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Original Article

EFEMP1 rs3791679 polymorphism was associated with susceptibility to glioma

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Abstract: We conducted a case-control study in a Chinese population, and investigated the association between four SNPs (rs3791679, rs1346786, rs1344733 and rs727878) in *EFEMP1* and development of glioma. A case-control study was taken in the present study. The rs3791679, rs1346786, rs1344733 and rs727878 gene polymorphisms were analyzed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. A total of 159 patients with glioma and 364 controls were collected between July 2012 and June 2014. By unconditional logistic regression analysis, we found that individuals carrying the AA genotype and GA+AA genotype were associated with development of glioma when compared with the GG genotype, and the adjusted ORs (95% CI) were 2.13 (1.15-3.90) and 1.55 (1.04-2.32), respectively. However, we did not find that rs1346786, rs1344733 and rs727878 were significantly associated with development of glioma. Moreover, we found that the GA+AA genotype of rs3791679 was associations with a heavy increased risk of glioma in patients who have family history of cancers, and the OR (95% CI) was 6.81 (1.17-48.06). The results of our study suggested an association between the rs3791679 polymorphism and an elevated risk of glioma, especially in those with family history of glioma.

Keywords: EFEMP1, single nucleotide polymorphisms, glioma

Introduction

Gliomas are central nervous system neoplasms derived from glial cells, and glioma is the most frequently type of brain tumors worldwide. Gliomas account for more than 70% of all malignant brain tumors [1]. It is reported that about 80% of patients with gliomas die within one year after initial diagnosis [2]. Many environmental and lifestyle factors including several occupations, Ionizing radiation, cellular phones, smoking, and diet have been considered to be associated with an increased glioma risk [3, 4]. However, the mechanisms underlying glioma tumorigenesis remain poorly understood. Not all individuals who are exposed to high doses of ionizing radiation and other risk factors of gliomas developed gliomas [5], which suggest that genetic factors may contribute to the development of glioma. Increasing evidences have reported that inherited risks may play an important role in the susceptibility to glioma, such as *XRCC1*, *LIG4*, *XRCC4*, *PTGS2*, *ERCC1*, *ERCC2* and *TGF-β1* [6-10].

EFEMP1 is located in chromosome 2 and encodes a member of the fibulin family of extracellular matrix glycoproteins. Like all members of this family, the encoded protein contains tandemly repeated epidermal growth factor-like repeats followed by a C-terminus fibulin-type domain. Different members of the fibulin family showed different functions, either tumor-suppressive or oncogenic activity. The *EFEMP1* may play a role in the nature of many malignant tumors and interacts with its partners and modulates their functions [11, 12]. So far, only one study reported the association between *EFEMP1* variations and risk of glioma [13]. Therefore, we conducted a case-control study in a Chinese population, and investigated the association between four SNPs (rs3791679, rs1346786, rs1344733 and rs727878) in *EFEMP1* and development of glioma.

Table 1. Characteristics of patients with glioma and control subjects

Parameters	Patients	%	Controls	%	χ^2-test	*P* value
Age, years	57.32±11.70		55.14±12.10			
Gender						
Female	63	39.62	157	43.13		
Male	96	60.38	207	56.87	0.56	0.46
Smoking status						
Never	100	62.89	238	65.38		
Ever	59	37.11	126	34.62	0.30	0.58
Drinking status						
Never	101	63.52	247	67.86		
Ever	58	36.48	117	32.14	0.93	0.33
Family history of cancer						
No	145	91.19	344	94.51		
Yes	14	8.81	20	5.49	1.20	0.16
Histology type						
Glioblastoma	52	32.70				
Astrocytoma; oligodendroglioma and mixed glioma	107	67.30				
WHO						
I-II	66	41.51				
III-IV	93	58.49				

Material and methods

Patients

A case-control study was taken in the present study. A total of 159 patients who were histopathologically diagnosed to be glioma were collected at Nanfang Hospital between July 2012 and June 2014. The tumors were graded according to the World Health Organization (WHO) classification.

A group of 364 control subjects was randomly selected from the trauma outpatients and the annual check-up visitors in our hospital during July 2012 and June 2014. All the control subjects were free of glioma. The controls with a self-reported history of cancer or central nervous system-related diseases and previously receiving radiotherapy and chemotherapy for certain diseases were excluded from this hospital.

At recruitment, all participants were interviewed by trained nurses to collect detailed demographic information, such as smoking, drinking and family history of cancer. The clinical characteristics of patients with glioma were collected from medical records, such as histology types and tumor grade. Smoking status was based on self-reported smoking, and the subjects who have never smoked less than 100 cigarettes in their lives were classified as non-smokers. Drinking status was defined as non-drinker and drinker (drinks per day).

Blood samples and signed informed consent forms were obtained from enrolled individuals prior to their participation in the study. The study protocol was approved by the Clinical Research Ethics Committee of the Jiujiang First People's Hospital.

