.749, 95% CI: 0.563-0.997, $p = 0.047$). Other haplotypes
189 did not exhibit an association with MPA. The SNP loci of the *PIK3CA* genes rs1607237 and rs9838117
190 also formed three haplotypes (Table 4). The results showed that the C-G haplotype was the most
191 commonly observed haplotype in the cases (73.3%) and in the healthy controls (66.7%) and was
192 associated with an increased risk of MPA (OR = 1.427, 95% CI: 1.050–1.939, and $p = 0.023$). The T-G
193 haplotype was significantly associated with a reduced risk of MPA (OR = 0.520, 95% CI: 0.339–0.799,
194 and $p = 0.0025$).
195

Stratification analysis based on age, sex and ethnicity
197   The analysis results showed that age, sex, and ethnicity significantly affected the association
198 between *ULK1* and *PIK3CA* SNPs and MPA risk. The mutations of *ULK1* rs4964879 (with GA genotype
199 in the overdominant model, OR = 1.65, 95% CI: 1.01–2.69, and $p = 0.046$) and rs9481 (with GG
200 genotype in the recessive model, OR = 1.88, 95% CI: 1.01–3.51, and $p = 0.047$) were associated with a
201 higher incidence of MPA in the population aged < 60 years; *PIK3CA* rs1607237 was associated with a
202 decreased MPA risk under the codominant model (CT versus CC, OR = 0.23, 95% CI: 0.10–0.53, and p
203 < 0.001), the dominant model (CT/TT versus CC, OR = 0.41, 95% CI: 0.20–0.85, and $p = 0.016$), and
204 the overdominant model (CT versus CC/TT, OR = 0.21, 95% CI: 0.09–0.49, and $p < 0.001$) in the
205 population $\geqq 60$ years (Table 5). The results were adjusted by sex.
206   The results also showed that sex significantly affected the association between SNPs and MPA risk
207 (Table 6). *ULK1* rs4964879 in females under the dominant model (GA/GG versus AA, OR = 1.69, 95%
208 CI: 1.02–2.82, and $p = 0.042$) and the overdominant model (GA versus AA/GG, OR = 1.74, 95% CI:
209 1.05–2.88, and $p = 0.031$), which could increase MPA risk. Marginal evidence revealed that rs9481 in

210 females under the regressive model increased MPA risk ($p = 0.05$). *PIK3CA* rs1607237 was associated
211 with a decreased MPA risk in the population, independent of sex. The results were adjusted by age.
212   In addition, the results showed that the Han population with the *ULK1* rs4964879 mutation had a
213 higher incidence of MPA with the GA genotype under the dominant model (GA/GG versus AA, OR =
214 1.78, 95% CI: 1.09–2.90, and $p = 0.02$, Table 7) and the overdominant model (GA versus AA/GG, OR =
215 1.70, 95% CI, 1.05–2.74, and $p = 0.03$); the Han population with *PIK3CA* rs1607237 could significantly
216 decrease MPA risk with the CT genotype in the codominant model (CT versus CC, OR = 0.42, 95% CI:
217 0.24–0.73, and $p = 0.0068$), the dominant model (CT/TT versus CC, OR = 0.48, 95% CI: 0.29–0.78, and
218 $p = 0.0031$) and the overdominant model (CT versus CC/TT, OR = 0.44, 95% CI: 0.26–0.76, $p = 0.0026$).
219 The results were adjusted by sex and age.
220
221 **Interaction of gene alleles with clinical characteristics**
222   Generalized multifactor dimensionality reduction (GMDR) was used to analyze the interaction
223 between the alleles of the *ULK1* gene (rs10902469, rs12303764, rs4964879, rs7300908, rs7138581, and
224 rs9481) and *PIK3CA* gene (rs1607237 and rs9838117). The interaction showed that rs4964879 and
225 rs1607237 were the best models for MPA prediction (cross-validation consistency: 10/10). The risk of
226 MPA in the "high-risk" combination was 2.27 times that in the "low-risk combination" (Figure 2), but a
227 margin testing p value was observed ($p = 0.0547$).
228

