Association of Genetic Polymorphisms of *EGFR* with Glioma in a Chinese Population

Xinyu Wang,¹ Huawei Zhang,² Donghai Wang,¹ and Xingang Li¹

Objective: Several studies indicated that genetic polymorphisms in the epidermal growth factor receptor (*EGFR*) gene were associated with glioma risk. However, the relationship between *EGFR* genetic polymorphisms and glioma remains unclear in the Chinese population. **Methods:** We designed a case–control study by selecting 300 histologically confirmed adult glioma patients and 300 cancer-free controls to analyze the distribution of *EGFR* genotype. Two single-nucleotide polymorphisms, rs730437 and rs1468727, were genotyped by using the polymerase chain reaction–restriction fragment length polymorphism method. **Results:** We found that the CC genotype of rs1468727 was more common in the glioma group than in the control group ($p=0.021$). We also found that the C allele frequency was higher in the glioma group than that in the control group ($p=0.005$; odds ratio [OR]=1.38, 95% confidence interval [CI]: 1.101–1.740). For rs730437, we found both the AA genotype ($p=0.011$) and A allele frequency ($p=0.003$; OR=0.703; 95% CI: 0.558–0.886) were significantly lower in the glioma patients than in the control subjects, respectively. Haplotype analysis showed that the A T haplotype frequency was higher in the control group than in the glioma group ($p=0.005$; OR=0.722; 95% CI: 0.575–0.908). However, the CC haplotype frequency was higher in the glioma group than in the control group ($p=0.003$; OR=1.423; 95% CI: 1.129–1.793). **Conclusion:** Our study indicates that polymorphisms in the *EGFR* gene are associated with glioma susceptibility in the Chinese population.

Introduction

GLIOMA IS THE MOST COMMON type of brain tumor worldwide and its incidence rate is about six per 100,000 annually (Okada *et al.*, 2013). However, the mechanism of glioma development remains unclear. Previous studies suggested that a variant of risk factors may result in glioma development, including exposure to ionizing radiation (Amirian *et al.*, 2010), genetic polymorphisms (Liang *et al.*, 2013; Liu *et al.*, 2013; Zeybek *et al.*, 2013), and a history of familial cancer (Goodenberger and Jenkins, 2012; Cheng *et al.*, 2013).

The epidermal growth factor receptor (*EGFR*) is involved in human tumors by regulating important cellular processes (Guo *et al.*, 2013). Previous studies have reported single-nucleotide polymorphisms (SNPs) in the *EGFR* gene to be associated with several kinds of cancers, including lung cancer (He *et al.*, 2013), breast cancer (Connor *et al.*, 2013), and esophageal cancer (Lee *et al.*, 2011). However, the relationship between *EGFR* polymorphisms and glioma remains unclear. Several studies have suggested that regulation of the *EGFR* pathway plays an important role in glioma progression (Bhowmick *et al.*, 2004), and several SNPs in the *EGFR* gene may be related to glioblastoma risk (Andersson *et al.*, 2010; Wibom *et al.*, 2012). Previously, Hou *et al.*

(2012) have investigated the association of *EGFR* haplotype with glioma and found that several haplotypes were associated with glioma risk. Costa *et al.* (2011) performed a case–control study in Portugal and found that *EGFR* polymorphisms were risk factors for gliomas.

A previous study (Liu *et al.*, 2012) indicated that two SNPs (rs1468727 and rs730437) were found to be associated with glioma. However, in another study (Hou *et al.*, 2012), these two SNPs were not found associated with the risk for glioma. Therefore, the relationship between these two SNPs should be further clarified. The SNP rs1468727 is in intron 13 and rs730437 is in intron 4 of the *EGFR* gene. Previous study suggested that rs1468727 and rs730437 may have a tight linkage with other functional SNPs. Therefore, in the present study, we performed a case–control study to further reveal the relationship between these two SNPs and the risk for glioma in the Chinese population.

