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Original Article

Association between XRCC1 Arg399Gln polymorphism and glioma risk in a Chinese population: a case-control study

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Abstract: Aim: In China, the incidence rates of glioma tend to be increased, however, the genetic contribution to its etiology is not well-understood. The aim of this study is to evaluate the association of XRCC1 Arg399Gln polymorphism with glioma risk in a Chinese population. Materials and methods: We conducted a case-control study on 387 patients with glioma and 400 cancer-free controls between 2004 and 2014. Peripheral blood samples of both groups were processed for DNA extraction and genotyping of the XRCC1 Arg399Gln polymorphism using PCR-RFLP. Comparison of the distribution of Arg399Gln genotypes in the study groups was performed by means of 2-sided contingency tables using the χ^2 test. Hazard ratios (HRs) were estimated by Cox proportional hazard regression model. Results: When the AA genotype was used as the reference group, the GG genotype was associated with significantly increased risk for glioma (adjusted OR = 3.18, 95% CI = 1.38-3.88; P = 0.017). Under the dominant model of inheritance, the AG + GG genotype was associated with significantly increased risk for glioma (adjusted OR =2.33, 95% CI = 1.12-5.81; P = 0.023). When the A allele was used as the reference group, the G allele was associated with increased glioma risk (adjusted OR, 2.44, 95% CI, 1.76-4.18; P = 0.003). Conclusion: Our data suggests that XRCC1 Arg399Gln polymorphism contribute to increased risk of glioma, which may be susceptibility biomarkers for glioma.

Keywords: Genetic susceptibility, molecular, epidemiology, XRCC1, glioma

Introduction

In China, in 2000, the annual incidence rate of brain tumors was less than 3.9 per 100,000 in men and 2.8 per 100,000 in women, and the incidence rates tend to be increased in large cities in China [1]. However, the mechanism of glioma development remains unclear. Previous studies suggested that a variant of risk factors may result in glioma development, including exposure to ionizing radiation [2], genetic polymorphisms [3-5], and a history of familial cancer [6, 7].

X-ray cross-complementing group 1 gene (XRCC1) is located on chromosome 19q13.2 consisting of 17 exons and finally translates to a 70-kDa protein which contains 633 amino acids [8, 9]. XRCC1 acts as a nonenzymatic, scaffold protein by recruiting and interacting with a variety of proteins important to the BER pathway, such as DNA glycosylase MPG, OGG1 [10]. Polymorphisms of XRCC1 were found to have reduced recruitment of XRCC1 interacting proteins, and deteriorated overall efficiency of DNA damage restoration [11]. Currently, it is reported that there are eight validated single nucleotide polymorphisms (SNPs) of XRCC1 gene. However, SNPs of XRCC1 in codon 194 (exon 6, C to T, Arg to Trp), codon 280 (exon 9, G to A, Arg to His) and codon 399 (exon 10, G to A, Arg to Gln) are the most common ones [12]. Previous study has indicated that Arg399Gln in XRCC1 could impair the DNA repair capability and increase the susceptibility to cancer [12]. Therefore, the polymorphism of XRCC1 Arg399Gln may have an impact on the susceptibility of glioma. In the present study, we investigated the association between XRCC1 Arg399Gln polymorphism and glioma risk in a Chinese population.

Materials and methods

Study population

387 patients diagnosed with glioma were recruited at the Department of Neurosurgery, Qilu

Table 1. Characteristics of glioma cases and controls

Characteristic	Glioma patients (n = 387)		Controls (n = 400)		*P* value
	N	%	N	%	
Age					
< 60	138	35.66	156	39.00	0.339
≥ 60	249	64.34	244	61.00	
Gender					
Male	187	48.32	199	49.75	0.722
Female	200	51.68	201	50.25	
Tobacco use					
Never	41	10.59	40	10.00	0.815
Ever	346	89.41	360	90.00	
Alcohol use					
Never	76	19.64	83	20.75	0.518
Ever	311	80.36	337	84.25	

Hospital of Shandong University between June 2004 and March 2014. All patients who voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. 400 healthy controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of the hospital. Exclusion criteria for the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. In addition, all members in the two groups completed a short questionnaire. Written informed consent was obtained from each participant, and the study protocol was approved by the Ethics Board of Qilu Hospital of Shandong University.

