Role of *IL-8* gene polymorphisms in glioma development in a Chinese population

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ABSTRACT. This case-control study aimed to investigate the role of -251 T>A (rs4073) and -781 C>T (rs2227306) polymorphisms in the interleukin-8 (*IL-8*) gene in the development of glioma in a Chinese population. One hundred and twenty-seven glioma patients and 284 healthy control subjects were recruited to this study between February 2013 and December 2014. The *IL-8* -251 T>A (rs4073) and -781 C>T (rs2227306) polymorphisms were genotyped by polymerase chain reaction coupled with restriction fragment length polymorphism. The patients and control subjects were comparable by gender (χ^2 = 1.24, P = 0.27), tobacco smoking status (χ^2 = 0.80, P = 0.37), alcohol consumption status (χ^2 = 0.97, P = 0.32), and family history of cancer (χ^2 = 1.54, P = 0.22). The age of glioma patients was statistically lower than that of control subjects (t = 2.87, P = 0.002). The chi-square test revealed the lack of any statistically significant differences in the genotype distributions of *IL-8* rs4073 (χ^2 = 0.89, P = 0.64) and rs2227306 (χ^2 = 2.58, P = 0.28) between the glioma patients and control subjects. Unconditional logistic regression analysis revealed that the

IL-8 rs4073 and rs2227306 gene polymorphisms did not contribute to the development of glioma. In conclusion, we determined that there is a lack of evidence suggesting a significant association between the *IL-8* rs4073 and rs2227306 gene polymorphisms and the development of glioma in a Chinese population.

Key words: IL-8; Polymorphism; Glioma; Chinese population

INTRODUCTION

Glioma accounts for more than 70% of all brain tumors (Wen and Kesari, 2008). Tumors in the central nervous system have been estimated to be the fifth and second most common type of tumors affecting adults and children, respectively. Glioma has a poor prognosis and is correlated with high mortality, despite the development of advanced treatment strategies for this disease (Ohgaki and Kleihues, 2005; Jakola et al., 2012). Moreover, the etiology of this disease remains unclear, exposure to high doses of ionizing radiation contributes to glioma development (Schwartzbaum et al., 2006). Several studies conducted so far have shown that genetic factors such as *XRCC1*, *CCDC26*, *CASP8*, *D302H*, *LIG4*, *XRCC4*, *PTGS2*, and *TGF-β1* play a critical role in the development of glioma (Li et al., 2015; Cacina et al., 2015; Cui, 2015; Lin et al., 2015; Lu et al., 2015; Su et al., 2015; Vieira de Castro et al., 2015).

Previous studies have indicated that IL-8 is correlated with many autoimmune diseases and infectious diseases, as well as tumors (Xie, 2001; Brat et al., 2005). Approximately 1% of the population shows genetic variations that determine the susceptibility of an individual to cancers (Liu et al., 2006). IL-8 is encoded by the *IL-8* gene, which is located on chromosome 4q13-21 and is composed of four exons, three introns, and the proximal promoter region (Mukaida et al., 1989). Here, we conducted a case-control study to investigate the role of *IL-8* -251 T>A (rs4073) and -781 C>T (rs2227306) gene polymorphisms in the development of glioma in a Chinese population.

MATERIAL AND METHODS

Patients

We recruited 127 glioma patients and 284 healthy controls from Nanfang Hospital between February 2013 and December 2014. All patients were diagnosed by pathological examinations conducted independently by two pathologists. The control subjects were recruited from among individuals who underwent a regular health examination at the hospital. All control subjects were confirmed to be free of glioma or any central nervous system tumors on performing computed tomography or magnetic resonance imaging examination. Study subjects with a history of cancer (or with recurrent cancer), or with serious kidney and liver diseases were excluded from this study.

The demographic characteristics of the glioma patients and control subjects, including the age, gender, and tobacco smoking and alcohol consumption status, and the family history of cancer, were collected through a questionnaire. Data regarding the clinical characteristics of glioma, including histological type (high and low grades) and WHO tumor stage (I-II and III-IV stages), were collected from the patient medical records.

