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Original Article

Association between *STAT5* polymorphisms and glioblastoma risk in Han Chinese population

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ABSTRACT

The aim of this study was to investigate the associations between *STAT5* gene polymorphisms and glioblastoma (GBM) risk predisposition. We undertook a case–control study to analyze two *STAT5* polymorphisms (*STAT5a* rs11079041 and *STAT5b* rs2293157) in a Han Chinese population, by extraction of genomic DNA from the peripheral blood of 328 patients with glioma and 342 control participants, and performed *STAT5* genotyping using DNA sequencing. The obtained results indicated that overall, no statistically significant association was observed in *STAT5a* rs11079041. Nevertheless, *STAT5b* rs2293157 G/T genotype was at increased risk of glioma ($P = 0.001$). Furthermore, rs2293157T allele was more significantly prognostic in patients suffering from glioblastoma compared to other subtypes of gliomas ($P < 0.001$; odds ratio (OR) = 5.14, CI 95%: 2.70–9.79). These findings led us to conclude that polymorphism in *STAT5b* rs2293157 G/T was observed to be associated with susceptibility of glioblastoma. Nevertheless, further investigation with a later confirmation in another ethnical or geographical cohort is required.

Introduction

Astrocytoma grade IV, also known as glioblastoma (GBM), is the most frequent type of brain tumor and is invariably associated with a poor prognosis [1,2]. In spite of some therapeutic improvements from multiple treatment modalities, the typical median survival remains only 12–15 months [1,2]. Therefore, enhancing our knowledge of molecular biology of glioblastoma will aid to cover the gaps in comprehending the pathogenesis of this malignant tumor and potentially improve patients' clinical outcome. And currently, genetic susceptibility, with regard to specific genotypes, suggests the combination of some genetic alleles in the etiology of this kind of neoplasm [3,4].

Signal transducers and activators of transcription (STAT) is a family of transcription factors and is involved in a wide variety of cellular physiological processes, including differentiation, survival, or cell growth [5–7]. To date, seven *STAT* genes have been identified. STAT5 is a latent cytoplasmic protein that comprises two highly homologous isoforms, 94 kDa STAT5a and 92 kDa STAT5b [8]. Although these two STAT proteins share a considerable functional overlap, gene-disruption experiments have revealed that STAT5a and STAT5b are functionally not redundant [9–11].

STAT5 has been shown to regulate proliferation and inhibition of apoptosis in several cancer cells [12]. For instance, STAT5 activation has been shown primarily in hematopoietic malignancies, which are associated with Bcl-Abl fusion protein [13]. In a previous study, Li and colleagues reported that STAT5 was correlated with aggressiveness of prostate cancer [14]. In another work, Liang et al. suggested that blocking STAT5b activity in glioblastoma cells is a potential novel therapeutic approach, since STAT5b is implicated in many areas of tumor progression, including cell growth, cell cycle regulation, invasion and migration [15].

Currently, many groups are carrying out association studies between single nucleotide polymorphisms (SNPs) of genes involved in the STAT5 pathway and risk of malignancies [16–18]. Among them, rs11079041 located in *STAT5a* intron 1 and rs2293157 located in *STAT5b* intron 8 (http://www.ncbi.nlm.nih.gov/SNP) attracted our attention. In one study, interaction analyses of *STAT5* SNPs (rs11079041|rs2293157) showed that there were inferior associations in chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML) compared to the control group. However, linkage disequilibrium existed between rs11079041 and rs2293157 in both leukemia and control groups [17].

Single-nucleotide polymorphisms are the most common human genetic variation and may contribute to an individuals'

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susceptibility to cancer. Thus, in this study, in order to clarify the association between *STAT5a* rs11079041 and *STAT5b* rs2293157 polymorphisms and glioblastoma risks, we have performed a hospital-based case–control study on Han Chinese population.

Materials and methods

Subjects

A total of 328 cases of patients with glioma and 342 healthy controls were qualified for this study. All samples were collected before any kind of therapeutic measures between April 2009 and November 2011 at Department of Neurosurgery of Qilu Hospital of Shandong University, and Department of Neurosurgery of Affiliated Hospital of Binzhou Medical College. The patients were all Han Chinese from the same geographical region. Tumor histological type and grade were determined according to the WHO criteria [19]. The patient samples were collected after the diagnosis was confirmed by histopathological analysis after operative therapy. None of the patients had received radiotherapy or chemotherapy prior to surgery. Written informed consent was obtained from all participants. The study protocol was approved by the Ethics Committee of Qilu Hospital of Shandong University and Binzhou Medical College in accordance with the Declaration of Helsinki (2000).

DNA extraction

Blood samples from patients with gliomas and the control group were collected by Vacutainer and transferred to ethylenediaminetetraacetic acid (EDTA) tubes. Genomic DNA from whole blood cells was extracted using a QIAamp Blood kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. DNA concentration and purity of each sample were measured by ultraviolet spectrophotometer (Eppendorf, Hamburg, Germany). DNA samples were routinely stored at −20 °C.

