Effect of miR-34b/c rs4938723 T > C on pediatric glioma susceptibility

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Abstract
Gliomas, the most common type of primary intracranial tumor, arise from the brain's glial cells and constitute 30% of all brain and central nervous system tumors. MiR-34b/c plays an important role in colorectal cancer and other cancers, but its role in glioma is unknown. In this study, we attempted to assess the effect of rs4938723 T > C in miR-34b/c on the susceptibility of glioma Chinese children. Odds ratios and 95% confidence intervals (CIs) were calculated to evaluate the association between the selected polymorphism and glioma susceptibility. Our results showed that there is no significant association between this polymorphism and the risk of glioma in Chinese children. However, stratified analysis showed that the rs4938723 TC/CC genotype significantly reduced the risk of glioma in participants under 60 months of age (adjusted OR = 0.56, 95% CI = 0.35–0.88, p = .013). Overall, our study indicates that miR-34b/c rs4938723 T > C polymorphism may have a weak influence on glioma susceptibility. Nevertheless, these findings need to be validated in well-designed studies with larger sample sizes and different populations.

Keywords
glioma, miR-34b/c, polymorphism, rs4938723, susceptibility

Abbreviations: CI, confidence interval; HWE, Hardy–Weinberg equilibrium; miRNA, microRNA; OR, odds ratio; SNP, Single nucleotide polymorphism.

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Glioma is the most common malignant brain cancer, and there are about 10,000 new cases of high-grade or malignant glioma each year.1 Gliomas of astrocytic, oligodendroglial, and ependymal origins account for more than 40% of primary brain tumors in China as well as in the West.2 Astrocytome was the most common glioma, followed by glioblastoma multiforme. Gliomas are more common in males, especially in glioblastoma multiforme and medulloblastoma. Most of the cases are between 20 and 50 years old, and the peak is between 30 and 40 years old. In addition, it is also common in children around 10 years old, which is another peak. In recent years, the incidence of glioma has increased dramatically worldwide.3 Surgery remains the dominant treatment, although other therapies, including targeted therapies, stem cell rescue, and post-operative AIDS such as radiation and chemotherapy and/or immunotherapy, are also used to keep patients alive.3-5 Today, little is known about the etiology of glioma. Apart from a few rare genetic syndromes6 and high-dose ionizing radiation (IR),7 there are no other identified causes or environmental risk factors of glioma.

Despite recent breakthroughs in treatment, the poor prognosis of gliomas has remained unchanged over the past three decades.8 To our knowledge, the etiology of gliomas has not been clarified. To develop effective treatments for glioma, it is critical to identify molecular markers that are closely related to the occurrence, progression, and metastasis of glioma. Genetic factors may play an important role in the occurrence of diseases. Regarding genetic factors, deletion of the gene encoding the tumor protein p53 (TP53) has been shown to be associated with poor prognosis in glioblastoma.9 P53 protein plays an essential role in basic cell functions such as cell cycle control, apoptosis, aging, DNA repair, and metabolism.10 P53 regulates the expression of miRNAs, such as the miR-34 family. Generally, members of the miR-34 family (miR-34a, miR-34b, and miR-34c) are considered as tumor suppressors. miR-34c has been shown to effectively reduce cell proliferation, cell cycle changes, cell apoptosis, and cell invasion of glioma cells.5 One study showed that METTL3 can activate Notch pathway and facilitate glioma occurrence through regulating its direct targets NOTCH3, DLL3, and HES1, and Notch pathway genes may serve as the potential treatment targets for glioma.11

MicroRNA (miRNA) is a class of small non-protein-encoded single-stranded RNA with about 22 nucleotides.12 miRNA participates in post-transcriptional regulation through various mechanisms, including promoting mRNA degradation, inhibiting translation, or leading to mRNA cleavage.13-14 In the past decade, numerous miRNAs have been identified. miRNAs are thought to be involved in many biological processes that play a key role in carcinogenesis, including cell differentiation, proliferation, and apoptosis. Mutations in the physiological processes of miRNA may lead to the occurrence and development of tumors. A potentially functional polymorphism rs4938723 T > C was found in the promoter region of pri-miR-34b/c, which was included in our previous study.15-18

In our previous study conducted recently, we firstly found that rs4938723 T > C polymorphism was associated with a significantly decreased neuroblastoma risk [37]. Here, we conducted a hospital-based case-control study aiming to verify the association between miR-34b/c rs4938723 T > C and glioma risk in Chinese children.

