

## Original Article

# Association between *RTEL1* polymorphisms and glioma susceptibility

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**Abstract:** Objective: The present study aimed to investigate the association between regulator of telomere elongation helicase1 (*RTEL1*) gene polymorphisms and the susceptibility to glioma. Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to detect the genotypes of *RTEL1* rs6010620, rs2297440, rs4809324 polymorphisms among 110 glioma patients and 134 healthy controls. Odds ratio (OR) with corresponding 95% confidence interval (CI) calculated by  $\chi^2$  test to represent the relevance strength of *RTEL1* polymorphisms and glioma. Hardy-Weinberg equilibrium (HWE) was checked in the control group by  $\chi^2$  test. The linkage disequilibrium and haplotype were analyzed by haploview software. Results: The genotype frequencies of *RTEL1* polymorphisms in control group were consistent with (HWE) ( $P>0.05$ ). GG genotype in rs6010620 polymorphism was associated with the increased risk of glioma (OR=2.706, 95% CI=1.019-7.187). Genotype CC of rs2297440 also significantly increased the susceptibility to glioma (OR=2.889, 95% CI=1.032-8.089). In haplotype analysis based on rs6010620, rs2297440, rs4809324, we found that haplotype G-C-T might be a risk factor for the development of glioma (OR=1.714, 95% CI=1.049-2.800). Conclusion: *RTEL1* rs6010620 and rs2297440 polymorphisms might be the risk factors for glioma, but not rs4809324. The three polymorphisms presented the interaction and affected the occurrence of glioma together.

**Keywords:** *RTEL1*, polymorphisms, glioma

## Introduction

Glioma is a type of tumor disease in the central nervous system that derived from the lesion of glial cells [1]. Glioma generally occurs in brain (known as cerebral glioma), but also in spine and nerves of other parts like optic nerve [2]. It accounts for about 30 percentage of all brain and center nerves system tumors, what's more, the percentage is 80% in malignant tumor of brain [3]. Without complete capsule, glioma intrusively grows in brain by an interlocking pattern with normal tissues [4]. Glioma tends to relapse because of its growth characteristics. The influential factors of glioma have been found at present include genetic factors, environmental factors and infection [5-8]. As we all known, genetic factors play an important role in the incidence of glioma. Reports related to glioma emerge in endlessly and mainly focus on gene polymorphism, such as *TGFB1*, *TERT*, *RECC* [9-11].

Regulator of telomere elongation helicase1 (*RTEL1*) is encoded by *RTEL1* gene located in chromosome 20q13.33. Recently, scientists from Cancer Research UK have proved that *RTEL1* related to DNA repairing may be a key factor in the prevention of tumors [12]. *RTEL1* not only takes charge of maintaining the ends of chromosomes (the structure containing the genetic material DNA), but also plays an important role in entire genome [13]. *RTEL1* works with proliferating cell nuclear antigen (PCNA) in the following processes: when PCNA forms a ring around DNA, *RTEL1* helps PCNA to remove nodes and in DNA duplication, *RTEL1* helps to unwind DNA chain. All these courses are essential for accurate DNA replication in cell growth and division. If *RTEL1* cannot combine with PCNA due to its malfunction, DNA replication may go wrong, which leads to the development of cancer [14]. So far *RTEL1* was studied only in several diseases, such as familial interstitial pneumonia, gastric cancer, astrocytoma [15-

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**Table 1.** Primer sequences of *RTEL1* polymorphisms

Polymorphisms	Primer sequences	Length
Rs6010620	Forward 5'-ACAGCGGTGTCACCAAGC-3'	164 bp
	Reverse 5'-GCTCCTCCCAGGTCTCAA-3'	
Rs2297440	Forward 5'-CCCTACACCACCTGTTTCTGA-3'	150 bp
	Reverse 5'-CCACTGTCTTTGCGTCT-3'	
Rs4809324	Forward 5'-TCAGAAGCAGCCACTCCC-3'	166 bp
	Reverse 5'-AACACGGCACAAGCAGAAC-3'	

17]. But the study of the association between *RTEL1* and glioma is relatively small.

In present study, we selected three polymorphisms rs6010620, rs2297440, rs4809324 in *RTEL1* to assess the effect on the risk of glioma. PCR-RFLP method was used to genotyping in three polymorphisms based on 110 patients with glioma and 134 healthy controls.

