RESEARCH ARTICLE

Comprehensive Study on Associations Between Nine SNPs and Glioma Risk

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Abstract

Aim: Glioma cancer is the most common type of adult brain tumor. Recent genome-wide association studies (GWAS) have identified various new susceptibility regions and here we conducted an extensive analysis of associations between 12 single nucleotide polymorphisms (SNPs) and glioma risk. Methods: A total of 197 glioma cases and 197 health controls were selected, and 9 SNPs in 8 genes were analyzed using the Sequenom MassARRAY platform and Sequenom Assay Design 3.1 software. Results: We found the MAF among selected controls were consistent with the MAF from the NCBI SNP database. Among 9 SNPs in 8 genes, we identified four significant SNP genotypes associated with the risk of glioma, C/C genotype at rs730437 and T/T genotype at rs1468727 in ERGF were protective against glioma, whereas the T/T genotype at rs1799782 in XRCC1 and C/C genotype at rs861539 in XRCC3 conferred elevated risk. Conclusion: Our comprehensive analysis of nine SNPs in eight genes suggests that the rs730437 and rs1468727 in ERGF, rs1799782 in XRCC1 gene, and rs861539 in XRCC3 gene are associated with glioma risk. These findings indicate that genetic variants of various genes play a complex role in the development of glioma.

Keywords: SNPs - EGRF - XRCC1 - XRCC3 - glioma risk

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Introduction

Tumors of the central nervous system (CNS) account for about 2% of all the cancers, and affect an estimated 4.2/105 to 5.4/105 people per year worldwide (Parkin et al., 2005). Gliomas are the most common CNS tumors, and accounts for 80% of all primary malignant brain tumors. Gliomas remain incurable in most cases, despite great improvement in diagnosis and treatment methods. This disease is generally associated with poor survival relative to other types of brain tumors (Bondy et al., 2008). Previous studies reports the inherited factors are involved in the susceptibility of this type of cancer, and a majority of the inherited risk is due to the co-inheritance of multiple low risk genetic variants. Recent genome-wide association studies (GWAS) have identified various new susceptibility regions (Wrensch et al., 2009; Rajaraman et al., 2012). Genes involved in the cell cycle and DNA repair, have been proposed to play role in glioma pathogenesis and progression, such as glutathione S-transferases(GSTs), excision repair cross-complementing rodent repair deficiency complementation group 1(ERCC1), X-ray repair cross-complementing groups 1(XRCC1) and X-ray repair cross-complementing groups 3 (XRCC3) (Wrensch et al., 2004; Custódio et al., 2011; Zhou et al., 2011; Yao et al., 2012).

In our study, we performed an extensive analysis study in Chinese population by using a case-control design. A total of 9 single nucleotide polymorphisms (SNPs) which suggested to be association with development of glioma cancer in previous studies were collected from these Chinese population. We investigated and validated this potential association with glioma risk.

Materials and Methods

Participants

This case-control study was conducted in the Affiliated Hospital of Inner Mongolia Medical University and Nanfang Hospital Between Oct. 2007 and Jan. 2012, 213 hospital patients with newly diagnosed, histologically confirmed primary gliomas whose first visit fell within two months of initial diagnosis were asked to participate in the study. Of 213 patients, 197 cases were successfully interviewed and approved to participate in our study, for a participation rate of 92.5%. Those who consented were interviewed and provided 2 ml blood samples. 197 controls were selected from among inpatients from the orthopedics, dermatology, and digestive departments; controls had to lack a prior history of cancer, and were frequency matched to the cases by age (within 5 years) and sex.

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Table 1. Basic Characteristics of Cases and Controls

Characteristics	Cases N=312	%	Controls N=312	%	P value
Sex					
Male	185	59.2	171	54.7	0.26
Female	127	40.8	141	45.3	
Age	0		0		
<=50	167	53.6	179	57.5	0.33
>50	145	46.4	133	42.5	
Mean age		51.7±8.1		52.0±8.5	
Histological type	0				
Astrocytoma	181	58.1			
Ependymoma	24	7.8			
Glioblastoma	39	12.4			
Oligodendroglioma	11	3.4			
Other	57	18.3			

Table 2. Genotype Characteristics of the 12 SNPs

Single nucleotide polymorphism	Minor Alleles	MAF[a]			HWE (P value)[b]	
		Case	Control	From dbSNP	Case	Control
TERT (rs2853676)	G	0.261	0.239	0.23	0.078	0.454
ERBB2 (rs2952155)	T	0.401	0.381	0.375	<0.05	0.064
VEGFR (rs3828550)	T	0.323	0.351	0.352	<0.05	0.062
EGFR (rs4947986)	G	0.358	0.332	0.333	<0.05	0.056
GSTP (rs1695)	G	0.297	0.323	0.325	<0.05	0.1
ERGF (rs730437)	C	0.379	0.449	0.452	<0.05	<0.05
ERGF (rs1468727)	T	0.249	0.297	0.289	0.924	0.839
XRCC1 (rs1799782)	T	0.219	0.145	0.13	0.128	0.103
XRCC3 (rs861539)	C	0.322	0.261	0.25	0.813	0.092