Discussion

230   In this study, polymorphisms in the autophagy-related genes *ULK1* and *PIK3CA* and their
231 association with MPA were examined. The results showed that the *ULK1* SNPs rs4964879 and rs9481
232 were risk factors for MPA, and P*IK3CA* rs1607237 was a protective factor for MPA.
233   Autophagy is a fundamental intracellular biological process of eukaryotic cells that is essential for
234 the activation of innate and adaptive immune responses, including self-antigen presentation, phagocytosis,
235 maintenance of lymphocyte homeostasis, and regulation of cytokine production (Ye et al. 2019). It is well
236 established that the mammalian target of rapamycin (mTOR)/ULK1 pathway is one of the main regulators
237 of autophagy. Inhibition of mTOR results in dephosphorylation of ULK1 and upregulates autophagy, and
238 it is positively modulated through the PI3K/AKT pathway and negatively modulated by adenosine
239 monophosphate-activated protein kinase (Mohamed et al. 2021). Increasing studies have demonstrated
240 that autophagy is involved in the biology of neutrophils, which play a critical role in the acute injury of
241 AAV by releasing proteolytic enzymes via degranulation, producing reactive oxygen species and
242 extruding neutrophil extracellular traps (NETs) (Al-Hussain et al. 2017; Skendros et al. 2018). Li-Li Sha
243 et al. proved that autophagy activity is elevated in neutrophils treated with ANCAs, and the NET
244 formation rate increases or decreases in neutrophils pretreated with an autophagy inducer or inhibitor,
245 respectively (Sha et al. 2016). Sha Tang et al. also demonstrated that NET formation is associated with
246 autophagy-related signaling in human neutrophils with AAV (Tang et al. 2015).
247   In this study, six SNPs (rs10902469, rs12303764, rs4964879, rs7300908, rs7138581, and rs9481) in
248 *ULK1* between healthy controls and MPA patients were evaluated. Our initial single SNP analysis
249 detected a significant difference in the genotypic distribution (rs4964879, A > G) between the two groups.
250 Subsequently, rs4964879 (codominant, dominant and overdominant model) and rs9481 (recessive model)
251 of the *ULK1* gene were significantly associated with the risk of MPA. In addition, the association
252 between *ULK1* gene polymorphisms and MPA risk was influenced by age, sex and ethnicity. Our findings

253 indicated that rs4964879 and rs9481 variations (A>G) in the *ULK1* gene may be able to increase
254 susceptibility to MPA.

255  *ULK1*, which is a serine/threonine protein kinase, plays a central role in initiating autophagy. It has
256 been reported that the knockdown of *ULK1* in HEK293 cells is sufficient to inhibit the autophagy
257 response (Chan et al. 2007). Mouse embryonic fibroblasts derived from ULK1$^{-/-}$ and ULK2$^{-/-}$ mice
258 blocked autophagy during amino acid starvation (Cheong et al. 2011). As expected by the role of ULK1
259 kinase in autophagy initiation, genetic variation in *ULK1* could result in autophagy disorder. David J.
260 Horne et al. found that ULK1-deficient cells present decreased cytokine secretion and autophagy activity.
261 The study was also the first to report that the rs12297124 minor allele of the *ULK1* gene contributes to an
262 80% reduction in latent tuberculosis infection risk in Asian participants (Horne et al. 2016). The *ULK1*
263 SNPs rs4964879 and rs9481 reported in this study are located in intron and 3'UTR regions, respectively.
264 Although introns are untranslated regions in mRNAs, mutations in introns may affect the binding of
265 transcription factors and change the splicing modes or transcription of the *ULK1* gene, ultimately altering
266 the sequence of amino acids (Kawasaki et al. 2018). The 3'UTR plays an important role in mRNA
267 transport, stability and posttranscriptional regulation. Trans-acting factors or microRNAs bind to cis-
268 acting elements in the 3'UTR of the target transcript and regulate protein synthesis by affecting
269 transcription factors. Sequence variations in mRNA introns or 3'UTR regions in *ULK1* may cause
270 abnormal expression of the gene (Zhang et al. 2019). Considering the role of ULK1 in the autophagy
271 pathway, we speculate that *ULK1* (rs4964879 and rs9481) variations may lead to abnormal expression of
272 ULK1 and then initiate the autophagy response, eventually increasing the susceptibility to MPA.

273  In the present study, another autophagy-related gene, *PIK3CA* (rs1607237, C>T), showed significant
274 differences in the allele frequency and genotypic distribution between the patients with MPA and the
275 healthy controls. A subject with at least one T allele has approximately half the risk for MPA compared
276 with a subject with a CC genotype (TT+CT vs. CC: OR 0.56, 95% CI: 0.37–0.83). Increasing findings
277 confirm that polymorphisms in the PI3K/AKT signaling pathway are related to the regulation of cell
278 proliferation, survival and death. Similar to the results of our study, a case-control study conducted by
279 Xing et al. found that the *PIK3CA* polymorphism is a defense factor against follicular thyroid cancer
280 (Xing et al. 2012). *PIK3CA* rs1607237 is also significantly associated with a small decrease in breast
281 cancer risk (Stevens et al. 2011). SNP rs1607237 is in the intron of *PIK3CA* gene. Although no published
282 literature has reported the feature of *PIK3CA* rs1607237, given the location of this SNP, we speculate that
283 SNPs may affect the transcription of the *PIK3CA* gene by interrupting the process of translation and
284 splicing. The *PIK3CA* mutation increases the expression of the p110α catalytic subunit of PI3K and then
285 activates AKT through the PI3K/AKT signaling pathway. As mentioned above, the PI3K/AKT pathway
286 positively regulates the mTOR/ULK1 pathway, and the activation of AKT may decrease the autophagy
287 response. However, further research will be needed to provide strong evidence for this speculation.

