Assessing the association between *EFEMP1* rs3791679 polymorphism and risk of glioma in a Chinese Han population

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ABSTRACT. In this study, we assessed the association between the *EFEMP1* rs3791679 polymorphism and glioma risk in a Chinese Han population. A total of 94 glioma patients and 206 healthy controls who conformed to the inclusion and exclusion criteria were recruited from Baogang Hospital between March 2012 and October 2014. The *EFEMP1* rs3791679 gene polymorphism was assessed using a polymerase chain reaction-restriction fragment length polymorphism assay and the results were statistically analyzed using SPSS Statistics 17.0. The results of unconditional logistic regression analysis revealed that the GG genotype of *EFEMP1* rs3791679 was positively correlated

with increased susceptibility to glioma (adjusted OR = 2.09, 95%CI = 1.21-7.81). Moreover, the GG genotype of *EFEMP1* rs3791679 was correlated with higher risk of glioma compared to the AA+GA genotype (OR = 2.60, 95%CI = 1.08-6.28) in the regressive model. In conclusion, we report that the *EFEMP1* rs3791679 polymorphism influences glioma susceptibility in the Chinese Han population.

Key words: Chinese Han population; *EFEMP1*; Glioma; rs3791679

INTRODUCTION

Gliomas are the most common primary malignant tumors of the central nervous system, and account for 24% of all brain tumors (Ostrom et al., 2013). The annual incidence of brain tumor in a Chinese population during 2012 was 1.9 for every 100,000 men and 2.5 per 100,000 women (IARC, 2012). Moreover, the etiology of glioma remains poorly understood: gliomas may be caused by both intrinsic and environmental factors. A number of studies conducted over the past few years revealed that hereditary factors, such as single nucleotide polymorphisms, play an important role in modifying glioma susceptibility.

Epidermal growth factor-containing fibulin-like extracellular matrix protein 1 (*EFEMP1*), located on chromosome 2, encodes a member of the fibulin family of extracellular matrix glycoproteins. Previous studies have reported that the *EFEMP1* gene is involved in the pathogenesis of human cancer, and modifies the functions of its receptors (Song et al., 2010). So far, only one study has reported the role of polymorphisms in the *EFEMP1* gene in the development of glioma (Zhang et al., 2015). Therefore, in this study, we assessed the correlation between the *EFEMP1* rs3791679 gene polymorphism and glioma risk in a Chinese Han population.

MATERIAL AND METHODS

Subjects

We conducted a hospital-based case-control study; ethnic Han Chinese glioma patients (N = 94) were recruited from the Department of Neurosurgery at Baogang Hospital between March 2012 and October 2014. All glioma patients were pathologically confirmed and newly diagnosed. The tumor type and stage was determined based on the criteria employed by the World Health Organization (WHO). Patients with a history of other cancers or prior chemotherapy were excluded from this study.

Ethnic Han Chinese control subjects (N = 206) were selected from among patients who received regular health check-ups at our hospital between March 2012 and October 2014. Subjects with a previous history of glioma and other malignant tumors were excluded from our study.

The demographic and clinical characteristics of glioma patients and controls, including the age, gender, smoking status, alcohol consumption, were collected from a structured questionnaire or from medical records. This case-control study was approved by the Ethics Committee of Baogang Hospital; written informed consent was obtained from all subjects.

Genotyping methods

Total genomic DNA was isolated from whole blood obtained from all patients and controls, using the Tiangen Blood mini kit (Tiangen Co. Ltd., Beijing, China), following the manufacturer instructions. The *EFEMP1* rs3791679 polymorphism was analyzed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The PCR primers for each polymorphism were designed by the Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA). The PCR conditions were set as follows: one cycle of denaturation at 94°C for 5 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min; and a final extension at 72°C for 5 min. The PCR products were digested using the appropriate enzyme and genotyped. A single-blind method was adopted for genotyping (without clinical data). The genotyping quality was monitored by repeating 10% of the assays from different batches.

Statistical analysis

The distribution of demographic characteristics between control individuals and glioma patients was examined using the Student *t*-test and the chi-square test. Genotype distribution of *EFEMP1* rs3791679 was assessed for conformance with the Hardy-Weinberg equilibrium (HWE); these results were confirmed by the chi-square test. The association between the *EFEMP1* rs3791679 gene polymorphism and glioma risk is reported as adjusted odds ratios (ORs) and their 95% confidence intervals (CIs). Multiple-logistic regression models were constructed to calculate the ORs (95%CI) after adjusting for potential confounding factors. Statistical analyses were performed on the SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, USA) software platform. P values <0.05 were considered to be statistically significant.

RESULTS

The mean age of the included glioma patients (N = 96) and healthy controls (N = 206) was 48.53 ± 10.34 and 46.65 ± 11.60 years, respectively (Table 1). The glioma patients were comparable with the control subjects in terms of age (t = 1.34, P = 0.09), gender (χ^2 = 1.33, P = 0.73), tobacco smoking status (χ^2 = 1.33, P = 0.73), alcohol consumption status (χ^2 = 0.40, P = 0.53), and family history of cancer (χ^2 = 0.48, P = 0.49).

Table 1. Demographic characteristics of included subjects.