### Materials and methods

#### Subjects

110 glioma patients diagnosed by pathology in the case group were all Chinese Han people hospitalized in Renmin Hospital of Wuhan University, including 64 males and 46 females with an average age of  $44.8 \pm 14.2$ . According to the pathological grading criteria established by World Health Organization (WHO) in 1997, 62 cases of our patients were classified into I-II grade and 48 cases III-IV grade. 134 healthy people (78 males and 56 females) were enrolled simultaneously as controls with an average age of  $43.6 \pm 12.4$  and they had no family history of tumors. No sex or age significant difference existed between two groups. Our research was approved by the Research Ethics Committee of Renmin Hospital of Wuhan University. Data and blood collection obtained the informed consensus from every subject and were conducted following various ethics rules. All subjects were not related by blood each other.

#### Specimen collection and genome DNA extraction

3 mL fasting venous blood was collected from every participant the next morning after their hospitalization and put into vacuum ACD anti-coagulant tubes. Genome DNA was extracted through routine phenol/chloroform method at the same day and preserved at  $-20^{\circ}\text{C}$  for later.

#### Genotyping

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to perform genotyping. PCR primers were designed according to the reference by Primer Premier 5.0 software. The primer sequences were listed in **Table 1**. PCR amplification reaction was performed in a total volume of 20  $\mu\text{L}$  solution, including 10.0  $\mu\text{L}$  PCR Master Mix (Shanghai Shenyong Biotech Co., Ltd), each 0.2  $\mu\text{L}$  of forward and reverse primers (10  $\mu\text{M}$ ), 1.5  $\mu\text{L}$  DNA template and 8.1  $\mu\text{L}$  sterile deionized water. The amplification conditions were: initial denaturation at  $94^{\circ}\text{C}$  for 2 minutes; followed by 32 cycles of denaturation at  $94^{\circ}\text{C}$  for 30 second, annealing for  $56^{\circ}\text{C}$  for 45 seconds and extension at  $72^{\circ}\text{C}$  for 45 seconds, and a final extension at  $72^{\circ}\text{C}$  for 10 minutes.

PCR products were digested with *Avall*, *Xbal*, *Alul* restriction endonucleases (NEB Company). A total of 10  $\mu\text{L}$  enzyme digestion reaction mixtures contained 3  $\mu\text{L}$  PCR products, 1 U corresponding restriction endonuclease, 1  $\mu\text{L}$  10  $\times$  NEB buffer and the rest volume of sterile deionized water. The digestion was performed at  $37^{\circ}\text{C}$  for 6 hours. The enzyme-digested products were analyzed with polyacrylamide gel electrophoresis (PAGE) method and visualized by silver nitrate staining to determine the genotypes.

#### Statistical analysis

SPSS software (Version 18.0) was used for statistical analysis.  $\chi^2$  test was utilized to assess the Hardy-Weinberg equilibrium (HWE) in the control group. Odds ratio (ORs) and 95% confidence intervals (CIs) were used to represent the relative risk (RR) of glioma and were also calculated by the chi-squared test. Haploview software was conducted to calculate the linkage disequilibrium and haplotype. When  $P < 0.05$ , it was statistical significance.

### Results

#### HWE test

The genotypes distributions of *RTEL1* rs6010620, rs2297440, rs4809324 polymorphisms in the control group were all consistent

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**Table 2.** Comparison of *RTEL1* polymorphisms between case and control groups

Genotype/Allele	Case (n=110, %)	Control (n=134, %)	OR (95% CI)	P value	
Rs6010620	AA	51 (46.36)	69 (51.49)	1.000 (Ref.)	-
	AG	45 (40.91)	58 (43.28)	1.050 (0.617-1.786)	0.858
	GG	14 (12.73)	7 (5.23)	2.706 (1.019-7.187)	0.040
	A	147 (66.82)	196 (73.13)	1.000 (Ref.)	-
Rs2297440	G	73 (33.18)	72 (26.87)	1.315 (0.893-1.938)	0.165
	TT	54 (49.09)	72 (53.73)	1.000 (Ref.)	-
	TC	43 (39.09)	56 (41.79)	1.024 (0.602-1.742)	0.931
	CC	13 (11.82)	6 (4.48)	2.889 (1.032-8.089)	0.037
Rs4809324	T	151 (68.64)	200 (74.63)	1.000 (Ref.)	-
	C	69 (31.36)	68 (25.37)	1.344 (0.905-1.997)	0.143
	TT	85 (77.27)	99 (73.88)	1.000 (Ref.)	-
	TC	22 (20.00)	33 (24.63)	0.776 (0.421-1.432)	0.417
	CC	3 (2.73)	2 (1.49)	1.747 (0.285-10.702)	0.541
	T	192 (87.27)	231 (86.19)	1.000 (Ref.)	-
	C	28 (12.73)	37 (13.81)	0.910 (0.538-1.542)	0.727

**Table 3.** The haplotypes analysis of *RTEL1* polymorphisms rs6010620, rs2297440, rs4809324

Haplotypes	Case (2n=220, %)	Control (2n=268, %)	OR (95% CI)	P value
A-T-T	147 (68.06)	196 (74.24)	1.000 (Ref.)	-
G-C-T	45 (20.83)	35 (13.26)	1.714 (1.049-2.800)	0.030
G-C-C	24 (11.11)	33 (12.50)	0.970 (0.550-1.711)	0.915

with HWE ( $P>0.05$ ). This result showed that our population had a good representativeness.

