

## Original Article

# Relationship between angiotensinogen genetic polymorphism and glioma in Chinese population

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**Abstract:** Objective: To investigate the relation between angiotensinogen gene (AGT) polymorphism and glioma in a Chinese population. Methods: Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect AGT M235T polymorphism in 243 cases of nerve glioma patients and 325 cases of healthy controls. The M235T genotype distribution was compared between the case and control group. Results: Compared with the TT genotype, MM and MT genotypes frequency significantly increased in gliomas patients (both  $P < 0.05$ ). Compared with the T allele, M allele significantly increased the risk of gliomas ( $P < 0.05$ , OR=1.436, 95% CI: 1.102-1.871). Conclusion: AGT M235T polymorphism was associated with the risk for glioma in Chinese population.

**Keywords:** Angiotensinogen, polymorphism, glioma

## Introduction

Glioma is one of the common intracranial tumors. Currently, the exact etiology and pathogenesis are not fully understood. Studies show that a variety of factors including environmental and genetic factors are involved in its incidence and development [1-5]. Human angiotensinogen (AGT), as one member of renin-angiotensin-aldosterone system (RAAS), not only is involved in regulating blood pressure and stabilizing fluid balance, but also can inhibit tumor cell proliferation, migration and tumor vascularization [6-8]. AGT gene exon 2 has a point mutation, which can transform the No. 235 methionine (Met) into a threonine (Thr). Several studies have investigated the correlation between AGT M235T gene polymorphism and various tumors [9-11]. However, the association of association of AGT M235T gene polymorphism with glioma remains unclear. In this study, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to study the relationship between AGT M235T gene polymorphism and glioma in Chinese Han population, in order to provide a theoretical basis for further investigating the role of AGT in glioma pathophysiology.

## Materials and methods

### Subjects

568 peripheral blood samples were collected from unrelated Han individuals in Huashan Hospital, Fudan University School of Medicine, which were divided into glioma group and normal control group. There were 243 cases in glioma group, including 140 males and 103 females, with an average age of 43.3 years and median age of 45 years. All cases were confirmed by pathological diagnosis, including 142 cases of pathological I~II grade, 101 cases of III-IV grade. There were 325 cases in normal control group, including 176 males and 149 females, with an average age of 44.0 years, median age of 44 years. All were normal hospital medical specimens, without cancer and other neurological diseases. The characteristics of included population were shown in **Table 1**.

### DNA extraction and primer synthesis

2 ml peripheral blood were drawn from glioma patients and normal control subjects; whole blood genomic DNA extraction kit (Beijing

**Table 1.** The characteristics of participants

Item	Control group (N=243)	Giloma group (N=325)	P
Sex (M/F)	140/103	176/149	0.223
Age (year)	43.3±11.5	44.0±11.1	0.654
Smoking (n, %)	98 (40.3)	133 (40.9)	0.766
Family cancer history (n, %)	43 (17.6)	61 (18.7)	0.334

and clinical characteristics in glioma group and control group were compared using the  $\chi^2$  test or *t* test.  $P < 0.05$  indicated a statistically significant difference.

## Results

### Hardy-Weinberg equilibrium test

Hardy-Weinberg equilibrium test showed that AGT M235T genotype distribution was in line with genetic equilibrium both in glioma group and control group (data not shown).

### Genotype and allele frequency distribution between case and control group

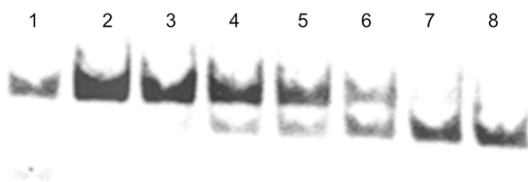
The comparison of AGT M235T Polymorphism between control and glioma groups was shown in **Table 2**. TT, MT and MM genotype frequencies in the control group were 56.0%, 40.9%, and 10%, respectively. Their frequencies in glioma group were 45.2%, 48.1%, and 6.7%, respectively. Compared with the TT genotype, MT and MM genotypes significantly increased the risk of glioma (MT vs. TT: OR=1.455, 95% CI: 1.033-2.051,  $P=0.032$ ; MM vs. TT: OR=2.647; 95% CI: 1.160-6.039,  $P=0.021$ ). Frequencies of T and M alleles in the control group were 0.76 and 0.24 respectively; their frequencies in glioma group were 0.69 and 0.31, respectively. The difference between the two groups was statistically significant (M allele vs. T allele: OR=1.436; 95% CI: 1.102-1.871,  $P=0.007$ ).

### Logistic regression analysis

Logistic regression analysis of genotypes indicated that M allele was associated with increased glioma risk compared with T genotype (Adjusted OR=1.465, 95% CI: 1.101~3.033,  $P=0.014$ , **Table 3**).