a, Minor Allele Frequency; b, Hardy-Weinberg equilibrium

Genotyping

9 kinds of candidate SNPs in 8 genes were selected from polymorphisms with previously established association with adult's glioma (Andersson et al., 2010; Hu et al., 2011; Zhou et al., 2011). Genomic DNA was extracted from whole blood by using TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). A multiplexed SNP single base extension (SBE) assays was designed by using Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA) according to the manufacturer's instructions. Genotyping was performed by using a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). Data management and analysis were performed by using Sequenom Assay Design 3.1 software as previous described (Thomas et al., 2007; Gabriel et al., 2009). For quality control, and a random sample of 10% of cases and controls was genotyped again by different researchers. The reproducibility was 100%.

Statistical analysis

Statistical analysis was performed by using Stata 8.0 (StataCorp, College Station, USA). Continuous variables were expressed as mean±standard deviation (SD), while categorical variables were shown as frequencies and percentages. Demographic characteristics were compared between cases and controls by means of a chi-square test and Student's t test. The genotype frequencies in control subjects for each SNP were tested for departure from Hardy-Weinberg equilibrium (HWE) using the chi-square test. Genotype frequencies and allele frequencies of glioma patients and controls were compared by using the chi-square test. Odds ratio (ORs) and 95% confidence intervals were calculated by using unconditional logistic regression analysis with adjustment for age and sex. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by using conditional logistic regression adjusted for sex and age. All comparisons were two-sided, and P < 0.05 was regarded as statistically significant.

Table 3. Association Between 9 SNP Genotypes and the Risk of Glioma

Single nucleotide polymorphism	No.(frequency) Cases		Controls		OR[1]	OR[2]
TERT (rs2853676)						
A/A	177	56.7	183	58.8	1.0 (Ref.)	1.0 (Ref.)
A/G	108	34.5	108	34.7	1.03(0.73-1.47)	1.12(0.78-1.53)
G/G	27	8.8	20	6.5	1.40(0.74-2.73)	1.46(0.82-2.84)
ERBB2 (rs2952155)						
C/C	130	42.7	145	45.1	1.0 (Ref.)	1.0 (Ref.)
C/T	114	34.4	105	33.6	1.21(0.84-1.76)	1.25(0.85-1.79)
T/T	68	22.9	62	21.3	1.22(0.79-1.90)	1.27(0.84-2.18)
VEGFR (rs3828550)						
C/C	223	50.4	214	48.3	1.0 (Ref.)	1.0 (Ref.)
C/T	154	34.7	147	33.2	1.01(0.74-1.36)	1.24(0.80-1.43)
T/T	66	14.9	82	18.5	0.78(0.52-1.15)	0.93(0.68-1.37)
EGFR (rs4947986)						
A/A	147	47.1	157	50.2	1.0 (Ref.)	1.0 (Ref.)
A/G	107	34.3	104	33.2	1.10(0.76-1.59)	1.15(0.77-1.64)
G/G	58	18.6	52	16.6	1.19(0.75-1.89)	1.23(0.78-1.96)
GSTP (rs1695)						
A/A	160	51.4	153	48.9	1.0 (Ref.)	1.0 (Ref.)
A/G	118	37.8	117	37.6	0.96(0.68-1.37)	0.98(0.71-1.44)
G/G	34	10.8	42	13.5	0.77(0.45-1.32)	0.80(0.49-1.43)
ERGF (rs730437)						
A/A	144	46.3	126	40.3	1.0 (Ref.)	1.0 (Ref.)
A/C	99	31.6	92	29.6	0.94(0.63-1.39)	0.90(0.61-1.25)
C/C	69	22.1	94	30.1	0.64(0.43-0.97)	0.55(0.36-0.88)
ERGF (rs1468727)						
C/C	249	56.3	236	53.2	1.0 (Ref.)	1.0 (Ref.)
C/T	166	37.5	152	34.3	1.03(0.77-1.39)	0.98(0.63-1.28)
T/T	27	6.2	55	12.5	0.47(0.27-0.78)	0.44(0.23-0.73)
XRCC1 (rs1799782)						
C/C	294	66.3	334	75.5	1.0 (Ref.)	1.0 (Ref.)
C/T	105	23.6	89	20.1	1.34(0.96-1.88)	1.40(1.02-2.15)
T/T	45	10.1	19	4.4	2.69(1.50-4.98)	2.66(1.48-4.88)
XRCC3 (rs861539)						
T/T	223	50.4	254	57.3	1.0 (Ref.)	1.0 (Ref.)
T/C	154	34.7	147	33.3	1.19(0.88-1.61)	1.23(0.92-1.73)
C/C	66	14.9	42	9.4	1.79(1.14-2.81)	1.83(1.22-2.94)

[1]Non-adjusted; [2]Adjusted for sex and age

Results

The mean ages of the cases and controls were 52.6±9.1 years and 51.7±9.3 years, respectively. There were no significant differences in the age or sex distribution between the two groups (P>0.05). Of all the glioma patients, 58.4% were astrocytoma.