Genotyping

Analysis of the *STAT5* SNPs, rs11079041 located in STAT5a intron 1 and rs2293157 located in STAT5b intron 8, were performed using multiplex polymerase chain reaction (PCR) with an ABI premix. Primers for polymerase chain reaction (PCR) and single base extension were designed using the Assay Designer software package (Sequenom). Primers for STAT5a (rs11079041) were: forward 5′-CTCGATCCGATGACCTGAGT-3′; reverse 5′-CGCATCGTATTACTAGTTCTAC-3′. Primers for STAT5b (rs2293157) were: forward 5′-GCTACTACTTTCCTGCCTGC-3′; reverse 5′-CTCGTACATTACCGCATCGGTTC-3′. Genomic DNA from whole blood was used as a PCR template in a total reaction volume of 10 μL that contained 10 pmol designed primers. PCR was performed as follows: one cycle at 94 °C for 10 min, 35 cycles at 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 30 s, the final extension was at 72 °C for 10 min. PCR products were analyzed on a 3% agrose gels stained by addition of ethidium bromide, photographs were taken under ultraviolet light using a transilluminator. Subsequently, PCR product was sequenced in an ABI PRISM 3100 sequencer using BigDye Terminator v3.1 Cycle Sequencing method (Applied Biosystems, USA) as recommended by the manufacturer.

Statistical analysis

Statistical calculations were performed using the SPSS Statistics 13.0 for Windows software package (SPSS Inc., Chicago, Ill). Frequency of mutations was compared with the χ^2 test. The *P* values obtained were 2-tailed, and significance was assumed less than 0.05. The Hardy–Weinberg equilibrium (HWE) was verified for the different polymorphisms studied; *P* value >0.05 implicated that the distribution did not deviate from the equilibrium. The crude and adjusted odds ratio (OR) and the corresponding 95% confidence intervals (CI) were calculated using unconditional multiple logistic regression.

Table 1
Characteristics of patients with glioma and controls.

Variable	Patients (n = 328)	Controls (n = 342)	*P*
Gender (%)			0.95
Male	192 (58.5)	201 (58.8)	
Female	136 (41.5)	141 (41.2)	
Age, mean (SD) in years	45.3 (6.4)	42.9 (7.5)	0.31
Smoking status (%)			0.30
Never	216 (65.9)	238 (69.6)	
Ever	112 (34.1)	104 (30.4)	
Drinking (%)			0.26
Never	167 (50.9)	189 (55.3)	
Ever	161 (49.1)	153 (44.7)	
Family history of cancer (%)			0.39
No	291 (88.7)	296 (86.5)	
Yes	37 (11.3)	46 (13.5)	
Histology (%)			
Astrocytomas[a]	127 (38.7)		
Glioblastoma	115 (35.1)		
Other gliomas[b]	86 (26.2)		

[a] Astrocytomas including diffuse astrocytomas, anaplastic astrocytomas and astrocytomas with a partially oligodendroglial differentiation.
[b] Other gliomas including oligodendrogliomas, enpendymomas, or mixed glioma.

Results

Characteristics of study population

The details of selected characteristics of 328 glioma patients and 342 control subjects are shown in Table 1. The differences in the distributions of gender, age, smoking status, drinking status and family history of cancer between patients and controls were not statistically significant (*P* = 0.95, 0.31, 0.30, 0.26 and 0.39, respectively). The mean age of patients (45.3 years) at diagnosis was slightly older than the reference age for controls (42.9 years). 58.5% cases and 58.8% controls were male. Among 328 glioma patients, there were 115 glioblastomas, 127 astrocytomas and 86 other gliomas (Table 1).

STAT5 polymorphisms in glioma

The gene polymorphisms of *STAT5* gene rs11079041 and rs2293157 were successfully amplified in the majority of patients and control cases; however, 3–6 samples failed for glioma patients and 4–6 samples for control subjects, as shown in Table 2. The number of patients with *STAT5* gene polymorphisms of rs11079041T/A and rs2293157 G/T were 150/322 cases and 172/325 cases, respectively. The genotypic distributions of all the two gene polymorphisms in patients and controls were in Hardy–Weinberg equilibrium (all *P* > 0.05). Overall, no statistically significant association was observed in *STAT5* rs11079041T/A polymorphism. Individuals with *STAT5* rs2293157 G/T genotype were more susceptible to glioma (*P* = 0.001, OR = 1.24).

STAT5 rs2293157 and risk of glioblastoma stratified by histology

Next, we performed the stratified analysis for *STAT5* rs2293157 by glioma histology. As shown in Table 3, statistically significant differences were observed in allele and genotype distributions of rs2293157 G/T between glioblastoma patients and control subjects (both are *P* < 0.001). Overall, the variant T allele was associated with an increased risk of glioblastoma compared with the G allele