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ORIGINAL RESEARCH

Genetic Variations of *CARMN* Modulate Glioma Susceptibility and Prognosis in a Chinese Han Population

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Background: This study aimed to evaluate the relationship between *CARMN* polymorphisms and glioma risk and prognosis in a Chinese Han population.

Methods: Seven single nucleotide polymorphisms (SNPs) in *CARMN* were genotyped among 592 glioma patients and 502 healthy controls. Log-additive models were used for risk assessment by the odds ratios (ORs) and 95% confidence intervals (CIs). Univariate and multivariate Cox regression analysis was applied to calculate Hazard ratios (HRs) and 95% CIs for prognosis assessment.

Results: *CARMN* rs13177623 was a protective factor for glioma susceptibility (OR = 0.78, p = 0.043). In addition, rs13177623, rs11168100, rs12654195 and rs17796757 were associated with the risk of glioma among the subgroup stratified by age or gender. We also found that $G_{rs13177623}G_{rs12654195}$ haplotype was related to the decreased risk of glioma (OR = 0.61, p = 0.005). Importantly, rs13177623 [overall survival (OS): HR = 0.83, p = 0.047, and progression free survival (PFS): HR = 0.82, p = 0.031], rs12654195 (OS: HR = 0.64, p = 0.005 and PFS: HR = 0.65, p = 0.007) and rs11168100 (OS: HR = 0.71, p = 0.035) were associated with a better prognosis for glioma, especially in grade I-II glioma. In patients with grade III-IV glioma, rs17796757 polymorphism presented an improved OS.

Conclusion: Our results firstly reported the contribution of *CARMN* variants (rs11168100, rs12654195, rs13177623, and rs17796757) to the susceptibility and prognosis of glioma in a Chinese Han population, which provided a novel insight on the relationship between *CARMN* gene and glioma tumorigenesis.

Keywords: glioma, *CARMN* variants, susceptibility, prognosis, genetic variations

Introduction

Glioma is the most common intracranial malignant tumor derived from glial cells, accounting for the majority of all primary brain and central nervous system tumors.[1] It is characterized by the significant mortality and morbidity of approximately 101,600 new cases and 61,000 deaths in China each year.[2] Malignant glioma is a devastating type of brain and other nervous system tumors because of its high malignancy, extremely high mortality rate, and recurrence risk.[3] Despite improvements in therapeutics including surgery in combination with chemo- and/or radiotherapy, the five-year relative survival rate following diagnosis of a malignant brain still grim.[4] The etiology of glioma remains poorly understood to date, but environmental exposure and genetic factors are identified to increase glioma risk. In recent years, the role of inherited genetic variants in glioma has been highly addressed, which revealed single nucleotide polymorphisms (SNPs) in genes contribute to the susceptibility and prognosis of glioma.[5–7]

Long non-coding RNAs (lncRNAs) are a class more than 200 nucleotides non-protein coding RNA, that regulate gene or miRNA expression at the transcriptional, post-transcriptional and epigenetic levels.[8] LncRNAs participate in different stages of glioma formation, invasion, and progression.[7] Recent evidence indicates that genetic variations in functional lncRNAs may play important roles in the occurrence and development of glioma, such as genetic polymorphisms in lncRNA-PTENP1 and lncRNA H19.[9,10]

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Cardiac mesoderm enhancer-associated non-coding RNA (CARMN) is a newly identified lncRNA, also named MIR143HG, and has been reported to be the precursor of miR-143 and miR-145, which linked to gliomagenesis.[11,12] MicroRNA-145-5p downregulation has been shown to play important roles in the oncogenesis and progression of many cancer types including glioblastoma.[13] Furthermore, miR-143 inhibited glioma cells migration and invasion through cytoskeletal rearrangement.[14] Ropivacaine suppressed glioma progression by regulating circSCAF11 and miR-145-5p.[15] These suggested *CARMN*, the host gene of miR-143 and miR-145, might have an important role in the occurrence and development of glioma. Nevertheless, no association studies between *CARMN* polymorphisms and glioma have been published to date.

Considering the effect of genetic variants on glioma, we hypothesized that *CARMN* polymorphisms might contribute to glioma development and prognosis. Here, we conducted a case–control study to evaluate the role of *CARMN* polymorphisms in glioma and found that four SNPs were significantly related to glioma risk and patients survival in a Chinese Han population.

