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# Association of three common single nucleotide polymorphisms of *SLC7A7* with the development of glioma in a Chinese population

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**ABSTRACT.** Gliomas are brain tumors that can be seriously damaging to human health. The *SLC7* family is involved in amino acid or peptide transportation. The relationship between *SLC7A7* polymorphisms and the development of glioma has been reported previously by a few studies. Therefore, we performed a hospital based case-control study to investigate the association of three common SNPs (rs12888930, rs12436190, and rs2065134) of *SLC7A7* with the development of glioma in a Chinese population. From January 2014 to December 2015, 122 patients with glioma and 252 individuals (controls) were recruited from the department of neurosurgery of Tangshan People's Hospital affiliated to North China University of Science and Technology. *SLC7A7* rs12888930, rs12436190, and rs2065134 genotyping was performed by the polymerase chain reaction-restriction fragment length polymorphism method. Multiple logistic regression analysis showed that a significantly higher risk of glioma was harbored by the GG and AG + GG genotypes than by the AA genotype; OR (95%CI) was 2.24 (1.18-4.22) and 1.59 (1.01-2.60), respectively. However, no significant

relationship was observed between *SLC7A7* rs12888930 and rs2065134 and the risk of glioma. In conclusion, this study reports a significant association between *SLC7A7* rs12436190 and the risk of glioma in a Chinese population.

**Key words:** Glioma; *SLC7A7*; Single nucleotide polymorphism; Polymorphism

## INTRODUCTION

The rate of incidence of glioma, a common type of brain tumor arising from neuroglia cells, keeps increasing (Bondy et al., 2008; Ferlay et al., 2012; IARC et al., 2011). The prognosis of glioma is poor, and the average overall survival of glioma patients is only 15 months. Therefore, early employment of glioma prevention strategies is necessary. It is well established that glioma is caused by multiple serious factors, including genetic and environmental factors (Bondy et al., 2008; Ostrom and Barnholtz-Sloan, 2011); however, the specific etiology of the tumor are not completely understood. Therapeutic or high-dose ionizing radiation exposure are the only established risk factors for glioma (Bondy et al., 2008; Ostrom and Barnholtz-Sloan, 2011). Moreover, recent genome-wide studies have reported that some genes could contribute to the development of glioma through influencing genomic stability, angiogenesis, and inflammation (Shete et al., 2009; Ghasimi et al., 2016).

The SLC group of proteins is the second largest category of the human genome transmembrane protein family (Cedernaes et al., 2011). It has been reported previously that the overexpression of SLC7 could contribute to the cellular growth and extension of cell cycles and thus, increase susceptibility to cancer (Wang et al., 2013). Thus far, only one study has reported the relationship between *SLC7A7* polymorphisms and the development of glioma (Fan et al., 2013). Therefore, we performed a hospital based case-control study to investigate the association of three common SNPs (rs12888930, rs12436190 and rs2065134) in *SLC7A7* with the development of glioma in a Chinese population. We also assessed the effect of gene-environment interactions in the risk of glioma.

## MATERIAL AND METHODS

### Subjects

A hospital-based case-control design was implemented in this study. A total of 122 patients with glioma were recruited, between January 2014 and December 2015, from the department of neurosurgery of Tangshan People's Hospital affiliated to the North China University of Science and Technology. The grade of glioma was diagnosed based on the pathological grading criteria established by World Health Organization (WHO) in 1997. Simultaneously, 252 subjects, designated as controls, were recruited from the physical examination center of Tangshan People's Hospital affiliated to North China University of Science and Technology. These study participants (controls) were confirmed to have no history of any malignant tumors, nervous system diseases, or serious kidney or liver diseases.

The demographic and clinical variables of patients with glioma and control subjects were collected from their medical records or face-to-face investigations with a questionnaire.

The demographic characteristics considered for the investigated subjects included sex, age, alcohol drinking and tobacco smoking habits, history of radiation exposure, family history of cancer, and WHO grade of glioma.

Signed consent forms were obtained from each study participant. The study received approval from the Research Ethics Committee of Tangshan People's Hospital affiliated to North China University of Science and Technology.