288  This study has several limitations, which should be mentioned. First, the number of participants was
289 relatively small because of the low incidence rate of MPA, especially for subpopulations after stratified
290 analysis, which may provide insufficient evidence to provide definitive conclusions. Second, some
291 follow-up information, such as the curative effect of glucocorticoids and immunosuppressants, renal
292 survival rate, relapse rate, and mortality, was lacking. Third, we did not perform studies to explore the
293 molecular mechanisms to verify the association between gene polymorphisms reported in this study and
294 MPA.

295

296 **Conclusions**

297   The present study indicated that the polymorphisms observed in *ULK1* (rs4964879 and rs9481) and
298   *PIK3CA* (rs1607237) were significantly associated with MPA risk in the Guangxi population. However,
299   the molecular mechanisms are still unclear, and studies designed with larger samples and basic research
300   are needed to confirm our conclusions and explore the mechanisms.
301

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306

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Figure 1

Graphical representation of the SNP locations and LD structure

A) LD plots containing 6 SNPs from *ULK1*; B) LD plots containing 2 SNPs from *PIK3CA*

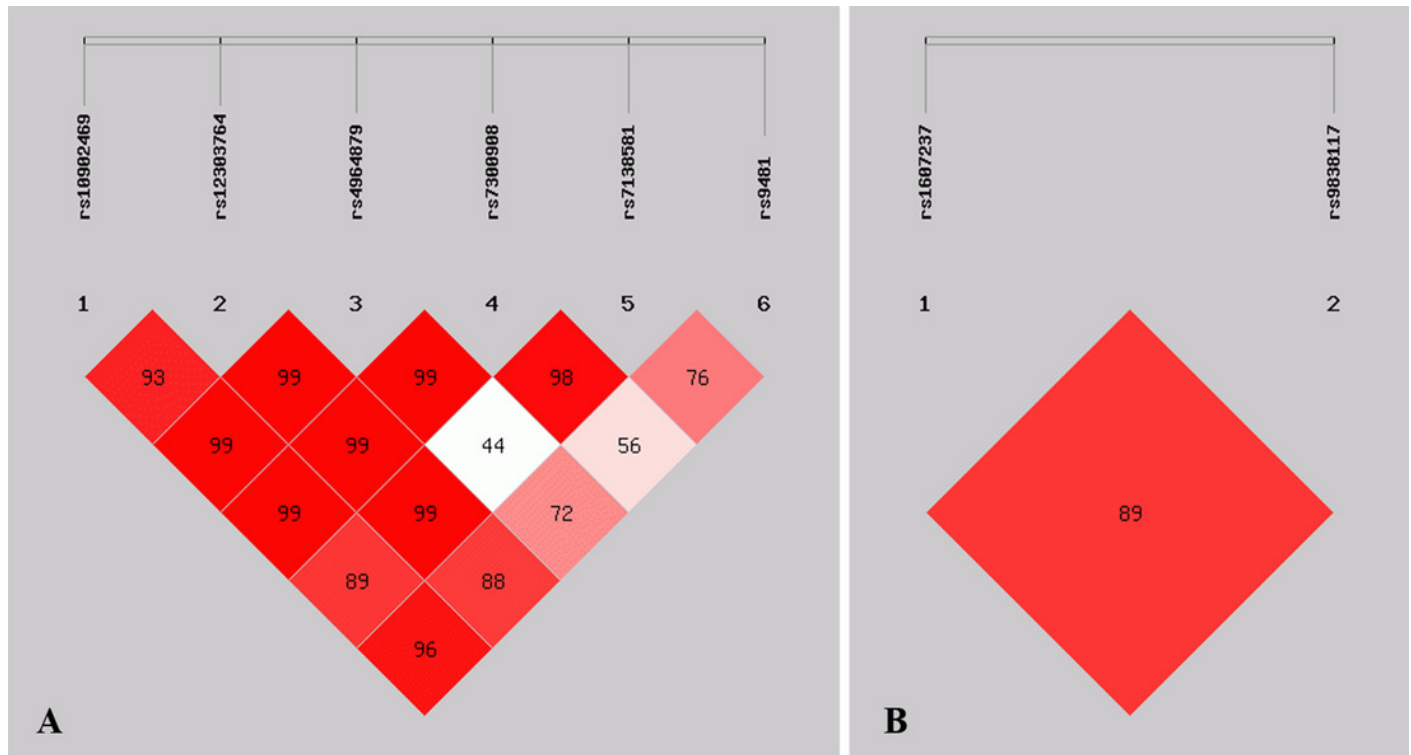
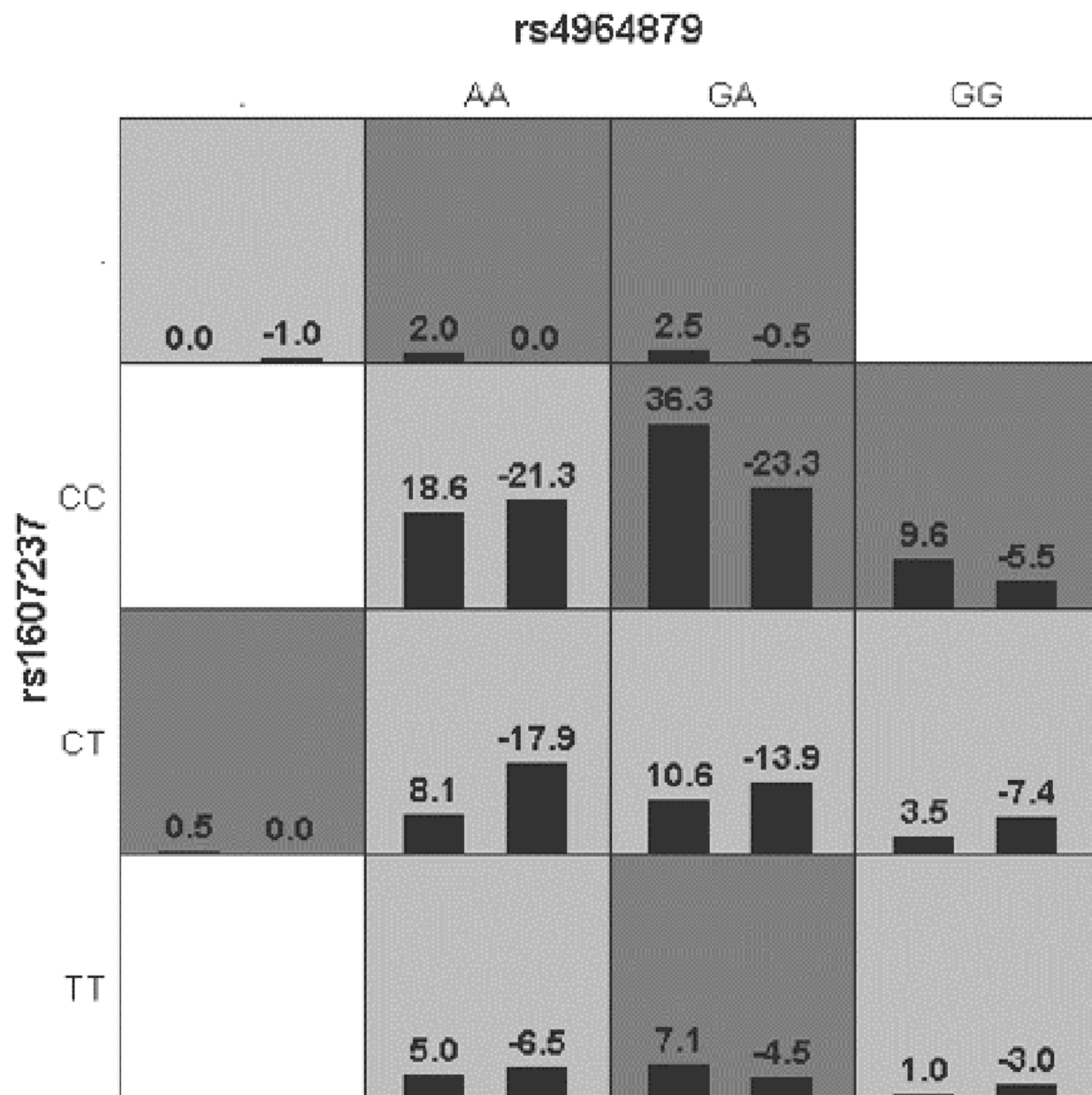