Materials and Methods

Study Subjects

In this study, 592 glioma patients and 502 healthy controls enrolled from the department of Neurosurgery at Xi'an Children's Hospital and Tangdu Hospital. All included patients had recently diagnosed and histopathologically confirmed glioma according to the World Health Organization (WHO) classification. All subjects had a Han Chinese ethnic background. All glioma patients were newly diagnosed and confirmed by histopathology. The blood samples were collected before radiotherapy and chemotherapy or surgery. Patients with a self-reported cancer history, serious systemic diseases or other complex diseases were excluded. Age and gender matched healthy controls were enrolled from annual checkup at the same hospitals. The controls had no any cancers or chronic diseases and no brain and central nervous system diseases. Demographic and clinical information was collected from structured questionnaires and/or medical records. All the patients were followed up every 3 months by return visit, telephone and letter. During the follow-up period, the survival time was recorded until death or the last follow-up. This study was approved by the institute ethics committee of the Xi'an Children's Hospital (No. 20200014) and in accordance with the Helsinki Declaration. Written informed consent was obtained from each participant.

Genotyping of CARMN Polymorphisms

Peripheral blood samples (5 mL) were collected from all of the study participants. Genomic DNA was extracted using the commercially available GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Co. Ltd., Xi′an, China), and stored at −80°C until analysis. The candidate variants in *CARMN* were selected based on a minor allele frequency (MAF) of > 5% in Chinese populations of the 1000 Genomes Project data (http://www.internationalgenome.org/), a pairwise linkage disequilibrium (LD) $r^2 \geq 0.80$, in conformance with Hardy–Weinberg equilibrium (HWE, $p > 0.05$) and the genotyping call rate > 95%. Seven *CARMN* SNPs (rs11168100, rs12654195, rs13177623, rs17796757, rs353299, rs353300 and rs353303) were included for genotyping in the current study.[16] Agena MassARRAY platform (Agena, San Diego, CA, USA) was applied to determine the genotypes of *CARMN* polymorphisms as described previously.[17,18] The MassARRAY platform is based on MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight) mass spectrometry in a high-throughput and cost-effective manner. Primers for amplification and extension were designed by Agena on-line design software (https://agenacx.com/online-tools/), as shown in Supplementary Table 1. The steps for SNPs genotyping were based on manufacturer's protocol, as following: 1) targeted regions for the multiplex assay were amplified by PCR; 2) PCR products were treated through shrimp alkaline phosphatase (SAP) to neutralize unincorporated nucleotides; 3) single base extension reaction were then performed to extend the PCR fragments by one base into the SNP site; 4) the mass of the resultant extended fragments were measured by MALDI-TOF, resulting in a spectrum of distinct mass peaks for the multiplex reaction. The process of genotyping was in double-blinded by two laboratory personnel. For quality control, 10% random sample was repeated genotyping, and the reproducibility was 100%.

Statistical Analyses

SPSS 18.0 (SPSS, Chicago, IL, USA) and PLINK 1.07 package was used for statistical analyses. Differences between cases and controls in demographic characteristics were evaluated by χ^2 test or independent samples *t*-test where appropriate. The frequencies of allele and genotype of *CARMN* polymorphisms in cases and controls were calculated by χ^2 test. HWE was tested for controls with the χ^2 test. The association between *CARMN* genetic variants and glioma risk was estimated by the odds ratios (ORs) and 95% confidence intervals (CIs) after adjusting for age and sex using logistic regression under allele genotype, dominant, recessive and log-additive models, respectively. The pairwise linkage disequilibrium (LD) were measured by the Lewontin's coefficient D' using the Haploview v4.2 software, and haplotype association tests for glioma susceptibility were carried out using logistic regression analysis. Univariate and multivariate Cox regression analysis was applied to calculate Hazard ratios (HRs) and 95% CIs for evaluating the association of *CARMN* polymorphisms with glioma prognosis. Survival analysis of glioma patients was assessed by Kaplan–Meier survival curves and the log rank test. A two-sided *p* values of <0.05 were regarded as statistically significant.

Results

Participant Characteristics

The subjects included 592 glioma samples (40.53 ± 13.90, 326 males and 266 females) and 502 cancer-free controls (40.46 ± 18.08, 275 males and 227 females). The frequency distribution of age and sex was matched between cases and controls ($p = 0.934$ and $p = 0.924$, respectively). Other clinical details of patients with glioma such as WHO grade, surgical method, radiotherapy, chemotherapy and survival condition were presented in Table 1.

Table 1 Features of Glioma Patients and Health Controls

Features	Cases (n = 592)	Controls (n = 502)	*p*
Age (Mean ± SD, years)	40.53 ± 13.90	40.46 ± 18.08	0.934[a]
≥ 40	329	249	
< 40	263	253	
Gender			
Male	326	275	0.924[b]
Female	266	227	
WHO grade			
I–II	378		
III–IV	214		
Surgical method			
STR & NTR	185		
GTR	407		
Radiotherapy			
No	58		
Conformal radiotherapy	159		
Gamma knife	375		
Chemotherapy			
No	349		
Yes	243		
Survival condition			
Survival	41		
Lost	24		
Death	527		

Notes: [a]*p* values was calculated by independent samples *t*-test. [b]*p* values was calculated by Chi-square tests.
Abbreviations: WHO, World Health Organization; NTR, near-total resection; STR, sub-total resection; GTR, gross-total resection.