## DNA extraction and genotyping

Five separate blood samples, collected from each participant after study enrollment, were kept in tubes with 10.0~12.5 IU/mL EDTA. Genomic DNA was extracted using Sangon DNA blood mini kit (Sangon, Shanghai, China) and kept at -80°C for later use. The primers for *SLC7A7* rs12888930, rs12436190, and rs2065134 were designed by Primer Premier 5.0 software. *SLC7A7* rs12888930, rs12436190, and rs2065134 were amplified and genotyped by the polymerase chain reaction-restriction fragment length polymorphism method. The reaction process included an initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 15 s, annealing at 62°C for 30 s, extension at 72°C for 1 min, and a final cycle of 72°C for 10 min. The enzyme reaction products were separated by agarose gel electrophoresis and observed by the gel imaging system for genotype determination.

## Statistical analysis

The differences in the demographic and clinical characteristics of glioma patients and controls were determined by Pearson's chi-square test or Fisher's exact test. The Hardy-Weinberg equilibrium (HWE) of genotype distributions was confirmed using the Pearson chi-square test with one degree of freedom. The relationship between *SLC7A7* rs12888930, rs12436190 and rs2065134 and the risk of glioma was assessed by multiple logistic regression analysis, and the results were expressed by the OR and 95%CI. The most common homozygote genotypes of *SLC7A7* rs12888930, rs12436190, and rs2065134 were designated as the reference group. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 17.0. (IBM Corp., Foster city, CA, USA). P values less than 0.05 were statistically significant.

# RESULTS

The demographic, lifestyle, and clinical variables of patients with glioma and controls are shown in Table 1. Using chi-square test, we found that glioma patients were more likely to have a history of radiation exposure in comparison to the control subjects ($\chi^2 = 13.28$, $P < 0.001$). However, no significant differences were observed based on age ($\chi^2 = 0.27$, $P = 0.60$), sex ($\chi^2 = 0.33$, $P = 0.57$), tobacco smoking ($\chi^2 = 0.21$, $P = 0.65$), alcohol consumption ($\chi^2 = 0.19$, $P = 0.66$), and family history of cancer ($\chi^2 = 2.28$, $P = 0.13$). There were 41 glioma patients at the I-II stage and 81 patients at the III-IV stage of cancer.

The distributions of *SLC7A7* rs12888930, rs12436190 and rs2065134 in glioma patients and controls are shown in Table 2. The chi-square test revealed significant differences in the genotype distributions of rs12436190 between glioma patients and control subjects ($\chi^2 = 7.27$, $P = 0.03$). However, the genotype distributions of *SLC7A7* rs12888930 ($\chi^2 = 1.75$, P =

0.42), and rs2065134 ($\chi^2$ = 0.71, P = 0.70) did not reveal significant differences between the two study groups. Genotype distributions of rs12888930 ($\chi^2$ = 3.02, P = 0.08) and rs12436190 ($\chi^2$ = 1.93, P = 0.16) were in line with the HWE, while the genotype distribution of rs2065134 ($\chi^2$ =) was not. Moreover, the minor allele frequencies of *SLC7A7* rs12888930, rs12436190 and rs2065134 were similar to those in the database of short genetic variation in NCBI (https://www.ncbi.nlm.nih.gov/snp/).