We assumed the minor allele frequencies (MAF) in cases and controls of each SNP in Table 2. We found the MAF among selected controls were consistent with the MAF from NCBI SNP database. Except for rs730437 in ERGF, eight SNPs were in line with the Hardy-Weinberg equilibrium among controls. However, rs2952155 in ERBB2, rs3828550 in VEGFR, rs4947986 in EGFR, rs1695 in GSTP and rs730437 in ERGF were not in line with Hardy-Weinberg equilibrium in cases (P<0.05).

Association results between 9 SNPs in 8 genes and the risk of glioma were showed in Table 3. We identified

four significant SNP genotypes associated with the risk of glioma, C/C genotype at rs730437 and T/T genotype at rs1468727 in ERGF were protective against glioma, with adjusted ORs and 95% CI of 0.55(0.36-0.88) and 0.44(0.23-0.73), respectively. However, the T/T genotype at rs1799782 in XRCC1 and C/C genotype at rs861539 in XRCC3 conferred elevated risk for glioma, with adjusted ORs and 95% CI of 2.66(1.48-4.88) and 1.83(1.22-2.94), respectively.

Discussion

This study is, to our knowledge, the first to perform a comprehensive tagging of nine SNPs of eight genes and estimates their association with glioma risk. Our study found a rs730437 and rs1468727 in ERGF gene were associated with a 45% and 56% reduction in the risk of developing glioma, while rs1799782 in XRCC1 gene, and rs861539 in XRCC3 gene were associated with an increased risk of glioma.

The epidermal growth factor receptor (EGFR) regulates important cellular processes and is implicated in human tumors. Previous studies have assessed the several SNPs in EGFR gene for the association with the risk of several cancers, such as lung cancer, gastric cancer, breast cancer, prostate cancer and esophageal cancer (Wong et al., 1992; Tokunaga et al., 1995; Kharrat et al., 2007; Upadhyay et al., 2008; Perez et al., 2010; Han et al., 2011; Yun et al., 2012). Somatic alterations of the EGFR gene are common in glioma and influence several mechanisms of malignant transformation (Wong et al., 1992). Previous studies have shown the regulation of EGFR pathway plays an important role in glioma progression, and certain EGFR genotypes may be related to glioblastoma risk, which suggested the polymorphisms in EGFR have a role in the carcinogensis of glioma (Hedman and Henriksson, 2007; Andersson et al., 2010). Andersson et al reported the genotype TT of rs1468727 was associated with a decreased risk of gliomain European population, with an OR of 0.61 (Andersson et al., 2010). Another study conducted in Chinese population also indicated the genotype TT of rs1468727 and rs740437 was potentially associated with an increased risk of glioma (Hou et al., 2012). In our study, the results showed TT of rs1468727 and rs740437 in EGFR was associated with a decreased risk of glioma, which is consistence with previous study.

Our study suggests two DNA repair genes, rs1799782 in XRCC1 and rs861539 in XRCC3, contribute to the glioma susceptibility. It is well known that the only established environmental risk factor are ionizing radiation and ultraviolet rays (Sadetzki et al., 2005), these two types of radiation cause an accumulation of DNA damage. XRCC1 and XRCC3 participates in the DNA double-strand break/recombination repair, previous studies showed these the polymorphisms of XRCC1 and XRCC3 have a role in the risk of various cancers, such as gastric cancer, ovarian cancer, pancreatic cancer, colorectal cancer and lung cancer (McWilliams et al., 2008; He et al., 2012; Liu et al., 2012; Wang et al., 2012; Wen et al., 2012; Zhao et al., 2012). Recently, several studies examined the association between rs1799782 in XRCC1 and rs861539 in XRCC3 and the risk of glioma (Felini et al., 2007; Kiuru et al., 2008; Hu et al., 2011; Zhou et al., 2011). All the studies are consistent with our results. A recent Finland study with 701 glioma and 1560 controls indicated rs861539 was associated with a three folds increased risk of glioma (Kiuru et al., 2008). Another Chinese study with 127 glioma cases showed that the homozygous T/T and heterozygotes C/T variants of XRCC1 codon 194 brought a 2.12-fold and 1.46-fold increased risk of glioma compared to the homozygous wild-type genotype (Hu et al., 2011). One meta-analysis reported that the XRCC3 241T allele is associated with increased risk for breast cancer risk in Asian and Caucasian populations (Lee et al., 2007). Polymorphisms in DNA repair genes may be associated with differences in repair of DNA damage, and thus influence the risk for developing tumors (Sreeja et al., 2008).

In conclusion, our comprehensive analysis of nine SNPs in the eight genes suggests that the rs730437 and rs1468727 in ERGF, rs1799782 in XRCC1 gene, and rs861539 in XRCC3 gene are associated with glioma risk. These findings indicate that genetic variants of various genes play a complex role in the development of glioma. Our study provides important information on the etiology of glioma. Furthermore, large-sample studies with standardized unbiases methods are needed to validate the results of our study.

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