Subjects and Methods

Ethics

This study was approved by the ethics committee of the Second Hospital of Shandong University. All the participants signed the informed consent before this study.

¹Department of Neurosurgery, Qilu Hospital of Shandong University, Jinan, China.

²Department of Ultrasound, Provincial Hospital Affiliated to Shandong University, Jinan, China.

TABLE 1. THE CHARACTERISTICS OF POLYMERASE CHAIN REACTION

SNPs	Primers	Endonuclease	Product length (bp)	Annealing temperature (°C)
rs730437	Forward: 5'GTCCCTGTCCCATTCC3' Reverse: 5'ATCCAAAGCCCTGTAGTT3'	AocI	325	55
rs1468727	Forward: 5'GATCCAGAAATATTTAGGAGC3' Reverse: 5'TTTCATCACCTTGCCCTCT3'	MaeI	207	56

SNPs, single-nucleotide polymorphisms.

Subjects

The glioma patients and the control subjects were enrolled in the Qilu Hospital, Shandong University from March 2009 to March 2013. All glioma patients ($n = 300$) had no previous history of other cancers or prior chemotherapy or radiotherapy. All patients were recently diagnosed and histologically confirmed to have glioma. We selected 300 healthy unrelated individuals as the control group recruited between March 2009 and March 2013 from the medical examination center at the same hospital. Subjects with chronic diseases and conditions involving the vital organs (heart, lung, liver, kidney, and brain) and severe endocrinological, metabolic, and nutritional diseases were excluded from this study. The purpose of the above exclusion procedures was to minimize the known environmental and therapeutic factors that influence the variation of human complex diseases.

Genotyping

There are 4538 SNPs for the human *EGFR* gene listed in the National Center for Biotechnology Information SNP database (www.ncbi.nlm.nih.gov/SNP). We selected two SNPs (rs730437 and rs1468727) to perform genotyping for the present study. Genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction Kit (Beijing Bioteke Co. Ltd). Genotyping was confirmed by the polymerase chain reaction-restriction fragment length polymorphism method. The primer sequences, endonucleases, and annealing temperatures are shown in Table 1.

Statistical analyses

SPSS 17.0 software was utilized to perform the data analysis. We utilized the χ^2 test to assess the Hardy–Weinberg equilibrium and to compare the difference between case and control group in allele and genotype distribution. We calculated the odds ratios (ORs) and 95% confidence intervals (95% CIs) by

using unconditional logistic regression. SHEsis software was utilized to perform linkage disequilibrium (LD) analysis and haplotype analysis. Haplotypes with a frequency of <0.02 were excluded. Statistical significance was established at $p < 0.05$.

Results

Subjects and general characteristics

In total, 600 subjects were recruited in this case–control study, including 300 glioma patients and 300 cancer-free controls. The clinical characteristics of these two groups are shown in Table 2. There were no significant differences between glioma patients and cancer-free controls in terms of age, sex, alcohol drinking, and smoking (all $p > 0.05$).

EGFR polymorphisms and glioma

We found that the CC genotype of rs1468727 was more common in the glioma group than in the control group ($p = 0.021$). We also found that the C allele frequency was higher in the glioma group than that in the control group ($p = 0.005$; OR = 1.38; 95% CI: 1.101–1.740). For rs730437, we found both AA genotype ($p = 0.011$) and A allele frequency ($p = 0.003$; OR = 0.703; 95% CI: 0.558–0.886) to be significantly lower in the glioma patients than in the control subjects, respectively (Table 3).

We found these two SNPs were in strong LD ($r^2 = 0.77$). Haplotype analysis showed that the A T haplotype frequency was higher in the control group than in the glioma group ($p = 0.005$; OR = 0.722; 95% CI: 0.575–0.908). However, the CC haplotype frequency was higher in the glioma group than in the control group ($p = 0.003$; OR = 1.423; 95% CI: 1.129–1.793) (Table 4).