**Table 1.** Demographic, lifestyle, and clinical variables of investigated patients and controls.

| Variables | Patients (N = 122) | % | Controls (N = 252) | % | $\chi^2$ value | P value |
|---|---|---|---|---|---|---|
| Age (in years) | | | | | | |
| <50 | 77 | 63.11 | 166 | 65.87 | | |
| ≥50 | 45 | 36.89 | 86 | 34.13 | 0.27 | 0.60 |
| Gender | | | | | | |
| Female | 48 | 39.34 | 107 | 42.46 | | |
| Male | 74 | 60.66 | 145 | 57.54 | 0.33 | 0.57 |
| Tobacco smoking | | | | | | |
| Never | 75 | 61.48 | 161 | 63.89 | | |
| Have smoked | 47 | 38.52 | 91 | 36.11 | 0.21 | 0.65 |
| Alcohol consumption | | | | | | |
| Never | 80 | 65.57 | 171 | 67.86 | | |
| Have consumed | 42 | 34.43 | 81 | 32.14 | 0.19 | 0.66 |
| Family history of cancer | | | | | | |
| No | 112 | 91.80 | 241 | 95.63 | | |
| Yes | 10 | 8.20 | 11 | 4.37 | 2.28 | 0.13 |
| Radiation exposure | | | | | | |
| No | 114 | 93.44 | 251 | 99.60 | | |
| Yes | 8 | 6.56 | 1 | 0.40 | 13.28 | <0.001 |
| WHO grade of glioma | | | | | | |
| I-II | 41 | 33.61 | | | | |
| III-IV | 81 | 66.39 | | | | |

WHO: World Health Organization.

**Table 2.** Genotype frequencies of *SLC7A7* rs12888930, rs12436190 and rs2065134.

| *SLC7A7* | Patients (N = 122) | % | Controls (N = 252) | % | $\chi^2$ value | P value | $\chi^2$ value for HWE | P for HWE | Minor allele frequency | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Controls | Database |
| rs128889300 | | | | | | | | | | |
| GG | 47 | 38.52 | 113 | 44.84 | | | | | | |
| GA | 52 | 42.62 | 102 | 40.48 | | | | | | |
| AA | 23 | 18.85 | 37 | 14.68 | 1.75 | 0.42 | 3.02 | 0.08 | 0.3492 | 0.3734 |
| rs12436190 | | | | | | | | | | |
| AA | 37 | 30.33 | 103 | 40.87 | | | | | | |
| AG | 52 | 42.62 | 108 | 42.86 | | | | | | |
| GG | 33 | 27.05 | 41 | 16.27 | 7.27 | 0.03 | 1.93 | 0.16 | 0.3770 | 0.3852 |
| rs2065134 | | | | | | | | | | |
| TT | 101 | 82.79 | 216 | 85.71 | | | | | | |
| TG | 16 | 13.11 | 29 | 11.51 | | | | | | |
| GG | 5 | 4.10 | 7 | 2.78 | 0.71 | 0.70 | 17.39 | <0.001 | 0.0853 | 0.0924 |

HWE: Hardy-Weinberg equilibrium.

The association between *SLC7A7* rs12888930, rs12436190 and rs2065134 and the risk of glioma are shown in Table 3. By multiple logistic regression analysis, we observed that the GG genotype of rs12436190 harbored a significantly higher risk of glioma compared to the AA genotype, with an OR (95%CI) of 2.24 (1.18-4.22). Moreover, we observed that the AG + GG genotype of rs12436190 was associated with an increased risk of glioma in comparison to the AA genotype, with an OR (95%CI) of 1.59 (1.01-2.60). However, no significant relationship was found between *SLC7A7* rs12888930 and rs2065134 and the risk of glioma.

**Table 3.** Association between *SLC7A7* rs12888930, rs12436190, and rs2065134 and the risk of glioma.

| SLC7A7 | Patients (N = 122) | % | Controls (N = 252) | % | OR (95%CI)[1] | P value |
|---|---|---|---|---|---|---|
| rs12888930 | | | | | | |
| GG | 47 | 38.52 | 113 | 44.84 | 1.0 (Ref.) | - |
| GA | 52 | 42.62 | 102 | 40.48 | 1.23 (0.74–2.03) | 0.40 |
| AA | 23 | 18.85 | 37 | 14.68 | 1.49 (0.76–2.90) | 0.20 |
| GA+AA | 75 | 61.47 | 139 | 55.16 | 1.30(0.82–2.07) | 0.25 |
| rs12436190 | | | | | | |
| AA | 37 | 30.33 | 103 | 40.87 | 1.0 (Ref.) | - |
| AG | 52 | 42.62 | 108 | 42.86 | 1.34 (0.79–2.29) | 0.25 |
| GG | 33 | 27.05 | 41 | 16.27 | 2.24 (1.18–4.22) | 0.007 |
| AG+GG | 85 | 69.67 | 149 | 59.13 | 1.59 (1.01–2.60) | 0.04 |
| rs2065134 | | | | | | |
| TT | 101 | 82.79 | 216 | 85.71 | 1.0 (Ref.) | - |
| TG | 16 | 13.11 | 29 | 11.51 | 1.18 (0.57–2.36) | 0.62 |
| GG | 5 | 4.1 | 7 | 2.78 | 1.53 (0.37–5.74) | 0.48 |
| TG+GG | 21 | 17.21 | 36 | 14.29 | 1.25 (0.66–2.32) | 0.46 |