Details of CARMN Genetic Polymorphisms

Seven genetic polymorphism in *CARMN* was genotyping and the call rate was > 99.7%. Details of *CARMN* genetic polymorphisms were displayed in Supplementary Table 2. The genotype frequencies of all variants in the controls were in HWE ($p > 0.05$), which suggesting selected samples could represent the whole population. We used HaploRegv4.1 to annotate the potential function of these selected SNPs (Supplementary Table 2). The results found that six intronic SNPs were associated with the regulation of promoter and/or enhancer histones, DNAse, proteins bound, or changed motifs, suggesting they might exert biological functions in this way in patients.

Genetic Effects CARMN Variants of on Glioma Susceptibility

The allele and genotype distribution for *CARMN* variants was summarized in Table 2 and Supplementary Table 3. Logistic regression analysis adjusted for age and sex was performed to examine the role of *CARMN* variants in glioma risk. We found that *CARMN* rs13177623 was a protective factor for glioma susceptibility, and GA-AA genotype of rs13177623 had a reduced glioma risk compared with GG genotype (OR = 0.78, 95% CI: 0.61–0.99, $p = 0.043$; Table 2). There was no statistically significant association between other *CARMN* variants (rs353299, rs353303, rs12654195, rs11168100, rs17796757 and rs353300) and risk for glioma (all p values > 0.05, Supplementary Table 3) in the overall participants.

We further investigated the correlation of *CARMN* variants with glioma risk by stratifying for age, sex and pathological grade. Stratified analyses by age (Table 3) displayed that rs13177623 had a lower risk of glioma (OR = 0.67, 95% CI: 0.48–0.94, $p = 0.022$) among the subgroup at age ≥ 40 years. *CARMN* rs11168100 and rs12654195 were associated with decreased the risk of glioma (OR = 0.47, 95% CI: 0.26–0.85, $p = 0.012$ and OR = 0.55, 95% CI: 0.31–0.96, $p = 0.034$, respectively), while rs17796757 increased the risk (OR = 1.50, 95% CI: 1.02–2.19, $p = 0.038$) among the subjects at age < 40 years. In stratified analyses by sex, rs13177623 was significantly associated with decreased risk in males under the allele (OR = 0.77, 95% CI: 0.60–0.99, $p = 0.045$) and dominant (OR = 0.72, 95% CI: 0.52–0.99, $p = 0.043$) models. However, no significant association was observed in females (all $p > 0.05$). These results suggested that *CARMN* rs13177623 polymorphism might be male specific for glioma risk. When stratified by the WHO grade, patients with III-IV glioma had a significantly lower frequency of rs13177623 GA genotype compared with patients with I-II glioma (OR = 0.66, 95% CI: 0.46–0.95, $p = 0.027$, Supplementary Table 4).

We also examined the impacts of the haplotypes on glioma susceptibility. Linkage disequilibrium (LD) is a nonrandom allele association, and generated by mutation and recombination. LD is measured by the LD coefficient D': D' = 1 is defined as complete linkage disequilibrium; D '= 0 is called linkage equilibrium; and D '< 1 indicated that gene recombination had occurred. If there is a linkage disequilibrium between SNPs, these SNPs can form a linkage disequilibrium block. As shown in Figure 1, three LD blocks (rs13177623–rs12654195, rs11168100–rs353303 and rs353300–rs353299) were constructed from the seven variants in *CARMN* by coefficient D' 0.97. In addition, we

Table 2 Correlation Between *CARMN* Variants and the Susceptibility to Glioma

SNP ID	Model	Genotype	Control	Case	OR (95% CI)	*p*
rs13177623	Allele	G	718	889	1	
		A	286	295	0.83 (0.69–1.01)	0.059
	Genotype	GG	256	338	1	
		GA	206	213	0.78 (0.61–1.01)	0.055
		AA	40	41	0.78 (0.49–1.24)	0.286
	Dominant	GG	256	338	1	
		GA-AA	246	254	**0.78 (0.61–0.99)**	**0.043**
	Recessive	GG-GA	462	551	1	
		AA	40	41	0.86 (0.55–1.35)	0.512
	Additive	GG+GA+AA	—	—	0.84 (0.69–1.01)	0.062

Notes: *p* values were calculated by logistic regression analysis with adjustments for age and gender. Bold $p < 0.05$ means the data is statistically significant.
Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Table 3 Correlation of *CARMN* Variants with Glioma Risk Stratified by Age and Gender