Figure 2

Distribution of high-risk and low risk genotypes in the best two-locus model

Dark gray and light gray boxed presented the high- and low- risk factor combinations, respectively. Left bars within each box represented case with positive score, right bars represented negative score. The higher the positive score, the greater the combination risk. GA in rs4964879 and CC in rs1607237 showed the most risk combinations.

Demographic characteristics of the study participants

1   Table 1 Demographic characteristic of the study participants.

Characteristic	MPA group (n=208)	control group (n=211)
Age (years)	54.6±14.9	51.2±12.6
<60	114 (54.8)	160 (75.8)
≥60	94 (45.2)	51 (24.2)
Gender (M/F)	78/130	83/128
Ethnicity (Han/Zhuang)	131/75	155/56
BVAS (mean ± SD)	16.8±4.43	-
Renal pathologic classification (Renal biopsy, n=89)		
Focal	36 (40.4%)	
Crescentic	9 (10.1%)	
Mixed	20 (22.5%)	
Sclerotic	24 (27.0%)	
Renal tubulointerstitial injury (Renal biopsy, n=89)		
Score 1	37 (41.6%)	
Score 2	41 (46.1%)	
Score 3	11 (12.4%)	

Basic information about SNPs in *ULK1* and *PIK3CA* and association with risk of AAV