DNA extraction and genotyping

DNA isolation was done using a DNA extraction kit (Zymo research) as per the manufacturer's protocol. Briefly, genomic lysis buffer was added to the samples and spun, and mixture was transferred to the Zymo spin column. Using the fast spin technology purified DNA was eluted. The region containing Arg→Gln substitution at codon 399 (in exon 10) was amplified by PCR to obtain an undigested fragment of 242 bp using the forward primer 5'-CCCCAAGTACAGCCA-GGTC-3'andthereverseprimer5'-TGTCCCGCTCCTCTCAG-TAG-3'. The PCR product was digested by MspI at 37°C for 1 hour according to the protocol provided by the manufacturer. It was resolved on 2% agarose using horizontal gel electrophoresis and staining with ethidium bromide. Arg/Arg genotype was digested to form 94 and 148 bp fragments while the Gln/Gln missed the MspI restriction site producing only one band. Heterozygous genotypes gave three bands.

Statistical analyses

The differences between cases and controls were assessed using Pearson's χ^2 test and t-tests, as appropriate. The Hardy-Weinberg equilibrium (HWE) was tested by a χ^2 test to compare the expected genotype frequencies with observed genotype frequencies in controls. Comparison of the distribution of Arg399Gln genotypes in the study groups was performed by means of 2-sided contingency tables using the χ^2 test. Hazard ratios (HRs) were estimated by Cox proportional hazard regression model. The SPSS 18.0 software was used to perform all statistical analyses. A $P < 0.05$ was considered statistically significant.

Results

Subjects and general characteristics

In total, 787 subjects were recruited in this case-control study, including 387 glioma patients and 400 cancer-free controls. The clinical characteristics of these two groups are shown in **Table 1**. There were no significant differences between glioma patients and cancer-free controls in terms of age (P = 0.339), sex (P = 0.722), tobacco use (P = 0.815), and alcohol drinking (P = 0.518).

Genotype and allele distribution of XRCC1 Arg-399Gln

The genotype distribution for XRCC1 Arg399Gln polymorphism was consistent with values predicted by Hardy-Weinberg equilibrium among controls (P > 0.05). **Table 2** summarizes the genotype and allele distributions of the XRCC1 Arg399Gln in the glioma case group and control group. Genotypes AA, AG and GG were detected in 45 (11.63%), 164 (42.38%) and

Table 2. Genotype and allele frequencies of XRCC1 Arg399Gln polymorphism in studied groups

Genotypes/Alleles	Glioma patients (n = 387)		Controls (n = 400)		*P* value
	N	%	N	%	
Arg399Gln polymorphism					
Genotype					
AA	45	11.63	211	52.75	< 0.001
AG	164	42.38	157	39.25	
GG	178	45.99	32	8.00	
Alleles					
A	254	32.82	579	72.38	< 0.001
G	520	67.18	221	27.63	

Table 3. The association of XRCC1 Arg399Gln polymorphism with glioma risk

General genotype	Patients	Controls	OR (95% CI)[1]	*P* value
AA	45	211	1.00 (Reference)	
AG	164	157	1.67 (0.93-3.72)	0.073
GG	178	32	3.18 (1.38-3.88)	0.017
Dominant genotype				
AA	45	211	1.00 (Reference)	
AG + GG	342	189	2.33 (1.12-5.81)	0.023
Recessive genotype				
AA + AG	209	368	1.00 (Reference)	
GG	178	32	1.98 (0.76-3.19)	0.129
Allele frequency				
A	254	579	1.00 (Reference)	
G	520	221	2.44 (1.76-4.18)	0.003

[1]Adjusted for sex, age, smoking status, and drinking status.

178 (45.99 of 387 glioma patients and in 211 (52.75%), 157 (39.25%) and 32 (8.00%) of 400 healthy control samples, respectively. There was significant difference in the distribution of XRCC1 Arg399Gln genotype between glioma patients and healthy controls (P < 0.001). The frequency of variant allele A was 254 (32.82%) and allele G was 520 (67.18%) in the glioma cases, and 579 (72.38%) and 221 (27.63%) in the controls, respectively. G allele frequency was significantly higher in cancer group as compared to control group (P < 0.001).