SNP ID	Model	OR (95% CI)	*p*	OR (95% CI)	*p*
Age (year)		≥ 40		< 40	
rs11168100	Allele	1.01 (0.79–1.30)	0.946	0.86 (0.66–1.12)	0.254
	Homozygote	1.32 (0.72–2.44)	0.372	0.54 (0.29–1.02)	0.057
	Heterozygote	0.85 (0.60–1.20)	0.344	1.38 (0.94–2.02)	0.101
	Dominant	0.91 (0.65–1.27)	0.583	1.15 (0.80–1.64)	0.462
	Recessive	1.44 (0.80–2.58)	0.226	**0.47 (0.26–0.85)**	**0.012**
	Additive	1.02 (0.79–1.31)	0.901	0.92 (0.70–1.20)	0.517
rs12654195	Allele	1.10 (0.86–1.41)	0.467	0.89 (0.69–1.15)	0.377
	Homozygote	1.52 (0.83–2.80)	0.173	0.66 (0.36–1.19)	0.164
	Heterozygote	0.94 (0.66–1.33)	0.710	1.46 (0.99–2.15)	0.057
	Dominant	1.02 (0.73–1.42)	0.923	1.22 (0.85–1.75)	0.284
	Recessive	1.58 (0.88–2.81)	0.123	**0.55 (0.31–0.96)**	**0.034**
	Additive	1.11 (0.86–1.43)	0.432	0.97 (0.74–1.26)	0.799
rs13177623	Allele	0.89 (0.69–1.16)	0.406	0.77 (0.58–1.02)	0.064
	Homozygote	1.59 (0.73–3.45)	0.241	0.56 (0.29–1.08)	0.084
	Heterozygote	**0.67 (0.48–0.94)**	**0.022**	1.05 (0.71–1.55)	0.810
	Dominant	0.74 (0.53–1.03)	0.076	0.92 (0.64–1.33)	0.663
	Recessive	1.90 (0.89–4.06)	0.098	0.55 (0.29–1.04)	0.067
	Additive	0.89 (0.68–1.17)	0.419	0.85 (0.65–1.12)	0.252
rs17796757	Allele	0.97 (0.75–1.24)	0.784	1.18 (0.91–1.54)	0.202
	Homozygote	1.24 (0.68–2.27)	0.486	1.09 (0.59–2.02)	0.778
	Heterozygote	0.79 (0.56–1.12)	0.186	**1.50 (1.02–2.19)**	**0.038**
	Dominant	0.86 (0.61–1.19)	0.358	1.41 (0.98–2.02)	0.063
	Recessive	1.39 (0.78–2.48)	0.266	0.89 (0.50–1.61)	0.709
	Additive	0.97 (0.76–1.26)	0.842	1.19 (0.90–1.56)	0.218
Gender		**Male**		**Female**	
rs13177623	Allele	**0.77 (0.60–0.99)**	**0.045**	0.92 (0.69–1.22)	0.554
	Homozygote	0.66 (0.36–1.22)	0.188	0.96 (0.47–1.99)	0.920
	Heterozygote	0.73 (0.52–1.02)	0.067	0.85 (0.59–1.24)	0.405
	Dominant	**0.72 (0.52–0.99)**	**0.043**	0.87 (0.61–1.24)	0.440
	Recessive	0.76 (0.42–1.37)	0.360	1.03 (0.51–2.09)	0.940
	Additive	0.78 (0.60–1.00)	**0.049**	0.92 (0.69–1.22)	0.555

Notes: *p* values were calculated by logistic regression analysis with adjustments for age and gender. Bold $p < 0.05$ means the data is statistically significant.
Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

found that $G_{rs13177623}G_{rs12654195}$ haplotype was related to the decreased risk of glioma (OR = 0.61, 95% CI: 0.43–0.86, $p = 0.005$, Table 4).

Genetic Effects CARMN Variants of on Glioma Prognosis

During follow-up, there were 527 patients died of glioma, 41 patients survived and 24 patients lost. We next explored the contribution of *CARMN* variants to the overall survival (OS) and progression free survival (PFS) of glioma patients. The Kaplan–Meier survival curves indicated that the genotype of rs12654195 variant might be associated with OS (Log-rank $p = 0.026$) and PFS (Log-rank $p = 0.027$) of glioma patients, as shown in Figure 2. In addition, rs17796757 polymorphism had the effect on OS (Log-rank $p = 0.039$) of patients with grade III–IV grade III–IV glioma, while rs12654195 variant on OS (Log-rank $p = 0.008$) and PFS (Log-rank $p = 0.011$) of patients with grade I-II glioma (Supplementary Figure 1).