1   Table 2 Basic information about SNPs in *ULK1* and *PIK3CA* and their association with the risk of AAV

Gene	SNP ID	Location	Alleles	MAF		*p* for allele frequencies	*p* for genotypes
				Case	Control		
ULK1	rs10902469	12:132378133	G>C	0.079	0.069	0.571	0.832
	rs12303764	12:132399065	T>G	0.200	0.185	0.566	0.678
	rs4964879	12:132400309	A>G	0.406	0.356	0.143	***0.019***
	rs7300908	12:132405421	C>T	0.099	0.090	0.673	0.897
	rs7138581	12:132406666	G>C	0.174	0.167	0.781	0.853
	rs9481	12:132407089	A>G	0.442	0.378	0.060	0.070
PIK3CA	rs1607237	3:178950297	C>T	0.240	0.325	***0.011***	***0.002***
	rs9838117	3:178952507	G>T	0.168	0.165	0.901	0.765

The genotype frequencies of the studied *ULK1* and *PIK3CA* gene SNPs in the cases and the healthy controls

1   Table 3 The genotype frequencies of the studied *ULK1* and *PIK3CA* gene SNPs in the cases and the healthy controls

SNP ID	Model	Genotype	Control n (%)	AVV n (%)	OR (95% CI)	*p*
ULK1 rs4964879	Codominant	AA	92 (44)	67 (32.4)	1.00	
		GA	85 (40.7)	112 (54.1)	**1.76 (1.15-2.70)**	***0.03***
		GG	32 (15.3)	28 (13.5)	1.19 (0.65-2.16)	
	Dominant	AA	92 (44)	68 (31.9)	1.00	
		GA+GG	117 (56)	145 (68.1)	**1.60 (1.07-2.40)**	***0.022***
	Recessive	AA+GA	177 (84.7)	184 (86.4)	1.00	
		GG	32 (15.3)	29 (13.6)	0.87 (0.50-1.51)	0.61
	Overdominant	AA+GG	124 (59.3)	97 (45.5)	1.00	
		GA	85 (40.7)	116 (54.5)	**1.68 (1.13-2.49)**	***0.0096***
ULK1 rs9481	Codominant	AA	81 (38.8)	73 (34.3)	1.00	
		AG	98 (46.9)	90 (42.2)	1.00 (0.65-1.54)	
		GG	30 (14.3)	50 (23.5)	1.76 (1.01-3.09)	0.086
	Dominant	AA	81 (38.8)	73 (34.3)	1.00	
		AG/GG	128 (61.2)	140 (65.7)	1.18 (0.79-1.76)	0.36
	Recessive	AA/AG	179 (85.7)	163 (76.5)	1.00	
		GG	30 (14.3)	50 (23.5)	**1.77 (1.06-2.94)**	***0.027***
	Overdominant	AA/GG	111 (53.1)	123 (57.8)	1.00	
		AG	98 (46.9)	90 (42.2)	0.83 (0.56-1.22)	0.34
PIK3CA rs1607237	Codominant	CC	101 (48.6)	138 (64.3)	1.00	
		CT	79 (38)	45 (22.6)	**0.47 (0.30-0.73)**	***0.0039***
		TT	28 (13.5)	26 (13.1)	0.78 (0.43-1.42)	
	Dominant	CC	101 (48.6)	128 (64.3)	1.00	
		CT/TT	107 (51.4)	71(35.7)	**0.55 (0.37-0.82)**	***0.0031***
	Recessive	CC/CT	180 (86.5)	173 (86.9)	1.00	
		TT	28 (13.5)	26 (13.1)	1.01 (0.57-1.81)	0.97
	Overdominant	CC/TT	129 (62)	154 (77.4)	1.00	
		CT	79 (38)	45 (22.6)	**0.49 (0.32-0.76)**	***0.0013***

The correlation between the haplotypes of *ULK1* and *PIK3CA* gene SNPs and the AAV susceptibility

1   Table 4 The correlation between the haplotypes of *ULK1* and *PIK3CA* gene SNPs and the AAV susceptibility

Gene	Haplotype	AAV (n=214)	Control (n=211)	OR (95% CI)	*p*
ULK1	C-T-A-C-C-G	27.6 (6.7%)	27.1(6.5%)	1.039 (0.599~1.799)	0.893
	G-G-G-G-G	80.8 (19.5%)	67.0(16%)	1.285 (0.897~1.841)	0.171
	G-T-A-C-G-A	203.7 (49.2%)	234.5(56.1%)	**0.749 (0.563~0.997)**	*0.047*
	G-T-G-C-G-A	13.9 (3.4%)	10.6 (2.5%)	1.343 (0.596~3.024)	0.475
	G-T-G-C-G-G	26.8 (6.5%)	20.7 (4.9%)	1.340 (0.741~2.422)	0.331
	G-T-G-T-C-G	30.8 (7.4%)	29.9 (7.1%)	1.054 (0.624~1.780)	0.844
PIK3CA	C-G	291.5 (73.3%)	278.7(66.7%)	**1.427 (1.050~1.939)**	*0.023*
	T-G	36.5 (8.8%)	68.3 (16.3%)	**0.520 (0.339~0.799)**	*0.0025*
	T-T	62.5 (15.7%)	66.7 (16.0%)	0.993 (0.681~1.446]	0.9688

Distribution of *ULK1* and *PIK3CA* polymorphisms in population of different ages and its association with risk of AAV

1   Table 5 Distribution of *ULK1* and *PIK3CA* polymorphisms in population of different ages and its association with the risk of AAV.