2 MATERIALS AND METHODS

2.1 Study population

In the current case-control study, 314 patients with glioma and 380 cancer-free controls were included, and the demographic characteristics of all participants are shown in Table S1. All participants or their guardians provide written informed consent prior to the study. The detailed information of selection criteria of study subjects was reported in our previous paper.19

2.2 SNP selection and genotyping

The rs4938723 T > C, located in the promoter region of pri-miR-34b/c, was selected for the study because of its well-known role in altering cancer susceptibility. Genotyping of SNP was performed with a TaqMan SNP Genotyping Assay (Applied Biosystems, USA), with details in a previous study.20 We first used a TIANamp Blood DNA Kit (TianGen Biotech Co. Ltd., Beijing, China) to extract genomic DNA from participants' blood. Then the genomic DNA was added into a 384-well plate. The reactions were set under the following conditions: denaturation at 95°C for 10 min, followed by 40 cycles each of denaturation at 95°C for 15 s, annealing and extension at 60°C for 60 s. Negative controls (with water) and duplicate test samples (10% of all the samples) were included in each 384-well plate. The 100% concordant of genotypes in replicates were achieved.

2.3 Statistical analysis

The goodness-of-fit χ² test was used to detect whether the selected SNP in the control group deviated from Hardy-Weinberg equilibrium (HWE). The demographic distribution and allele frequency of all cases were compared with the control group by the two-sided chi-square test. The relationship between miR-34b/c polymorphism and glioma susceptibility was evaluated by logistic regression analysis using odds ratios (ORs) and 95% confidence intervals (CIs). In addition, adjusted OR and the corresponding 95% CIs (adjusted for age and gender) were calculated by unconditional multifactor logistic regression analysis. Finally, stratified analysis was performed according to age, gender, tumor origin site, and clinical stage. All statistical analyses were processed in SAS software (version 9.4, SAS Institute, NC, USA). When the p value <.05, the results were considered statistically significant.
RESULTS

3.1 Associations between miR34b/c polymorphisms and glioma risk among Chinese children

The study included 314 glioma patients and 380 controls, as shown in Table 1. The genotype distribution of miR-34b/c rs4938723 T > C was compared between all cases and controls. The genotype frequency of selected SNP was consistent with that of HWE in control subjects (HWE = 0.763). There was no difference in age (p = .461) or gender (p = .379). Statistical analysis showed that no significant association was found between rs4938723 C variant allele and glioma risk in Chinese children (TC vs. TT: adjusted OR = 0.78, 95% CI = 0.57–1.08; additive model: adjusted OR = 0.85, 95% CI = 0.67–1.07; TC/CC vs. TT: adjusted OR = 0.78, 95% CI = 0.58–1.07).