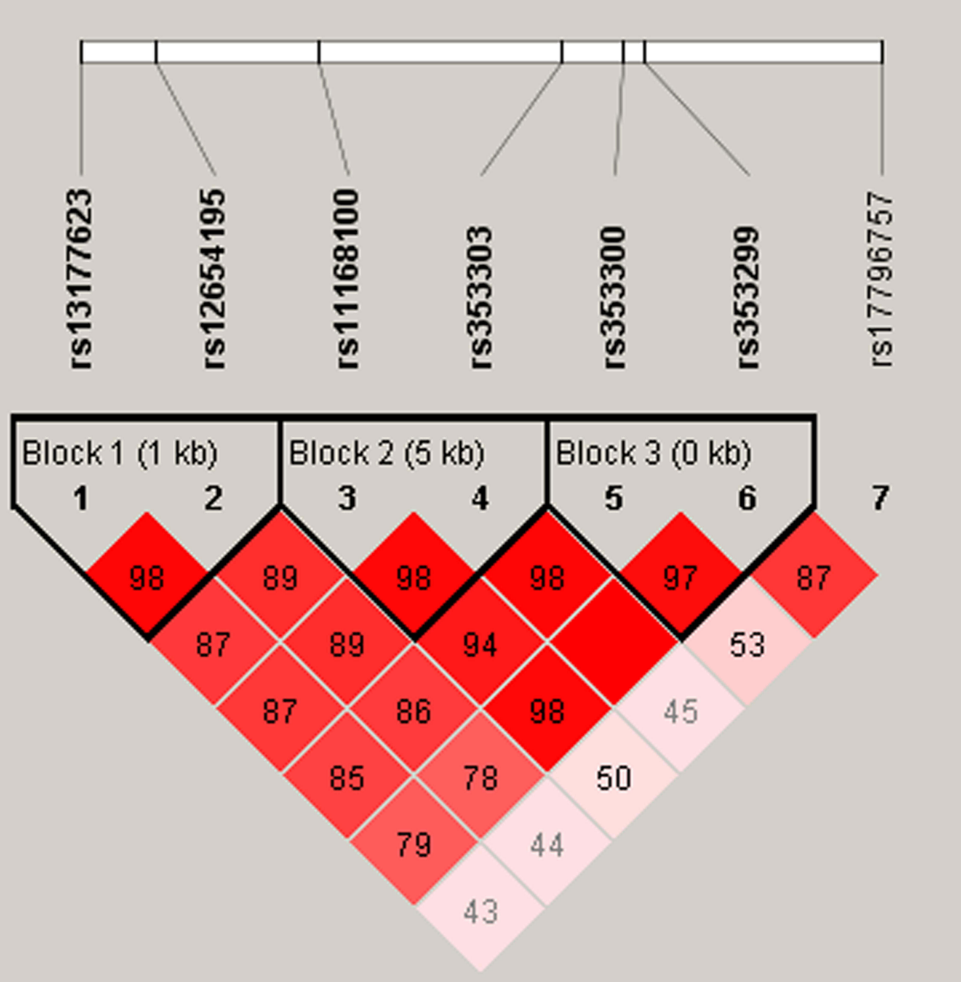

Figure 1 The linkage disequilibrium structure of seven SNPs in the *CARMN* gene. Three LD blocks (rs13177623–rs12654195, rs11168100–rs353303 and rs353300–rs353299) were constructed from the seven variants in *CARMN* by coefficient D' 0.97. The numbers in squares are D′ values.

The results of univariate Cox proportional hazard model revealed that GG genotype of rs12654195 had a better OS (HR = 0.71, 95% CI: 0.52–0.96, $p = 0.025$) and PFS (HR = 0.69, 95% CI: 0.51–0.95, $p = 0.021$) of glioma patients compared with TT genotype (Table 5). In patients with grade III–IV glioma, rs17796757 was significantly related to the

Table 4 Correlation of *CARMN* Haplotypes with Glioma Susceptibility

Blocks	SNPs	Haplotype	Frequency		Crude Analysis		Adjusted by Age and Gender	
			Case	Control	OR (95% CI)	p	OR (95% CI)	p
Block 1	rs13177623|rs12654195	AG	0.248	0.281	0.84 (0.70–1.02)	0.080	0.84 (0.70–1.02)	0.079
	rs13177623|rs12654195	GG	0.916	0.947	**0.61 (0.43–0.86)**	**0.005**	**0.61 (0.43–0.86)**	**0.005**
	rs13177623|rs12654195	GT	0.666	0.663	1.02 (0.85–1.22)	0.867	1.02 (0.85–1.22)	0.863
Block 2	rs11168100|rs353303	AG	0.408	0.413	0.98 (0.82–1.16)	0.797	0.98 (0.82–1.16)	0.796
	rs11168100|rs353303	TA	0.313	0.330	0.92 (0.77–1.11)	0.387	0.92 (0.77–1.11)	0.386
	rs11168100|rs353303	AA	0.723	0.744	0.90 (0.74–1.09)	0.272	0.90 (0.74–1.09)	0.270
Block 3	rs353300|rs353299	TT	0.854	0.861	0.95 (0.75–1.21)	0.677	0.95 (0.75–1.21)	0.678
	rs353300|rs353299	TC	0.338	0.351	0.95 (0.79–1.13)	0.538	0.94 (0.79–1.13)	0.534
	rs353300|rs353299	CC	0.515	0.508	1.03 (0.87–1.22)	0.732	1.03 (0.87–1.22)	0.727