SNP ID	Model	Geno type	Age<60 years			*P* value	Age≥ 60 years			*p* value
			Control	Case	OR (95% CI)		Control	Case	OR (95% CI)	
ULK1 rs4964879	Codominant	AA	72 (45%)	39 (34.2%)	1.00		20 (40.8%)	28 (30.1%)	1.00	
		GA	62 (38.8%)	58 (50.9%)	1.74 (1.02-2.96)	0.12	23 (46.9%)	54 (58.1%)	1.66 (0.78-3.53)	0.42
		GG	26 (16.2%)	17 (14.9%)	1.21 (0.59-2.49)		6 (12.2%)	11 (11.8%)	1.37 (0.43-4.35)	
	Dominant	AA	72 (45%)	39 (34.2%)	1.00		20 (40.8%)	28 (20.1%)	1.00	
		GA/GG	88 (55%)	75 (65.8%)	1.58 (0.96-2.60)	0.073	29 (59.2%)	65 (69.9%)	1.60 (0.77-3.30)	0.21
	Recessive	AA/GA	134 (83.8%)	97 (85.1%)	1.00		43 (87.8%)	82 (88.2%)	1.00	
		GG	26 (16.2%)	17 (14.9%)	0.90 (0.47-1.76)	0.77	6 (12.2%)	11 (11.8%)	1.01 (0.35-2.95)	0.98
	Overdominant	AA/GG	98 (61.2%)	56 (49.1%)	1.00		26 (53.1%)	39 (41.9%)	1.00	
		GA	62 (38.8%)	58 (50.9%)	*1.65 (1.01-2.69)*	*0.046*	23 (46.9%)	54 (58.1%)	1.53 (0.76-3.08)	0.23
ULK1 rs9481	Codominant	AA	63 (39.4%)	40 (35.1%)	1.00		18 (36.7%)	32 (34.4%)	1.00	
		AG	74 (46.2%)	47 (41.2%)	0.99 (0.57-1.70)	0.14	24 (49%)	40 (43%)	0.92 (0.42-1.99)	0.44
		GG	23 (14.4%)	27 (23.7%)	1.87(0.94-3.71)		7 (14.3%)	21 (22.6%)	1.73 (0.61-4.88)	
	Dominant	AA	63 (39.4%)	40 (35.1%)	1.00		18 (36.7%)	32 (34.4%)	1.00	
		AG/GG	97 (60.6%)	74 (64.9%)	1.20 (0.73-1.98)	0.47	31 (63.3%)	61 (65.6%)	1.10 (0.53-2.27)	0.8
	Recessive	AA/AG	137 (85.6%)	87 (76.3%)	1.00		42 (85.7%)	72 (77.4%)	1.00	
		GG	23 (14.4%)	27 (23.7%)	*1.88 (1.01-3.51)*	*0.047*	7 (14.3%)	21 (22.6%)	1.81 (0.71-4.65)	0.2
	Overdominant	AA/GG	86 (53.8%)	67 (58.8%)	1.00		25 (51%)	53 (57%)	1.00	
		AG	74 (46.2%)	47 (41.2%)	0.81 (0.49-1.32)	0.39	24 (49%)	40 (43%)	0.77 (0.38-1.54)	0.45
PIK3CA rs1607237	Codominant	CC	76 (47.8%)	63 (58.3%)	1.00		25 (51%)	65 (71.4%)	1.00	
		CT	58 (36.5%)	32 (29.6%)	0.67 (0.39-1.15)	0.23	21 (42.9%)	13 (14.3%)	*0.23 (0.10-0.53)*	*6e-04*
		TT	25 (15.7%)	13 (12%)	0.62 (0.29-1.32)		3 (6.1%)	13 (14.3%)	1.70 (0.44-6.51)	
PIK3CA rs1607237	Dominant	CC	76 (47.8%)	63 (58.3%)	1.00		25 (51%)	65 (71.4%)	1.00	
		CT/TT	83 (52.2%)	45 (41.7%)	0.65 (0.40-1.07)	0.09	24 (49%)	26 (28.6%)	*0.41 (0.20-0.85)*	*0.016*
	Recessive	CC/CT	134 (84.3%)	95 (88%)	1.00		46 (93.9%)	78 (85.7%)	1.00	
		TT	25 (15.7%)	13 (12%)	0.73 (0.35-1.49)	0.38	3 (6.1%)	13 (14.3%)	2.61 (0.70-9.71)	0.12

	Overdominant	CC/TT	101 (63.5%)	76 (70.4%)	1.00		28 (57.1%)	78 (85.7%)	1.00	
		CT	58 (36.5%)	32 (29.6%)	0.74 (0.44-1.24)	0.25	21 (42.9%)	13 (14.3%)	*0.21 (0.09-0.49)*	*2e-04*

Distribution of *ULK1* and *PIK3CA* polymorphisms in population of different genders and its association with risk of AAV

1   Table 6 Distribution of *ULK1* and *PIK3CA* polymorphisms in population of different sex and its association with the risk of AAV