### TABLE 1
miR-34b/c rs4938723 T > C polymorphism and glioma risk in Chinese children

| Genotype | Cases (N = 294) | Controls (N = 379) | \( p^a \) | Crude OR (95% CI) | \( p \) | Adjusted OR (95% CI)\(^a \) | \( p^b \) |
|----------|----------------|-------------------|---------|------------------|-------|---------------------------|-------|
| rs4938723 (HWE = 0.763) | | | | | | | |
| TT | 158 (53.74) | 181 (47.76) | 1.00 | 0.78 (0.57–1.08) | .133 | 0.78 (0.57–1.08) | .138 |
| TC | 109 (37.07) | 160 (42.22) | 0.81 (0.48–1.39) | .453 | 0.79 (0.46–1.36) | .402 |
| CC | 27 (9.18) | 38 (10.03) | | | | | |
| Additive | | | \(.183 \) | 0.85 (0.68–1.08) | \(.183 \) | 0.85 (0.67–1.07) | \(.166 \) |
| Dominant | | | | | | | |
| TT/TC | 267 (90.82) | 341 (89.97) | 1.00 | 0.81 (0.54–1.53) | .714 | 0.80 (0.52–1.49) | .640 |
| CC | 27 (9.18) | 38 (10.03) | | | | | |

Abbreviations: CI, confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odds ratio.
\(^a\)Adjusted for age and gender.
\(^b\)\(\chi^2\) test for genotype distributions between glioma patients and cancer-free controls.

### TABLE 2
Stratification analysis between miR34b/c rs4938723 T > C polymorphism and glioma risk

| Variables | Cases/Controls | Crude OR (95% CI) | \( p \) | Adjusted OR\(^a \) (95% CI) | \( p^b \) |
|-----------|---------------|------------------|-------|-----------------------------|-------|
| Age, month | | | | | |
| <60 | 73/74 | 55/100 | 0.56 (0.35–0.88) | .013 | 0.56 (0.35–0.88) | .013 |
| \(\geq60 \) | 85/107 | 81/98 | 1.04 (0.69–1.57) | .850 | 1.02 (0.68–1.55) | .909 |
| Sex | | | | | |
| Females | 68/78 | 70/85 | 0.95 (0.60–1.49) | .806 | 0.94 (0.60–1.48) | .788 |
| Males | 90/103 | 66/113 | 0.67 (0.44–1.01) | .057 | 0.67 (0.44–1.02) | .061 |
| Subtype | | | | | |
| Astrocytic tumors | 109/181 | 89/198 | 0.75 (0.53–1.05) | .097 | 0.75 (0.53–1.06) | .105 |
| Ependymoma | 32/181 | 26/198 | 0.74 (0.43–1.29) | .294 | 0.65 (0.37–1.16) | .144 |
| Neuronal and mixed | 10/181 | 14/198 | 1.28 (0.56–2.95) | .563 | 1.31 (0.57–3.04) | .524 |
| Embryonal tumors | 6/181 | 6/198 | 0.91 (0.29–2.89) | .878 | 1.11 (0.34–3.61) | .863 |
| Clinical stages | | | | | |
| I | 78/181 | 67/198 | 0.79 (0.54–1.15) | .217 | 0.80 (0.55–1.18) | .270 |
| II | 38/181 | 31/198 | 0.75 (0.45–1.25) | .265 | 0.74 (0.44–1.25) | .261 |
| III | 18/181 | 16/198 | 0.81 (0.40–1.64) | .563 | 0.72 (0.35–1.47) | .362 |
| IV | 24/181 | 21/198 | 0.80 (0.43–1.49) | .480 | 0.88 (0.47–1.66) | .694 |
| I + II | 116/181 | 98/198 | 0.77 (0.55–1.08) | .132 | 0.77 (0.55–1.08) | .136 |
| III + IV | 42/181 | 37/198 | 0.81 (0.50–1.31) | .382 | 0.81 (0.50–1.32) | .395 |

Abbreviations: CI, confidence interval; OR, odds ratio.
\(^a\)Adjusted for age and sex, omitting the corresponding stratify factor.
3.2 | Stratification analysis

We further demonstrate whether the association between rs4938723 T > C genotype and glioma risk varies by age, gender, tumor site, and clinical stage (Table 2). We observed a significantly reduced risk of glioma in the subgroup ≤60 months of age (adjusted OR = 0.56, 95% CI = 0.35–0.88, p = .013) for carriers of the rs4938723 TC/CC genotype compared with carriers of the TT genotype. No significant correlation was found between glioma risk and gender, tumor site, tumor stage, and other factors.