Signed informed consent forms were obtained from the patients and controls. The study was approved by the Ethics Committee of the Nanfang Hospital.

Genotyping methods

Genomic DNA was extracted using the TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to the manufacturer instructions. The *IL-8* -251 T>A (rs4073) and -781 C>T (rs2227306) polymorphisms were genotyped by polymerase chain reaction coupled with restriction fragment length polymorphism (PCR-RFLP). The PCR primers are summarized in Table 1. The *IL-8* rs4073 and rs2227306 were digested using *Mfe*I and *Bcl*I restriction enzymes, respectively. The cycling conditions were set as follows: initial denaturation at 95°C for 10 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 10 min.

Table 1. Primers used to genotype the *IL-8* rs4073 and rs2227306 polymorphisms.

Gene	SNP	Primers (5'-3')
IL-8 -251 T>A	rs4073	Forward: TAAAATACTGAAGCTCCACAATGG Reverse: ATCTTGTTCTAACACCTGCCACTCT
IL-8 -781 C>T	rs2227306	Forward: AAGGGTCAGTGTGGTATCACAGAG Reverse: GTCAGTCAGTCATAACTGACAAGC

Statistical analysis

The differences in demographic and lifestyle characteristics between groups were analyzed using the chi-square test or the Student *t*-test. Genotypes of the *IL-8* rs4073 and rs2227306 from the Hardy-Weinberg equilibrium (HWE) were analyzed using the Pearson chi-square test. The association between gene polymorphisms and development of glioma was determined using logistic regression analysis; the results are reported as odds ratios (OR) and their 95% confidence intervals (CIs). A P value <0.05 was considered to be a significant difference.

RESULTS

The baseline and clinical data of study subjects are summarized in Table 2. The patients and control subjects were comparable by gender ($\chi^2 = 1.24$, P = 0.27), tobacco smoking status ($\chi^2 = 0.80$, P = 0.37), alcohol consumption status ($\chi^2 = 0.97$, P = 0.32), and family history of cancer ($\chi^2 = 1.54$, P = 0.22). The age of glioma patients was lower compared to that of control subjects ($t = 2.87$, P = 0.002). Of the 127 glioma patients, 40 (31.50%) showed a high-grade tumor (histological type), while 87 (68.50%) patients showed low-grade tumor; 52 patients (40.94%) were at tumor stages I-II, and 75 (59.06%) displayed tumor stages III-IV.

The genotype distribution and their association with glioma risk are shown in Table 3. The chi-square test showed no statistically significant differences in the genotype distributions of *IL-8* rs4073 ($\chi^2 = 0.89$, P = 0.64) and rs2227306 ($\chi^2 = 2.58$, P = 0.28) between glioma patients and control subjects. The goodness-of-fit χ^2 test revealed that the genotype frequencies of *IL-8* rs4073 and rs2227306 did not deviate from the HWE (P values = 0.25 and 0.79, respectively). Unconditional logistic regression analysis revealed that the *IL-8* rs4073 and rs2227306 gene

polymorphisms did not contribute to the development of glioma (Table 3). Moreover, the allele frequencies of *IL-8* rs4073 and rs2227306 were not significantly associated with glioma risk.

Table 2. Demographic characteristics of glioma patients and control subjects.

Variables	Patients (N = 127)	%	Controls (N = 284)	%	Chi-square test or Student *t*-test	P value
Age (years)	41.4 0 ± 17.32		46.52 ± 16.42		2.87	0.002
Gender						
Male	79	62.20	160	56.34		
Female	48	37.80	124	43.66	1.24	0.27
Tobacco smoking						
Never	57	44.88	141	49.65		
Ever	70	55.12	143	50.35	0.80	0.37
Alcohol consumption						
Never	97	76.38	229	80.63		
Ever	30	23.62	55	19.37	0.97	0.32
Family history of cancer						
No	114	89.76	265	93.31		
Yes	13	10.24	19	6.69	1.54	0.22
Histology type						
High grade	40	31.50				
Low grade	87	68.50				
WHO tumor stage						
I-II	52	40.94				
III-IV	75	59.06				

Table 3. Association between *IL-8* rs4073 and rs2227306 gene polymorphisms and development of glioma.