SNP ID	Model	Geno-type	Male			p value	female			p value
			Control	case	OR (95% CI)		Control	Case	OR (95% CI)	
ULK1 rs4964879	Codominant	AA	31 (37.8%)	23 (29.1%)	1.00		61 (48%)	44 (34.1%)	1.00	
		GA	38 (46.3%)	46 (58.2%)	1.63 (0.82-3.26)	0.32	47 (37%)	67 (51.9%)	1.86 (1.08-3.20)	0.08
		GG	13 (15.8%)	10 (12.7%)	1.03 (0.38-2.77)		19 (15%)	18 (13.9%)	1.28 (0.60-2.73)	
	Dominant	AA	31 (37.8%)	23 (29.1%)	1.00		61 (48%)	44 (34.1%)	1.00	
		GA/GG	51 (62.2%)	56 (70.9%)	1.47 (0.76-2.86)	0.25	66 (52%)	85 (65.9%)	*1.69 (1.02-2.82)*	*0.042*
	Recessive	AA/GA	69 (84.2%)	69 (87.3%)	1.00		108 (85%)	111 (86%)	1.00	
		GG	13 (15.8%)	10 (12.7%)	0.76 (0.31-1.87)	0.56	19 (15%)	18 (13.9%)	0.93 (0.46-1.88)	0.84
	Overdominant	AA/GG	44 (53.7%)	33 (41.8%)	1.00		80 (63%)	62 (48.1%)	1.00	
		GA	38 (46.3%)	46 (58.2%)	1.61 (0.86-3.02)	0.13	47 (37%)	67 (51.9%)	*1.74 (1.05-2.88)*	*0.031*
ULK1 rs9481	Codominant	AA	29 (35.4%)	28 (35.4%)	1.00		52 (40.9%)	45 (34.9%)	1.00	
		AG	43 (52.4%)	36 (45.6%)	0.89 (0.45-1.76)	0.5	55 (43.3%)	51 (39.5%)	1.04 (0.60-1.82)	0.14
		GG	10 (12.2%)	15 (19%)	1.49 (0.57-3.90)		20 (15.8%)	33 (25.6%)	1.90 (0.95-3.78)	
	Dominant	AA	29 (35.4%)	28 (35.4%)	1.00		52 (40.9%)	45 (34.9%)	1.00	
		AG/GG	53 (64.6%)	51 (64.6%)	1.00 (0.52-1.92)	0.86	75 (59.1%)	84 (65.1%)	1.27 (0.76-2.12)	0.36
	Recessive	AA/AG	72 (87.8%)	64 (81%)	1.00		107 (84.2%)	96 (74.4%)	1.00	
		GG	10 (12.2%)	15 (19%)	1.60 (0.67-3.84)	0.25	20 (15.8%)	33 (25.6%)	*1.86 (0.99-3.47)*	*0.05*
	Overdominant	AA/GG	39 (47.6%)	43 (54.4%)	1.00		72 (56.7%)	78 (60.5%)	1.00	
		AG	43 (52.4%)	36 (45.6%)	0.79 (0.42-1.47)	0.51	55 (43.3%)	51 (39.5%)	0.83 (0.50-1.38)	0.48
PIK3CA rs1607237	Codominant	CC	39 (47.6%)	51 (66.2%)	1.00		62 (49.2%)	78 (63.4%)	1.00	
		CT	29 (35.4%)	15 (19.5%)	*0.40 (0.19-0.84)*	*0.042*	50 (39.7%)	30 (24.4%)	*0.50 (0.28-0.88)*	*0.048*
		TT	14 (17.1%)	11 (14.3%)	0.60 (0.25-1.47)		14 (11.1%)	15 (12.2%)	0.93 (0.41-2.11)	
PIK3CA rs1607237	Dominant	CC	39 (47.6%)	51 (66.2%)	1.00		62 (49.2%)	78 (63.4%)	1.00	
		CT/TT	43 (52.4%)	26 (33.8%)	*0.46 (0.24-0.88)*	*0.017*	64 (50.8%)	45 (36.6%)	*0.59 (0.35-0.99)*	*0.063*
	Recessive	CC/CT	68 (82.9%)	66 (85.7%)	1.00		112 (88.9%)	108 (87.8%)	1.00	
		TT	14 (17.1%)	11 (14.3%)	0.81 (0.34-1.91)	0.63	14 (11.1%)	15 (12.2%)	1.21 (0.55-2.66)	0.64

Overdominant	CC/TT	53 (64.6%)	62 (80.5%)	1.00		76 (60.3%)	93 (75.6%)	1.00	
	CT	29 (35.4%)	15 (19.5%)	*0.44 (0.21-0.91)*	*0.024*	50 (39.7%)	30 (24.4%)	*0.50 (0.29-0.88)*	*0.014*
	GA	59 (38.6%)	68 (52.3%)	*1.70 (1.05-2.74)*	*0.03*	26 (46.4%)	42 (5%)	1.42 (0.69-2.89)	0.34

Distribution of *ULK1* and *PIK3CA* polymorphisms in population of different ethnicity and its association with risk of AAV

1   Table 7 Distribution of *ULK1* and *PIK3CA* polymorphisms in population of different ethnicity and its association with the risk of AAV.