XRCC1 Arg399Gln polymorphism and glioma risk

When the AA genotype was used as the reference group, the AG genotype was not associated with risk (adjusted OR, 1.67, 95% CI, 0.93-3.72; P = 0.073), but the GG genotype was associated with significantly increased risk for glioma (adjusted OR = 3.18, 95% CI = 1.38-3.88; P = 0.017). Under the dominant model of inheritance, the AG + GG genotype was associated with significantly increased risk for glioma (adjusted OR = 2.33, 95% CI = 1.12-5.81; P = 0.023, shown in **Table 3**). When the A allele was used as the reference group, the G allele was associated with increased glioma risk (adjusted OR, 2.44, 95% CI, 1.76-4.18; P = 0.003, shown in **Table 3**).

Discussion

Gliomas are the most common primary tumors of the central nervous system, but in spite of the marked advances in the characterization of their molecular pathogenesis, these tumors remain incurable [13]. Estimated 5-year survival was 60% and 74% for biopsy and watchful waiting and early resection in low-grade gliomas, respectively [1]. Gliomas are classified as grade I to grade IV on the basis of histopathological and clinical criteria established by the World Health Organization (WHO) [14]. This group of tumors includes specific histological subtypes, the most common of which are the astrocytomas, glioblastoma, and other gliomas. Malignant gliomas account for approximately 70% of the 22,500 new cases of malignant primary brain tumors that are diagnosed in adults in USA each year [15]. Several occupations, environmental carcinogens, and diet have been reported to be associated with an elevated glioma risk, as well as genetic factors such as single nucleotide polymorphisms [1].

In humans, > 70 genes are involved in the five major DNA repair pathways: direct repair, base excision repair (BER), nucleotide excision repair

(NER), mismatch repair and double-strand break (DSB) repair [16]. Among them, BER gene, especially XRCC1, is the primary guardian against DNA damage [17]. The Arg399Gln polymorphism, located in the region of the BRCT-I interaction domain of XRCC1, has been extensively explored in its function and association with cancer risk. The meta-analysis conducted by Zhao et al found that there were no associations of Arg399Gln polymorphism with gastric cancer, no matter in the co-dominant model, dominant model or recessive model [18]. In an updated meta-analysis by Pan et al, the XRCC1 polymorphism Arg399Gln was significantly associated with HCC susceptibility in a homozygote model as well as in a dominant model (G/G vs. A/A, OR = 1.253, P = 0.028; G/G+A/G vs. A/A, OR = 1.281, P = 0.047, respectively), but not in a heterozygote model (A/G vs. A/A, OR = 1.271, P = 0.066) or a recessive model (G/G vs. A/G + A/A, OR = 1.049, P = 0.542) [19].

Several studies have investigated the association between XRCC1 Arg399Gln polymorphism and glioma risk. However, these studies generated contradictory instead of conclusive results. Wang et al. found that there was no association between Arg399Gln polymorphism and glioma risk [20-23]. But Yosunkaya et al. concluded that XRCC1 Arg399Gln polymorphism was a significant risk factor, and 399Gln (G) allele carried a 3.5 times greater risk for glioma [24]. Hu et al. demonstrated the codon 399 Gln/Gln and Arg/Gln genotypes being associated with a 2.24- and 1.67-fold increased risk in glioma [25]. In the present study, we investigated the association between XRCC1 Arg399Gln polymorphism and glioma risk in a Chinese population. We found that there was significant difference in the distribution of XRCC1 Arg399Gln genotype between glioma patients and healthy controls. G allele frequency was significantly higher in cancer group as compared to control group. When the AA genotype was used as the reference group, the AG genotype was not associated with risk, but the GG genotype was associated with significantly increased risk for glioma. Under the dominant model of inheritance, the AG + GG genotype was associated with significantly increased risk for glioma. When the A allele was used as the reference group, the G allele was associated with increased glioma risk. In conclusion, our data suggests that XRCC1 Arg399Gln polymorphism contribute to increased risk of glioma, which may be susceptibility biomarkers for glioma.

Disclosure of conflict of interest

None.

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