4 | DISCUSSION

miR-34b and miR-34c are members of the miR-34 family, and they share a common primary transcript (pri-miR-34b/c).21 miR-34b/c is located on human chromosome 11.22,23 The biological role of miR-34b/c has been well documented in several types of cancer. For example, Majid et al. found that miR-34b effectively reduces the incidence of prostate cancer through demethylation, active chromatin modification, and the AKT pathway.24 miR-34b/c has been documented to target TP53 and synergistically inhibit cell proliferation and adherent-independent growth.25 The findings of Wong et al.26 provide new insights into the tumor-suppressive effects of miR-34b/c in myeloma. However, studies on the role of miR-34b/c in glioma are still lacking. The rs4938723 T > C polymorphism located in the CpG island of pri-miR-34b/c was studied in detail. In a study conducted by Liu et al.27 in the Chinese population, they found that the CC and TC + CC genotypes of pri-miR-34b/c rs4938723 resulted in a higher susceptibility to hepatocellular carcinoma, respectively, compared with the TT genotype. Hashemi et al. reported that miR-34b/c rs4938723 C allele was associated with reduced risk of acute lymphoblastic leukemia in an Iranian population of 110 children with acute lymphoblastic leukemia and 120 healthy children.28 Polymorphisms can have different genetic effects on susceptibility to cancer, depending on cancer type, ethnicity, and region.27–29

In a study involving 393 cases and 812 controls, miR-34b/c rs4938723 T > C was shown to be protective against neuroblastoma.30 In 2019, Zhuo et al.31 further validated protective effect of miR-34b/c rs4938723 T > C on the risk of neuroblastoma in another Chinese sample (children from Hunan, China) This protective effect also appears in other cancer types, such as colorectal cancer,8 gastric cancer,32 and esophageal cancer.33 The protective effect of miR-34b/c rs4938723 T > C may be determined by genetic, environmental factors, and gene–environment interaction.34,35 In children younger than 60 months, glioma susceptibility is reduced, which is conducive to the prevention and treatment of glioma in children. The low-sample size may be the reason why other observations were not positive.

As a preliminary study, this work has several limitations. First, we did not obtain any positive results in the logistic regression analysis comparing cases and controls, which may be due to insufficient sample size. Second, since all the subjects were of Chinese descent, this conclusion is not universal. Further research is needed to extend it to foreign countries. In addition, here we only genotyped one SNP in miR-34b/c. More potentially functional polymorphisms in miR-34b/c await to be explored. The role of other important SNPs in miR-34b/c may be ignored, resulting in inaccurate results. Last, environmental factors and gene–environment interactions could not be assessed in the current study, with the absence of environmental data. The relationship between miR-34b/c and glioma from the mRNA level is warranted to be determined.

However, due to the above limitations, it is necessary to conduct multi-ethnic population studies and functional analysis of rs4938723 polymorphism. Identification of glioma susceptibility genes will provide a better understanding of the underlying mechanism of glioma and lay the foundation for the development of new genetic markers and monitoring procedures.

5 | CONCLUSIONS

In conclusion, we provide the possibility of miR-34b/c rs4938723 T > C in predicting glioma risk in Chinese children and the possibility of age factors in the population for glioma risk. Our study lays the foundation for future functional studies of miR-34b/c rs4938723 T > C in glioma risk in independent populations.

AUTHOR CONTRIBUTIONS

Xingyu Jia, Wenchao Chen, Wei Chen, Yuxiang Liao, Jingying Zhou, conceived and designed the study; Li Yuan, Jingying Zhou analyzed the data; Xingyu Jia drafted the manuscript; Huiran Lin, Jun Bian coordinated the study over the entire time. All authors reviewed the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

This study was approved by the Institutional Review Committee of Guangzhou Women and Children Medical Center.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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