Notes: *p* values were calculated using logistic regression analysis with and without adjustment by gender and age. Bold $p < 0.05$ indicates statistical significance.
Abbreviations: OR, odds ratio; CI, confidence interval.

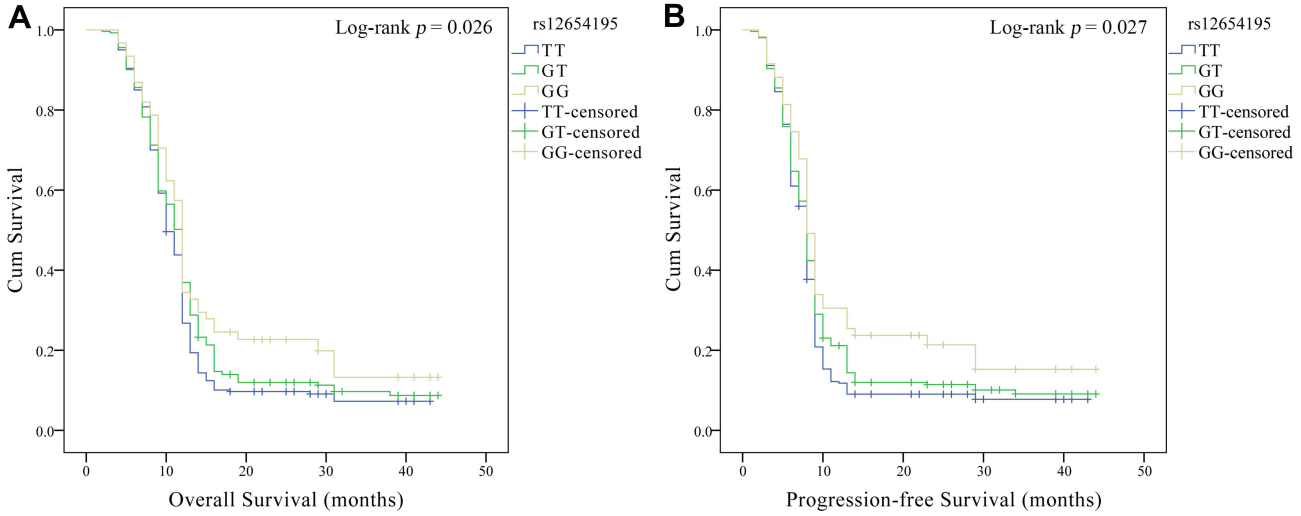

Figure 2 Kaplan–Meier survival curve for significant association of rs12654195 with OS (**A**) and PFS (**B**) of glioma patients.
Abbreviations: OS, overall survival; PFS, progression free survival.

improved OS (AT vs AA, HR = 0.71, 95% CI: 0.52–0.95, $p = 0.024$). In patients with grade I-II glioma, GT genotype and TT genotype of rs12654195 presented an increased OS (HR = 0.75, 95% CI: 0.59–0.94, $p = 0.011$, and HR = 0.66, 95% CI: 0.44–0.99, $p = 0.043$, respectively) and PFS (HR = 0.76, 95% CI: 0.61–0.96, $p = 0.020$, and HR = 0.66, 95% CI: 0.44–0.99, $p = 0.046$, respectively).