## Discussion

Renin-angiotensin-aldosterone system (RAAS) includes AGT, angiotensin I, angiotensin transferase, angiotensin II and its receptor. All the ingredients of RAAS can be detected in human malignant glioma. Shang et al [13] utilized angiotensin II in combination with chemotherapy to treat rat glioma and found that this method can increase the local blood flow in the tumor and prolong survival time, indicating that



**Figure 1.** Genotyping results by PCR-RFLP (1, 2, 3: TT genotype; 4, 5, 6: MT genotype; 7, 8: MM genotype).

Bioteke Company) was used to extract DNA. Primers were designed according to the literature [12]. Upstream: 5: CAGGGTGCTGTCCAC-ACTGGACCCC-3, Downstream: 5: CCGTGTGC-AGGGCCT GGCTCTCT-3, primers were synthesized by Shanghai Biological Engineering Co., Ltd (Shanghai, China).

### AGT M235T genotyping

Using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), we detected the genotype of AGT M235T. PCR reaction system was 10  $\mu$ l, containing 5.0  $\mu$ l of DNA Mix (purchased from Beijing Bioteke Company, China), 0.1  $\mu$ l of each primers (upstream and downstream), 0.8  $\mu$ l of genome DNA, with sterile deionized water to make up to 10  $\mu$ l. On the Eppendorf amplification instrument, PCR reactions were carried out under the following conditions: 94°C denaturation for 4 min; 94°C denaturation for 30 s, 66.5°C annealing for 45 s, 72°C extension for 90 s, 35 cycles. Final extension at 72°C for 10 min. PCR products were digested with the restriction enzyme TthIII I (Fermentas, USA). After digestion and electrophoresis, three genotypes were obtained, as shown in **Figure 1**.

### Statistical methods

SPSS11.5 statistical package (Chicago, IL, USA) was used to process data, and optimization-fitting  $\chi^2$  test was used to test that whether genotypes were in line with Hardy-Weinberg equilibrium. Genotype and allele frequencies

**Table 2.** Distribution of genotype and alleles

Genotype or Allele	Glioma group, n (%), (N=243)	Control group, n (%), (N=325)	OR (95% CI)	P
TT	110 (45.2)	182 (56.0)	1	
TM	117 (48.1)	133 (40.9)	1.455 (1.033-2.051)	0.032
MM	16 (6.7)	10 (3.1)	2.647 (1.160-6.039)	0.021
T	0.69	0.76	1	
M	0.31	0.24	1.436 (1.102-1.871)	0.007

**Table 3.** Logistic regression of the relation between gene polymorphism and glioma risk

Parameters	OR	95% CI	P value
AGT polymorphism	1.465	1.101~3.033	0.014
Smoking	1.192	0.901~3.201	0.219
Age	1.121	0.711~3.443	0.232
History of cancer disease	1.321	0.789~3.189	0.121

RAAS plays a very important role in the occurrence and development of glioma. AGT, one of the important members of the RAAS, not only is involved in regulating blood pressure and maintaining a stable internal environment, but also has significant anti-tumor effects. *Vivo* and *vitro* experiments have confirmed that overexpression of AGT can inhibit tumor cell proliferation and tumor angiogenesis, thereby delaying tumor growth, suggesting that AGT is expected to be a new target for cancer biotherapy [14-18].

Due to the clear anti-tumor effect of AGT, in recent years, a number of studies have investigated the correlation between AGT gene M235T polymorphisms and tumor. However, the results are not entirely consistent. In 2008, Vairaktaris et al [19] used PCR-RFLP method to analyze the polymorphism of AGT gene M235T in 163 cases of Germany and Greece oral cancer patients and 124 cases healthy control subjects. The authors found no significant differences between the two groups. Subsequently, studies have reported that AGT gene M235T polymorphism had no correlation with gastric cancer in Japan [20], colorectal cancer in Czech Republic [21] and breast cancer in Mexico [22]. However, Xi et al [23] conducted a meta-analysis to evaluate the correlation between AGT M235T polymorphism and breast cancer, and found that genetic variation increased the risk of breast cancer in the Caucasian population,

and MM genotype reduced the disease-free survival of breast cancer patients. Currently, the correlation between AGT M235T polymorphism and glioma is rarely reported in the literature internationally. In this study, a case-control study was conducted to investigate the correlation of AGT M235T gene polymorphism with glioma in Chinese Han population. We found that MT and MM genotypes and M allele significantly increased the risk of glioma. The reasons for these inconsistent findings may be as follows: 1) there are different genetic backgrounds and different environments between different ethnic; 2) AGT M235T polymorphism plays different roles in different tumors. Therefore, it is necessary to conduct collaborative research of multi-center, multi-ethnic and large-sample to verify the results of this study.

In summary, missense mutations lead to amino acid changes, resulting in changes in protein function, having an important significance for the study of genetic effects and mechanism of gene in related diseases. This study found that, the frequency distribution of AGT M235T missense mutation was associated with glioma susceptibility in Chinese Han population. The incidence and development of gliomas is the result of the interaction of environmental factors and host factors. Therefore, further study of the interaction between AGT M235T polymorphism and environmental exposure factors would contribute to the comprehensive understanding of the etiology of glioma and its complex pathological process, laying a foundation for the prevention and treatment of gliomas.

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#### Disclosure of conflict of interest

None.

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