Discussion

In the present study, we found that the *EGFR* genetic polymorphisms and haplotypes were associated with glioma. We performed a haplotype-based case–control study to reveal the relationship between *EGFR* polymorphism and glioma risk in the Chinese population. Using this method, we provided strong support that *EGFR* gene variations contributed to the susceptibility to glioma.

In our report, we found that two SNPs (rs1468727 and rs730437) in the *EGFR* gene were associated with glioma risk. The C allele of rs1468727 of the *EGFR* gene may increase the risk, but the A allele in rs730437 may decrease the risk of glioma. Previously, in a European population, the T allele of rs1468727 was found to be associated with a decreased risk of glioma (OR = 0.61; 95% CI: 0.40–0.93;

TABLE 2. THE CLINICAL CHARACTERISTICS OF GLIOMA CASES AND CONTROLS

Characteristics	Cases (n, %)	Controls (n, %)	p-Value
Number	300	300	
Gender (male)	221 (73.7)	226 (75.3)	0.876
Age (years)	55 ± 10.8	56 ± 10.7	0.894
Smoking	219 (73.0)	208 (69.3)	0.311
Alcohol drinking	143 (47.7)	141 (47.0)	0.879
Family history of cancer	44 (14.7)	—	

TABLE 3. GENOTYPE DISTRIBUTION OF EPIDERMAL GROWTH FACTOR RECEPTOR SINGLE-NUCLEOTIDE POLYMORPHISMS BETWEEN CASES AND CONTROLS

SNPs	Genotype and allele	Glioma (n = 300)	Control (n = 300)	OR (95% CI)	p-Value
rs730437	AA	89 (0.297)	121 (0.403)	0.703 (0.558–0.886)	0.011
	AC	151 (0.503)	138 (0.460)		
	CC	60 (0.200)	41 (0.137)		
	A	329 (0.548)	380 (0.633)		0.003
	C	271 (0.452)	220 (0.367)		
rs1468727	CC	70 (0.233)	48 (0.160)	1.38 (1.101–1.740)	0.021
	TC	148 (0.493)	144 (0.480)		
	TT	82 (0.273)	108 (0.360)		
	C	288 (0.480)	240 (0.400)		0.005
	T	312 (0.520)	360 (0.600)		

CI, confidence interval; OR, odds ratio.

TABLE 4. DISTRIBUTION OF HAPLOTYPES

Haplotypes	Glioma	Control	p	OR (95% CI)
AC	17.00 (0.028)	20.00 (0.033)	0.616	0.846 (0.439–1.631)
AT	312.00 (0.520)	360.00 (0.600)	0.005	0.722 (0.575–0.908)
CC	271.00 (0.452)	220.00 (0.367)	0.003	1.423 (1.129–1.793)

$p=0.017$), which was in line with our finding that rs1468727 was a susceptibility locus and the genotype C allele of this locus was a risk factor for glioma (Andersson *et al.*, 2010). For SNP rs730437, we found A allele carriers to have reduced risk for glioma. However, in the European population (Andersson *et al.*, 2010), the A allele was found to increase the risk for gliomas, which was not in line with our finding. This inconsistency may result from ethnic differences. In addition, these two polymorphisms are located at the intron region, which indicated that these two SNPs did not affect protein function. The rational explanation may be that these two SNPs have a tight linkage with other functional SNPs. Therefore, the exact location and biological functions of the real causal SNPs in the EGFR gene are of great interest and warrant further investigation.

We also found that the AT haplotype frequency was higher in the control group than in the glioma group ($p=0.005$; OR=0.722; 95% CI: 0.575–0.908) and the CC haplotype frequency was higher in the glioma group than in the control group ($p=0.003$; OR=1.423; 95% CI: 1.129–1.793). These results indicated that AT haplotype carriers have a reduced risk for gliomas, but the CC haplotype carriers had a 0.423-fold increased risk for glioma.

Conclusion

In conclusion, the present study suggested that genetic polymorphisms of *EGFR* were associated with glioma in the Chinese population. The haplotype CC may be a genetic marker for risk of glioma, but haplotype AT could be a protective factor for glioma.