Further, the correlation of CARMN variants and PFS or OS was evaluated using a multivariate Cox proportional hazard model, adjusted for age, gender, WHO grade, surgical method, radiotherapy and chemotherapy (Table 6). We found rs13177623 GA genotype carriers had an improved OS (HR = 0.83, 95% CI: 0.69–1.00, p = 0.047) and PFS (HR = 0.82, 95% CI: 0.68–0.98, p = 0.031) for glioma. Rs12654195 (GG vs TT, OS: HR = 0.64, 95% CI: 0.47–0.87, p = 0.005 and PFS: HR = 0.65, 95% CI: 0.48–0.89, p = 0.007) and rs11168100 (TT vs AA, OS: HR = 0.71, 95% CI: 0.51–0.98, p = 0.035) homozygous carriers were also associated with a better prognosis for glioma. For the subgroup of patients with grade III–IV glioma, rs17796757 polymorphism presented an increased OS (AT vs AA, HR = 0.70, 95% CI: 0.51–0.95, p = 0.025). For the subgroup of patients with grade I–II glioma, the heterozygous of rs13177623 and rs11168100 were significantly associated with improved OS (HR = 0.78, 95% CI: 0.62–0.98, p = 0.030 and HR = 0.73, 95% CI: 0.58–0.92, p = 0.008, respectively) and PFS (HR = 0.79, 95% CI: 0.63–0.99, p = 0.044 and HR = 0.76, 95% CI: 0.60–0.96, p = 0.020, respectively). In addition, improved OS and PFS for grade I–II glioma was also seen for the homozygote (OS: HR = 0.62, 95% CI: 0.42–0.94, p = 0.024, and PFS: HR = 0.62, 95% CI: 0.41–0.94, p = 0.024) and heterozygous (OS: HR = 0.70, 95% CI: 0.56–0.88, p = 0.002, and PFS: HR = 0.72, 95% CI: 0.57–0.91, p = 0.006) of rs12654195 variant.

Discussion

The present study explored the possible correlation of seven polymorphisms in *CARMN* with the risk and prognosis of glioma among a Han Chinese population. Our results revealed that rs11168100, rs12654195, rs13177623, and rs17796757 variants were associated with the susceptibility to glioma and the OS and PFS of patients. In addition, we also found that $G_{rs13177623}G_{rs12654195}$ haplotype was a protective factor for glioma susceptibility. To the best of our knowledge, this is the first to assess the role of *CARMN* polymorphisms in glioma risk and prognosis.

CARMN gene, located on chromosome 5q32, is affiliated with the non-coding RNA class.[19] The expression of CARMN was significantly dysregulated in various cancers and involved in carcinogenesis. For example, Lin et al reported that CARMN inhibited tumor proliferation and metastasis by suppressing MAPK and Wnt signaling pathways in hepatocellular carcinoma.[20] CARMN suppressed miR-21 through methylation to inhibit cell invasion and migration.[21] CARMN have reported expressing stably homologous miRNAs: miR-143 and miR-145.[22] Previous studies have demonstrated miR-143/145 regulate the proliferation, migration and invasion of glioma cells and could be potential

Table 5 Univariate Analysis of the Association Between *CARMN* Variants and OS and PFS of Glioma Patients

SNP ID	Genotype	OS					PFS				
		Total	Events	SR (1-/3-Year)	HR (95% CI)	*p*	Total	Events	SR (1-/3-Year)	HR (95% CI)	*p*
rs12654195	TT	260	237	0.267/0.073	1		259	236	0.118/0.077	1	
	GT	271	240	0.369/0.097	0.86 (0.72–1.03)	0.097	269	239	0.212/0.091	0.89 (0.74–1.06)	0.184
	GG	61	50	0.344/0.132	**0.71 (0.52–0.96)**	**0.025**	59	48	0.305/0.153	**0.69 (0.51–0.95)**	**0.021**
III–IV grade											
rs17796757	AA	98	93	0.245/0.041	1		97	92	0. 124/0.049	1	
	AT	90	80	0.356/0.097	**0.71 (0.52–0.95)**	**0.024**	88	79	0.170/0.097	0.81 (0.60–1.09)	0.161
	TT	26	24	0.385/0.051	0.75 (0.48–1.17)	0.208	26	24	0.247/0.041	0.82 (0.52–1.28)	0.383
I–II grade											
rs12654195	TT	158	144	0.224/0.072	1		157	143	0.104/0.076	1	
	GT	184	158	0.418/0.123	**0.75 (0.59–0.94)**	**0.011**	184	158	0.244/0.114	**0.76 (0.61–0.96)**	**0.020**
	GG	36	28	0.333/0.187	**0.66 (0.44–0.99)**	**0.043**	35	27	0.314/-	**0.66 (0.44–0.99)**	**0.046**

Notes: Log-rank *p* values were calculated using the Chi-Square test. Bold $p < 0.05$ indicates statistical significance.
Abbreviations: OS, overall survival; PFS, progression free survival; SR, survival rate; HR, hazard ratio; CI, confidence interval.