### *Relationship between RTEL1 polymorphisms and glioma risk*

As shown in **Table 2**, GG genotype frequency of *RTEL1* rs6010620 polymorphism was significantly higher in cases than controls and it might be a risk factor for glioma (OR=2.706, 95% CI=1.019-7.187). CC genotype in rs2297440 was also associated with the remarkably increased susceptibility to glioma (OR=2.889, 95% CI=1.032-8.089). However, there was no obvious difference between the case and control groups in rs4809324 polymorphism ( $P>0.05$ ).

### *Result of haplotype analysis*

We conducted the linkage disequilibrium and haplotype analyses in three polymorphisms rs6010620, rs2297440, rs4809324. Three

haplotypes A-T-T, G-C-T, G-C-C were checked and the rest was excluded because of the frequency less than 5%. The frequency of haplotype G-C-T was 1.741 times in cases compared with the control group and it might be a influence factor for the occurrence of glioma (OR=1.714, 95% CI=1.049-2.800) (**Table 3**).

### **Discussion**

Glioma is one of the most aggressive tumors and its sensitivity to treatment is extremely poor. Astrocytomas, oligodendrogliomas and mixed gliomas account for more than 80% of the adult central nervous system tumors [18]. Glioma is a special type of tumor occurred in brain, but in terms of pathogenesis, it has no essential difference from the tumors in other parts of the body. Data indicate that the incidence of glioma is associated with mostly genes related to tumors [19-23].

The occurrence of glioma can be attributed to genetic abnormalities and environmental impact, so the study emphasis is always on the exploration of the susceptible genes related to the onset and development of glioma [24]. 20q13/*RTEL1*, as an encoding helicase gene, maintains the stability of the genome DNA by inhibiting the homologous recombination [12, 13]. Genome-wide association study (GWAS) has found that *RTEL1* may closely related to the progress of astrocytoma and glioblastoma and it can be also used to evaluate patients prognosis [25, 26].

Two articles about genome-wide association studies on glioma in European descendants were published successively in the United States and the United Kingdom in 2009 [27]. One study by Shete et al., found 5 risk genes:

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*TERT*, *CCDC26*, *CDKN2A/B*, *PHLDB1* and *RTEL1* and the other one proved that three polymorphisms located in *CDKN2B* and *RTEL1*, two of which (rs142829 and rs6010620) were also reported by Shete et al., were strongly associated with high-grade gliomas ( $P < 10^{-7}$ ) [28].

Lots of studies have indicated that the mutation of *RTEL1* can induce errors in the process of DNA replication in human cells, which indicates that *RTEL1* takes part in the development of glioma [26, 29]. The *RTEL1* polymorphisms may affect the combination of many transcription factors and regulate transcriptional activity of *RTEL1*. Since different races have various potential genetic backgrounds, it is very important to comprehensively verify the correlation of glioma with the related polymorphisms contained in the *RTEL1* genome-wide association studies among Chinese Han people and to better understand the pathogenesis of glioma.

In present study, we designed a case-control experiment based on hospital glioma patients. *RTEL1* rs6010620 polymorphism was associated with the increased risk of glioma and this result was similar to previous relevance studies in other populations. Rs2297440 was also a risk factor in the development of glioma, however, the other polymorphism in *RTEL1* rs4809324 hardly behaved any association with glioma. Even so, the haplotype G-C-T of the three polymorphisms still significantly increased the risk of glioma, which suggested that among *RTEL1* polymorphisms presented the interaction.

In conclusion, our study explored the relationship between *RTEL1* polymorphisms and the risk of glioma. The outcome manifested that *RTEL1* rs6010620 and rs2297440 polymorphisms might be the risk factors in the development and progression of glioma. In the meanwhile, we also explored the interaction among *RTEL1* polymorphisms for the first time and haplotype G-C-T in rs6010620, rs2297440, rs4809324 also increased the susceptibility to glioma. But our results also have several limitations. For example, we not considered the influence from environment and races. So the result needs to be certified through further studies based on more races with larger sample scale, considering the interaction with environment.

### Disclosure of conflict of interest

None.

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