DNA extraction and genotyping

5 ml blood sample was collected from each patient with glioma and control subject, and the blood samples were collected in ethylene diamine tetra-acetic acid (EDTA)-coated tubes and stored at -20°C until use. The rs3791679, rs1346786, rs1344733 and rs727878 gene polymorphisms were analyzed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The primers of rs3791679, rs1346786, rs1344733 and rs727878 were designed using the Sequenom Assay Design 3.1 software. The reaction conditions were performed as follows:

Table 2. Distributions of *EFEMP1* polymorphisms and development of glioma

SNPs	Patients	%	Controls	%	χ^2-test	*P* value	Chromosome position	SNP location	Base change	Minor allele frequency		P for HWE
										In dbSNP database	In controls	
rs3791679												
GG	58	36.48	171	46.98	7.37	0.03	55950396	Intron10	G>A	0.2919	0.3187	0.17
GA	73	45.91	154	42.31								
AA	28	17.61	39	10.71								
rs1346786												
AA	51	32.08	129	35.44	0.54	0.76	55961837	Intron5	A>G	0.4569	0.4148	0.76
AG	76	47.80	168	46.15								
GG	32	20.12	67	18.41								
rs1344733												
AA	55	34.59	136	37.36	0.38	0.83	55981531	Intron4	A>G	0.4299	0.3984	0.83
AG	75	47.17	166	45.61								
GG	29	18.24	62	17.03								
rs727878												
AA	53	33.33	132	36.26	0.44	0.8	55973161	Intron4	A>G	0.4593	0.4121	0.80
AG	74	46.54	164	45.06								
GG	32	20.13	68	18.68								

one cycle of DNA denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, 55°C annealing step for 1 min with a 72°C extension step for 2 min, with a final extension step of 5 min at 72°C. The PCR products were analyzed by electrophoresis in a 2% agarose gel stained with ethidium bromide and visualized under UV light. For quality control, the genotyping analysis was done blind as regards the subjects.

Statistical analysis

The demographic and clinical characteristics of patients with glioma and control subjects were expressed by mean ± standard deviation or frequency and percentage. The differences between groups were compared by the *t*-test and chi-square test. The goodness-of-fit χ^2-test was taken to analyze the departures from the Hardy-Weinberg equilibrium (HWE) of genotype distributions in rs3791679, rs1346786, rs1344733 and rs727878. Unconditional logistic regression analysis was taken to analyze the association between rs3791679, rs1346786, rs1344733 and rs727878 polymorphisms and development of glioma, and the results were evaluated using the Odd's ratio (OR) and 95% confidence interval (95% CI). The major homozygous genotypes of the rs3791679, rs1346786, rs1344733 and rs727878 polymorphism were used as references. The interaction between gene polymorphism and environmental factors in the risk of glioma was analyzed using multiple logistic regression analysis. Statistical analysis was conducted using the SPSS 17.0 package (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to indicate a significant difference.

Results

The demographic and clinical characteristics of patients with glioma and control subjects are summarized in **Table 1**. The mean ages of patients with glioma and control subjects were 57.32±11.70 and 55.14±12.10, respectively. By a comparison of the demographic characteristics between patients and controls, we found no significant difference between the two groups. Of the 159 patients with glioma, 52 (32.70%) patients were glioblastoma, 107 (67.30%) were astrocytoma; oligodendroglioma and mixed glioma, 66 (41.51%) were at I-II tumor stage and 93 (58.49%) were at III-IV tumor stage.

The information of the four common SNPs in *EFEMP1* was shown in **Table 2**. By χ^2-test, we found a significant difference in the genotype distribution of rs3791679 between patients and controls (χ^2=7.37, P=0.03). By the goodness-of-fit χ^2-test, we found that the genotype distributions of rs3791679, rs1346786, rs13-

Table 3. Association between studies SNPs and development of glioma

SNP	Patients	%	Controls	%	OR (95% CI)	*P* value
rs3791679						
GG	58	36.48	171	46.98	1.0 (Ref.)	-
GA	73	45.91	154	42.31	1.41 (0.92-2.16)	0.11
AA	28	17.61	39	10.71	2.13 (1.15-3.90)	0.01
GA+AA	101	63.52	193	53.02	1.55 (1.04-2.32)	0.02
rs1346786						
AA	51	32.08	129	35.44	1.0 (Ref.)	-
AG	76	47.80	168	46.15	1.14 (0.73-1.78)	0.56
GG	32	20.12	67	18.41	1.20 (0.68-2.10)	0.5
AG+GG	108	67.93	235	64.56	1.15 (0.76-1.75)	0.48
rs1344733						
AA	55	34.59	136	37.36	1.0 (Ref.)	-
AG	75	47.17	166	45.60	1.18 (0.72-1.73)	0.6
GG	29	18.24	62	17.04	1.16 (0.65-2.05)	0.6
AG+GG	104	65.41	228	62.63	1.12 (0.75-1.70)	0.54
rs727878						
AA	53	33.33	132	36.26	1.0 (Ref.)	-
AG	74	46.54	164	45.06	1.12 (0.72-1.75)	0.59
GG	32	20.13	68	18.68	1.17 (0.67-2.05)	0.56
AG+GG	106	66.67	232	63.73	1.14 (0.76-1.72)	0.52

44733 and rs727878 were in line with Hardy-Weinberg equilibrium, and the *P* values were 0.17, 0.76, 0.83 and 0.80, respectively (**Table 2**). Moreover, the minor allele frequencies of rs3791679, rs1346786, rs1344733 and rs727878 were similar with those in dbSNP databases.