IL-8	Patients (N = 127)	%	Controls (N = 284)	%	Chi-square test	P value	P for HWE	OR (95%CI)	P value
rs4073									
TT	32	25.20	83	29.23				1.0 (Ref.)	-
TA	60	47.24	132	46.48				1.18 (0.69-2.04)	0.53
AA	35	27.56	69	24.30	0.89	0.64	0.25	1.32 (0.71-2.44)	0.35
Allele									
T	124	48.82	298	52.74				1.0 (Ref.)	-
A	130	51.18	270	47.79	0.93	0.33		1.16 (0.85-1.57)	0.33
rs2227306									
CC	54	42.52	139	48.94				1.0 (Ref.)	-
CT	55	43.31	118	41.55				1.20 (0.75-1.93)	0.43
TT	18	14.17	27	9.51	2.58	0.28	0.79	1.72 (0.82-3.53)	0.11
Allele									
C	163	64.17	396	69.72				1.0 (Ref.)	-
T	91	35.83	172	30.28	2.48	0.12		1.29 (0.93-1.78)	0.12

DISCUSSION

Here, we performed a study to assess the association between *IL-8* rs4073 and rs2227306 gene polymorphisms and glioma risk in a Chinese population; however, we observed no significant association between the *IL-8* rs4073 and rs2227306 gene polymorphisms and the development of glioma.

Several studies have reported the association between polymorphisms in the *IL-8* gene and the development of different types of cancers, including liver, gastric, lung, ovarian, and colorectal cancers (Burada et al., 2013; Yang et al., 2014; Koensgen et al., 2015; Kumar et al., 2015; Wang et al., 2014, 2015). A previous meta-analysis conducted by Yang et al. (2014)

indicated that the *IL-8* rs4073 gene polymorphism does not influence the susceptibility to oral cancer. In a case-control study comprising 205 patients with hepatocellular carcinoma (HCC) and 208 healthy controls, Wang et al. (2014) discovered no association between the *IL-8* -251 A/T, +781 C/T, -353 A/T, and +678 T/C polymorphisms and risk of HCC in a Chinese population. Burada et al. (2013), in a case-control study comprising 144 patients and 233 controls, reported that the *IL-8* rs4073 polymorphism does not influence the risk of colorectal cancer. On the other hand, another case-control study performed in an Indian population reported that the *IL-8* rs4073 gene polymorphism plays an important role in *Helicobacter pylori*-associated gastric carcinogenesis in northern India (Kumar et al., 2015). Wang et al. (2015) suggested that mutations in the *IL-8* gene confer an increased risk of lung cancer in Asians. Another study discovered an association between the *IL-8* +781 and +2767 polymorphisms and susceptibility to ovarian cancer in a German population (Koensgen et al., 2015).

A recent study reported that *IL-8* silencing could suppress the invasion of human glioma U87 cell line (Zhu et al., 2013). Another study revealed that the autocrine function of IL-8 contributes to the invasive phenotype of glioma (Zhang et al., 2015). Only one previous study comprising 300 glioma patients and 300 healthy controls reported an association between the occurrence of the AA genotype at *IL-8* rs4073 and increased risk of glioma (Liu et al., 2015). However, there is a lack of evidence confirming the association between the *IL-8* rs4073 and rs2227306 gene polymorphisms and glioma risk.

In conclusion, we did not observe a statistically significant association between the *IL-8* rs4073 and rs2227306 polymorphisms and development of glioma in a Chinese population. Further large-scale studies are required to confirm this finding.

Conflicts of interest

The authors declare no conflict of interest.

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