Table 6 Multivariate Analysis of the Association Between *CARMN* Variants and OS and PFS of Glioma Patients

SNP ID	Genotype	OS		PFS	
		HR (95% CI)	*p*	HR (95% CI)	*p*
rs13177623	GG	1		1	
	GA	0.83 (0.69–1.00)	**0.047**	**0.82 (0.68–0.98)**	**0.031**
	AA	0.72 (0.51–1.03)	0.070	0.75 (0.53–1.05)	0.096
rs12654195	TT	1		1	
	GT	0.87 (0.72–1.04)	0.129	0.84 (0.70–1.01)	0.059
	GG	**0.64 (0.47–0.87)**	**0.005**	**0.65 (0.48–0.89)**	**0.007**
rs11168100	AA	1		1	
	AT	0.88 (0.73–1.06)	0.167	0.84 (0.70–1.01)	0.067
	TT	**0.71 (0.51–0.98)**	**0.035**	0.74 (0.54–1.01)	0.060
III–IV grade					
rs17796757	AA	1		1	
	AT	**0.70 (0.51–0.95)**	**0.025**	0.75 (0.55–1.03)	0.079
	TT	0.70 (0.44–1.10)	0.123	0.75 (0.47–1.18)	0.213
I–II grade					
rs13177623	GG	1		1	
	GA	**0.78 (0.62–0.98)**	**0.030**	**0.79 (0.63–0.99)**	**0.044**
	AA	0.88 (0.56–1.38)	0.579	0.85 (0.54–1.33)	0.469
rs12654195	TT	1		1	
	GT	**0.70 (0.56–0.88)**	**0.002**	**0.72 (0.57–0.91)**	**0.006**
	GG	**0.62 (0.42–0.94)**	**0.024**	**0.62 (0.41–0.94)**	**0.024**
rs11168100	AA	1		1	
	AT	**0.73 (0.58–0.92)**	**0.008**	**0.76 (0.60–0.96)**	**0.020**
	TT	0.82 (0.53–1.27)	0.375	0.77 (0.50–1.21)	0.264

Notes: *p* values were calculated by Cox multivariate analysis with adjustments for gender, age, WHO grade, surgical method, use of radiotherapy and chemotherapy. Bold $p < 0.05$ indicates statistical significance.

Abbreviations: OS, overall survival; PFS, progression free survival; HR, hazard ratio; CI, confidence interval.

therapeutic target for anti-invasion therapies of glioma patients.[11,23] Recently, LncRNA CARMN inhibited the proliferation of glioblastoma cells by sponging miR-504.[24] These suggested that *CARMN* could be of pathogenic importance in glioma.

Our study was the first to evaluate the correlation of *CARMN* variants with susceptibility and prognosis of glioma. We found *CARMN* rs13177623 was related to the decreased risk of glioma. Previous studies have indicated that the incidence rates of glioma tended to be associated with age and gender.[25] Age stratified analysis showed rs13177623 had a lower risk of glioma at age ≥ 40 years, while rs11168100, rs12654195 and rs17796757 were associated with the susceptibility to glioma at age < 40 years. These indicate that the contribution of *CARMN* polymorphisms to glioma risk was associated with age exposures. In stratified analyses by gender, rs13177623 was significantly associated with decreased risk in males, but not in females, which suggesting the effect of rs13177623 polymorphism on glioma risk presented sex difference. Moreover, our study also evaluated the effect of *CARMN* polymorphisms on the prognosis of glioma patients. We found that rs13177623, rs12654195 and rs11168100 were associated with a better prognosis for glioma, especially in grade I–II glioma. In patients with grade III–IV glioma, rs17796757 polymorphism presented an improved OS. Previous studies supported that SNPs differentially might influence the expression and function of lncRNAs.[26–28] Therefore, *CARMN* variants might contribute to the risk and prognosis of glioma by affecting the function of *CARMN*. However, further functional study is necessary to explore the role of these polymorphisms in the etiology of glioma.

Inevitably, our study had several limitations. Firstly, the inherent selection bias cannot be exclude because this study based on a hospital-based case–control study. Therefore, we recruited subjects matched by age, gender, and residential

area to reduce the bias. Secondly, we did not assess the potential function of these polymorphisms in *CARMN*. Further functional experiments should be required to investigate the role of *CARMN* variants in glioma occurrence and development. Thirdly, some environmental factors such as occupational exposure and dietary were not available; the interaction of these factors with *CARMN* genotypes should be performed in a larger survey.

Conclusion

In summary, we firstly reported the contribution of *CARMN* variants (rs11168100, rs12654195, rs13177623, and rs17796757) to the susceptibility and prognosis of glioma in a Chinese Han population. Our study provides a novel insight on the relationship between *CARMN* gene and glioma tumorigenesis. These findings add to the growing body of evidence linking lncRNAs polymorphisms to glioma etiology. In addition, further studies are required to validate our results.

Data Sharing Statement

All the data regarding the findings are available within the manuscript. Anyone who is interested in the information should contact the corresponding author.

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Disclosure

The authors declared no conflicts of interest in this work.

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