By unconditional logistic regression analysis, we found that individuals carrying the AA genotype and GA+AA genotype were associated with development of glioma when compared with the GG genotype, and the adjusted ORs (95% CI) were 2.13 (1.15-3.90) and 1.55 (1.04-2.32), respectively (**Table 3**). However, we did not find that rs1346786, rs1344733 and rs727878 were significantly associated with development of glioma.

By stratification analysis, we found that the GA+AA genotype of rs3791679 was associations with a heavy increased risk of glioma in patients who have family history of cancers, and the OR (95% CI) was 6.81 (1.17-48.06) (**Table 4**). However, we did not find significant association of rs3791679 polymorphism with smoking status and drinking status in the risk of glioma.

Discussion

It is well known that individuals may not develop the same type of cancer despite being exposed to similar environmental conditions. Therefore, genetic variations may play an important role in the development of cancers. In the present study, we conducted a case-control study to investigate the association between four common SNPs in *EFEMP1* and development of glioma in a Chinese population, and we found that the rs3791679 polymorphism was associated with susceptibility to glioma.

EGF-containing fibulin-like extracellular matrix protein1 is also called *EFEMP1*. Fibulins are a seven-member family of secreted glycoproteins, and they have a role in repeating epidermal growth-factor-like domains and a unique C-terminal structure [12]. Glycoproteins include a complex network structure, support and connect the organizational structure, regulate tissue and cell physiological activity, and thus contribute the formation and development of cancers [12]. Previous studies have reported that the members of the fibulin family have a role in tumor-suppressive and oncogenic activity [11, 12].

Currently, many studies have reported the association between low expression of *EFEMP1* and development of cancers, such as prostate cancer, hepatocellular carcinoma and non-small cell lung cancer [14-17]. Almeida et al. reported that *EFEMP1* promoter methylation is associated with development of prostate cancer, and this epigenetic alteration of *EFEMP1* is associated with prostate carcinogenesis [14]. Nomoto et al. have reported that downregulation of *EFEMP1* expression was associated with promoter hypermethylation, and was a marker of worse prognosis in hepatocellular carcinoma [15]. Lang et al. have reported that *EFEMP1* has a role in suppressing the lung cancer growth and invasion [17]. However, some stud-

Table 4. Stratification analysis of rs3791679 polymorphism in the development of glioma

Variables	Patients		Controls		OR (95% CI)	*P* value
	GG	GA+AA	GG	GA+AA		
Smoking status						
Never	38	62	112	126	1.45 (0.88-2.41)	0.13
Ever	20	39	59	67	1.77 (0.89-3.57)	0.08
Drinking status						
Never	37	64	114	133	1.48 (0.90-2.46)	0.1
Ever	21	37	57	60	1.73 (0.87-3.50)	0.09
Family history of cancer						
No	55	90	159	185	1.41 (0.93-2.14)	0.09
Yes	3	11	12	8	6.81 (1.17-48.06)	0.01

ies reported that the high expression of *EFEMP1* was associated with development of breast cancer and pancreatic cancers [18, 19]. En-lin et al. have reported that over expression was significantly correlated with lymph node metastasis, vascular invasion and poor survival of cervical cancer [18]. Seeliger et al. overexpression of *EFEMP1* has protumorigenic effects on pancreatic cancer in vivo and in vitro [19]. These previous studies have suggested that gene expression of *EFEMP1* was associated with the development and prognosis of cancers.

For the association between *EFEMP1* polymorphisms and susceptibility to glioma, only one previous study reported their association [13]. Zhang et al. conducted a study in a Chinese population, and they found that *EFEMP1* rs3791679, rs134678, rs1346787 and rs3791675 polymorphisms contributed to the susceptibility of glioma [13]. In our study, we reported that rs3791679 polymorphism was associated with the development of glioma, but no significant association was found between rs1346786, rs1344733 and rs727878 polymorphisms and susceptibility to glioma. Moreover, our study found that rs3791679 has interaction with family history of cancer in the glioma risk, which suggests that rs3791679 may be an inherited factor for the development of glioma.

Two limitations in our study should be taken into consideration. First, patients with glioma and control subjects were collected from the same hospital, which would cause selection bias in our study. However, the genotype distributions of rs3791679, rs1346786, rs1344733 and rs727878 in *EFEMP1* did not deviated from the Hardy-Weinberg equilibrium, which suggests that the study participants could represent the general population. Second, the sample size of the glioma patients is small, which could results in a lack of statistical power. Therefore, further studies with more sample size and more ethnicities are needed to confirm our results.

In conclusion, the results of our study suggested an association between the rs3791679 polymorphism and an elevated risk of glioma; in addition, rs3791679 interacts with the family history of cancer in contributing to the development of glioma. Future studies using larger sample sizes, and employing either similar or different analytical strategies may help in elucidating the impact of *EFEMP1* gene polymorphisms on the risk of glioma.

Disclosure of conflict of interest

None.

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