Variables	Patients (N = 94)	%	Controls (N = 206)	%	*t*-test or χ^2 test	P value
Age (years)	48.53 ± 10.34		46.65 ± 11.60		1.34	0.09
Gender						
Female	38	40.43	98	47.57		
Male	56	59.57	108	52.43	1.33	0.25
Tobacco smoking						
No	62	65.96	140	67.96		
Yes	32	34.04	66	32.04	0.12	0.73
Alcohol consumption						
No	67	71.28	154	74.76		
Yes	27	28.72	52	25.24	0.40	0.53
Family history of cancer						
No	86	91.49	193	93.69		
Yes	8	8.51	13	6.31	0.48	0.49

The characteristics of the *EFEMP1* rs3791679 polymorphism in glioma patients and control subjects are summarized in Table 2. *EFEMP1* rs3791679 was located at intron 10, at the chromosomal position 55950396. Thirty-seven (39.36%), 43 (45.74%), and 14 (14.89%) glioma patients and 106 (51.46%), 87 (42.23%), and 13 (6.31%) control subjects expressed the AA, GA, and GG genotypes, respectively. The genotype frequencies did not differ significantly between the glioma patients and control subjects ($\chi^2 = 7.45$, P = 0.02). The P values of HWE in the glioma patients and control subjects were 0.79 and 0.38, respectively.

Table 2. Genotype characteristics of the *EFEMP1* rs3791679 polymorphism between glioma patients and control subjects.

EFEMP1 rs3791679	Chromosome position	SNP location	Base changes	Patients	%	Controls	%	χ^2 test	P value	P for HWE	
				(N = 94)		(N = 206)				Patients	Controls
AA				37	39.36	106	51.46				
GA				43	45.74	87	42.23				
GG	55950396	Intron 10	G>A	14	14.89	13	6.31	7.45	0.02	0.79	0.38

HWE, Hardy-Weinberg equilibrium

Unconditional logistic regression analysis revealed that the GG genotype of *EFEMP1* rs3791679 was positively correlated with susceptibility to glioma (adjusted OR = 2.09, 95%CI = 1.21-7.81) (Table 3). The GG genotype of the *EFEMP1* rs3791679 polymorphism was related to a higher risk of glioma compared to the AA+GA genotype (OR = 2.60, 95%CI = 1.08-6.28) in the regression model.

Table 3. Association between the *EFEMP1* rs3791679 gene polymorphism and risk of glioma.

EFEMP1 rs3791679	Patients	%	Controls	%	OR (95%CI)	P value
	(N = 94)		(N = 206)			
Co-dominant						
AA	37	39.36	106	51.46	1.0 (Ref.)	-
GA	43	45.74	87	42.23	1.42 (0.81-2.47)	0.19
GG	14	14.89	13	6.31	2.09 (1.21-7.81)	0.01
Dominant						
AA	37	39.36	106	51.46	1.0 (Ref.)	-
GA+GG	57	60.63	100	48.54	1.63 (0.97-2.77)	0.06
Recessive						
AA+GA	80	85.1	193	93.69	1.0 (Ref.)	-
GG	14	14.89	13	6.31	2.60 (1.08-6.28)	0.02

DISCUSSION

Increasing number of studies has revealed that progressive genomic changes potentially influence the cell phenotypes and assist the development of pre-neoplastic lesions into glioma. Genetic polymorphisms and somatic mutations in factors such as interleukin-8 (IL-8), IL-10, IL-12p40, IL-13, XRCC1, ERCC2, and VEGFR2 are associated with the risk of glioma (Jia et al., 2015; Li et al., 2015; Liu et al., 2015; Shamran et al., 2015; Wang et al., 2015; Xu et al., 2015). Multiple-gene alterations can induce genetic and molecular aberrations, which can also lead to the pathogenesis of glioma. In this study, we investigated the role of the *EFEMP1* rs3791679 gene polymorphism in the development of glioma in a Chinese population, and identified an association between the GG and GA+GG genotypes of *EFEMP1* rs3791679 and

increased risk of glioma in the Chinese population.

Previous studies have reported that the *EFEMP1* gene plays a role in several types of cancers, such as lung cancer, liver cancer, breast cancer, prostate cancer, and nasopharyngeal cancer (Yue et al., 2007; Sadr-Nabavi et al., 2009; Hwang et al., 2010; Nomoto et al., 2010; Kim et al., 2011; Zhang et al., 2011). Yue et al. (2007) reported that alterations in the expression of *EFEMP1* could inhibit lung cancer-cell growth, resulting in a high tumor grade. Sadr-Nabavi et al. (2009) investigated the role of *EFEMP1* in human breast cancer, and reported an association between reduced *EFEMP1* expression and the development of sporadic breast cancer and epigenetic alterations. Hwang et al. (2010) reported that fibulin-3 could suppress cell migration and invasion in nasopharyngeal cancer cells in a Taiwanese population. Furthermore, Nomoto et al. (2010) reported that *EFEMP1* was significantly down-regulated in tumor tissues, and that promoter methylation of the *EFEMP1* gene could be a marker for the prognosis of hepatocellular carcinoma. Kim et al. (2011) reported that epidermal *EFEMP1* is a lead candidate methylation marker for prostate cancer and Zhang et al. (2011) found that methylation of the *EFEMP1* gene contributes to the pathogenesis of non-small cell lung cancer.

So far, only one study has reported an association between the *EFEMP1* gene polymorphisms and susceptibility to glioma (Zhang et al., 2015). This study, comprising 979 glioma patients and 1007 control subjects selected from a Chinese population, reported that the *EFEMP1* rs3791679 polymorphism was associated with an increased risk of glioma. These results were corroborated by the results of our study, which showed that the *EFEMP1* rs3791679 polymorphism contributed to the development of glioma.

In conclusion, our results indicated that the *EFEMP1* rs3791679 gene polymorphism could influence the susceptibility of a Chinese Han population to glioma. Further large-scale studies are required to confirm our findings.

Conflicts of interest

The authors declare no conflict of interest.

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