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Author Disclosure Statement

The authors declared that no competing interests exist.

References

- Amirian E, Liu Y, Scheurer ME, *et al.* (2010) Genetic variants in inflammation pathway genes and asthma in glioma susceptibility. *Neuro Oncol* 12:444–452.
- Andersson U, Schwartzbaum J, Wiklund F, *et al.* (2010) A comprehensive study of the association between the EGFR and ERBB2 genes and glioma risk. *Acta Oncol* 49:767–775.
- Bhowmick DA, Zhuang Z, Wait SD, Weil RJ (2004) A functional polymorphism in the EGF gene is found with increased frequency in glioblastoma multiforme patients and is associated with more aggressive disease. *Cancer Res* 64:1220–1223.
- Cheng HB, Xie C, Zhang RY, *et al.* (2013) Xeroderma pigmentosum complementation group f polymorphisms influence risk of glioma. *Asian Pac J Cancer Prev* 14:4083–4087.
- Connor AE, Baumgartner RN, Baumgartner KB, *et al.* (2013) Epidermal growth factor receptor (EGFR) polymorphisms and breast cancer among Hispanic and non-Hispanic white women: the Breast Cancer Health Disparities Study. *Int J Mol Epidemiol Genet* 4:235–249.
- Costa BM, Viana-Pereira M, Fernandes R, *et al.* (2011) Impact of EGFR genetic variants on glioma risk and patient outcome. *Cancer Epidemiol Biomarkers Prev* 20:2610–2617.
- Goodenberger ML, Jenkins RB (2012) Genetics of adult glioma. *Cancer Genet* 205:613–621.
- Guo H, Xing Y, Liu R, *et al.* (2013) –216G/T (rs712829), a functional variant of the *EGFR* promoter, is associated with the pleural metastasis of lung adenocarcinoma. *Oncol Lett* 6:693–698.
- He C, Zheng L, Xu Y, *et al.* (2013) Highly sensitive and non-invasive detection of epidermal growth factor receptor T790M mutation in non-small cell lung cancer. *Clin Chim Acta* 425:119–124.

- Hou WG, Ai WB, Bai XG, *et al.* (2012) Genetic variation in the EGFR gene and the risk of glioma in a Chinese Han population. *PLoS One* 7:e37531.
- Lee JM, Yang SY, Yang PW, *et al.* (2011) Polymorphism in epidermal growth factor receptor intron 1 predicts prognosis of patients with esophageal cancer after chemoradiation and surgery. *Ann Surg Oncol* 18:2066–2073.
- Liang HJ, Yan YL, Liu ZM, *et al.* (2013) Association of XRCC3 Thr241Met polymorphisms and gliomas risk: evidence from a meta-analysis. *Asian Pac J Cancer Prev* 14:4243–4247.
- Liu HB, Peng YP, Dou CW, *et al.* (2012) Comprehensive study on associations between nine SNPs and glioma risk. *Asian Pac J Cancer Prev* 13:4905–4908.
- Liu P, Huang Z, Chen Z, *et al.* (2013) Silver nanoparticles: a novel radiation sensitizer for glioma? *Nanoscale* 5:11829–11836.
- Okada H, Scheurer ME, Sarkar SN, Bondy ML (2013) Integration of epidemiology, immunobiology, and translational research for brain tumors. *Ann N Y Acad Sci* 1284:17–23.
- Wibom C, Ghasimi S, Van Loo P, *et al.* (2012) EGFR gene variants are associated with specific somatic aberrations in glioma. *PLoS One* 7:e47929.
- Zeybek U, Yaylim I, Ozkan NE, *et al.* (2013) Cyclin D1 gene G870A variants and primary brain tumors. *Asian Pac J Cancer Prev* 14:4101–4106.

Address correspondence to:

Xingang Li, MD

Department of Neurosurgery

Qilu Hospital of Shandong University

No. 107 Jinan West Wenhua Road

Jinan 250012

China

E-mail: liuqcsu44@163.com