[1]Adjusted for sex, age, and history of radiation exposure.

Additionally, we analyzed the gene-environment interaction between rs12436190 polymorphism and environmental factors in the risk of glioma (Table 4). We did not find evidence for any interaction between them; however, we observed a marginal non-significant association between rs12436190 polymorphism and a family history of cancer in the risk of glioma (Correlation coefficient = 0.08; P = 0.06).

**Table 4.** Interaction between rs12436190 polymorphism and environmental factors in the risk of glioma.

| Variables | rs12436190 | |
|---|---|---|
| | Correlation coefficient | P value |
| Age (in years) | 0.053 | 0.47 |
| Sex | 0.039 | 0.66 |
| Family history of cancer | 0.085 | 0.06 |
| Radiation exposure | 0.034 | 0.69 |

## DISCUSSION

Genetic factors, such as SNPs, have been regarded as part of the etiology of cancers. Therefore, we performed a hospital based case-control study to analyze the association between *SLC7A7* rs12888930, rs12436190 and rs2065134 and the risk of glioma in a Chinese population. We observed that the frequency of GG and AG + GG genotypes of *SLC7A7* rs12436190 was found to be higher in glioma patients than in healthy controls. Therefore, individuals with the GG and AG + GG genotypes of *SLC7A7* rs12436190 were significantly associated with susceptibility to glioma. These statistically significant results indicated towards the possible role of *SLC7A7* rs12436190 in the pathogenesis of glioma.

The overexpression of *SLC7A7* could cause lysinuric protein intolerance (Borsani et al., 1999). Some previous studies have shown that the abnormal gene expression regulation of *SLC7A7* is associated with many malignant tumors. The up-regulated gene expression of *SLC7A7* is associated with radiation-induced radioresistance in non-small cell lung cancer (Xie et al., 2011). Cheng et al. (2010) indicated that the overall expression of *SLC7A7* is related to chemoresistance in ovarian cancer.

Only two previous studies have reported the association between *SLC7A7* expression and the risk and prognosis of glioma. Fan et al. (2013) performed a study of 119 patients

with pathologically confirmed glioblastoma and 16 controls and found that overexpression of *SLC7A7* is significantly associated with poor prognosis in patients with glioblastoma. Fan et al. (2013) also reported that rs12888930 and rs2065134 polymorphisms of *SLC7A7* were significantly associated with the development of glioma in a Chinese population. In the present study, we observed that rs12436190 was associated with the etiology of glioma, which is partly in line with previous results. Moreover, our paper is the first to report that the AG + GG genotypes of rs12436190 contribute more to the development of glioma in comparison to the AA genotype. The inconsistencies in the results of these studies could be attributed to the differences in population, sample size, and study design.

Two limitations should be mentioned. Firstly, all the study participants had been recruited from only one hospital located in a single city in China, and thus, they are not representative of glioma patients and healthy controls of other ethnicities. Secondly, the low prevalence of glioma meant that only 122 glioma patients were included in the study. This limited sample size may have reduced the statistical power of differentiating between the two investigated groups. Therefore, results from future studies incorporating larger samples sizes are required to verify our results.

In conclusion, our study reports a significant association between the GG and AG + GG genotypes of *SLC7A7* rs12436190 and the risk of glioma in a Chinese population. More studies with larger sample size and better design need to be conducted.

## Conflicts of interest

The authors declare no conflict of interest.

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