SNP ID	Model	Geno type	Ethnicity = Han			*p* value	Ethnicity = Zhuang			*p* value
			Control	Case	OR (95% CI)		Control	Case	OR (95% CI)	
ULK1 rs4964879	Codominant	AA	73 (47.7%)	43 (33.1%)	1.00		19 (33.9%)	24 (32%)	1.00	
		GA	59 (38.6%)	68 (52.3%)	1.88 (1.12-3.17)	0.054	26 (46.4%)	42 (56%)	1.24 (0.56-2.76)	0.48
		GG	21 (13.7%)	19 (14.6%)	1.48 (0.71-3.07)		11 (19.6%)	9 (12%)	0.67 (0.23-1.98)	
	Dominant	AA	73 (47.7%)	43 (33.1%)	1.00		19 (33.9%)	24 (32%)	1.00	
		GA/GG	80 (52.3%)	87 (66.9%)	*1.78 (1.09-2.90)*	*0.02*	37 (66.1%)	51 (68%)	1.07 (0.50-2.28)	0.86
	Recessive	AA/GA	132 (86.3%)	111 (85.4%)	1.00		45 (80.4%)	66 (88%)	1.00	
		G/G	21 (13.7%)	19 (14.6%)	1.06 (0.54-2.07)	0.87	11 (19.6%)	9 (12%)	0.58 (0.22-1.55)	0.28
	Overdominant	AA/GG	94 (61.4%)	62 (47.7%)	1.00		30 (53.6%)	33 (44%)	1.00	
		GA	59 (38.6%)	68 (52.3%)	*1.70 (1.05-2.74)*	*0.03*	26 (46.4%)	42 (5%)	1.42 (0.69-2.89)	0.34
ULK1 rs9481	Codominant	AA	60 (39.2%)	47 (36.1%)	1.00		21 (37.5%)	25 (33.3%)	1.00	
		AG	72 (47.1%)	54 (41.5%)	0.94 (0.56-1.59)	0.18	26 (46.4%)	32 (42.7%)	1.07 (0.49-2.37)	0.62
		GG	21 (13.7%)	25 (22.3%)	1.74 (0.88-3.45)		9 (16.1%)	18 (24%)	1.61 (0.59-4.41)	
	Dominant	AA	60 (39.2%)	47 (36.1%)	1.00		21 (37.5%)	25 (33.3%)	1.00	
		AG/GG	93 (60.8%)	83 (63.9%)	1.12 (0.69-1.83)	0.64	35 (62.5%)	50 (66.7%)	1.22 (0.58-2.54)	0.6
	Recessive	AA/AG	132 (86.3%)	104 (77%)	1.00		47 (83.9%)	57 (76%)	1.00	
		GG	21 (13.7%)	31 (23%)	1.80 (0.96-3.35)	0.064	9 (16.1%)	18 (24%)	1.55 (0.62-3.86)	0.34
	Overdominant	AA/GG	81 (52.9%)	79 (58.5%)	1.00		30 (53.6%)	43 (57.3%)	1.00	
		AG	72 (47.1%)	56 (41.5%)	0.79 (0.49-1.27)	0.33	26 (46.4%)	32 (42.7%)	0.91 (0.44-1.87)	0.8
PIK3CA rs1607237	Codominant	CC	76 (49.7%)	86 (68.2%)	1.00		25 (45.5%)	40 (56.3%)	1.00	
		CT	59 (38.6%)	27 (21.4%)	*0.42 (0.24-0.73)*	*0.0068*	20 (36.4%)	18 (25.4%)	0.59 (0.26-1.36)	0.47
		TT	18 (11.8%)	13 (10.3%)	0.68 (0.31-1.49)		10 (18.2%)	13 (18.3%)	0.81 (0.30-2.18)	
	Dominant	CC	76 (49.7%)	86 (68.2%)	1.00		25 (45.5%)	40 (56.3%)	1.00	
		CT/TT	77 (50.3%)	40 (31.8%)	*0.48 (0.29-0.78)*	*0.0031*	30 (54.5%)	31 (43.7%)	0.67 (0.32-1.37)	0.27
	Recessive	CC/CT	135 (88.2%)	113 (89.7%)	1.00		45 (81.8%)	58 (81.7%)	1.00	
		TT	18 (11.8%)	13 (10.3%)	0.91 (0.43-1.97)	0.82	10 (18.2%)	13 (18.3%)	0.97 (0.38-2.51)	0.96

Overdominant	CC/TT	94 (61.4%)	99 (78.6%)	1.00		35 (63.6%)	53 (74.7%)	1.00	
	CT	59 (38.6%)	27 (21.4%)	*0.44 (0.26-0.76)*	*0.0026*	20 (36.4%)	18 (25.4%)	0.63 (0.28-1.38)	0.24