RESEARCH ARTICLE

Association Between Genetic Polymorphism of *XRCC1* Gene and Risk of Glioma in a Chinese Population

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Abstract

Background: Gliomas are the most common type of primary brain tumor in adults, and the X-ray repair complementing group 1 gene (XRCC1) is an important candidate gene influencing its risk. The objective of this study was to detect the influence of XRCC1 genetic polymorphisms on glioma risk. Materials and Methods: A total of 629 glioma patients and 641 cancer-free subjects were enrolled in this case-control study. The genotypes of the c.1471G>A genetic polymorphism were determined by created restriction site-polymerase chain reaction (CRS-PCR) and DNA sequencing methods. The influence of the XRCC1 genetic polymorphism on glioma risk was evaluated by association analysis. Results: Our data indicated that the alleles/genotype of this genetic variant was statistically associated with glioma risk. The AA genotype was statistically associated with the increased risk of glioma compared to the GG wild genotype (odds ratios (OR) = 1.89, 95% CI 1.25-2.87, *P* = 0.003). The allele-A may contribute to increased the susceptibility to glioma (OR = 1.23, 95% CI 1.04-1.46, *P* = 0.017). Conclusions: These preliminary findings indicate that the c.1471G>A genetic polymorphism of XRCC1 has the potential to influence glioma susceptibility, and might be used as molecular marker for assessing glioma risk.

Keywords: Glioma - XRCC1 gene - genetic polymorphism - molecular marker - risk factors

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Introduction

Gliomas are the most common type of primary brain malignancy in adults, and approximately 70 % of adult malignant brain tumours (Wen et al., 2008; Jacobs et al., 2012; Ricard et al., 2012; Wei et al., 2013). Gliomas are a multi-factorial disorder, which causes by the interaction of several genetic and environmental factors (Kiuru et al., 2008), while it has been generally accepted that the genetic factors may play key roles in the development of glioma (Liu et al., 2009; Melin, 2011; Zhang et al., 2012). Recently, several studies reported that the X-ray repair complementing group 1 gene (XRCC1) is an important candidate gene for influencing the risk of glioma (Wang et al., 2004; Kiuru et al., 2008; Liu et al., 2009; Yosunkaya et al., 2010; Zhou et al., 2011; Zhang et al., 2012; Jiang et al., 2013; Luo et al., 2013; Wei et al., 2013). The XRCC1 gene, which locates at chromosome 19q13.2, encodes an enzyme involved in the base excision repair (BER) pathway (Tudek, 2007; Mutamba et al., 2011; Zhang et al., 2012). Genetic polymorphisms in XRCC1 gene (such as Arginine (Arg)194 Tryptophan (Trp), Arg280 Histidine (His) and Arg399 Glutanine (Gln)) have been reported to be potential influence on the altered risk of glioma (Kiuru et al., 2008; Liu et al., 2009; Rajaraman et al., 2010; Yosunkaya et al., 2010; Hu et al., 2011; Zhou et al., 2011; Liu et al., 2012; Sun et al., 2012; Wang et al., 2012; Jiang et al., 2013; Luo et al., 2013; Pan et al., 2013; Wei et al., 2013). However, the results from these observations were conflicting rather than conclusive (Wang et al., 2004; Felini et al., 2007; Kiuru et al., 2008; Liu et al., 2009; Rajaraman et al., 2010; Yosunkaya et al., 2010; Hu et al., 2011; Melin, 2011; Zhou et al., 2011; Jacobs et al., 2012; Liu et al., 2012; Sun et al., 2012; Wang et al., 2012; Zhang et al., 2012; Jiang et al., 2013; Luo et al., 2013; Pan et al., 2013; Wei et al., 2013). Up to date, no related studies about the potential association between the c.1471G>A genetic polymorphism of XRCC1 gene and the risk of glioma have not been analyzed in any population. Therefore, considering of the importance of the XRCC1 gene for influencing the risk of glioma, we conducted a case-control study to assess the distribution of c.1471G>A genetic polymorphism of XRCC1 gene and its genetic influence on glioma risk.

Materials and Methods

Study subjects

This case-control study was conducted in Chinese Han populations from the First Affiliated Hospital of Dalian Medical University. The 629 patients (males = 376, females = 253) were enrolled, which newly diagnosed

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Table 1. The General Characteristics of Study Subjects

Characteristics	Cases (n)		Controls (n)		χ^2-value	*P*-values
Number	629	49.53	641	50.47		
Age (years)					1.1469	0.2842
Mean ± SD	53.22 ± 13.66		54.75 ± 14.11			
< 45	234	37.2	220	34.32		
≥ 45	395	62.8	421	65.68		
Gender (n)					0.7136	0.3982
Male	376	59.78	398	62.09		
Female	253	40.22	243	37.91		
Tobacco smoking (n)					3.4922	0.0617
Yes	345	54.85	318	49.61		
No	284	45.15	323	50.39		
Alcohol drinking (n)					3.7495	0.0528
Yes	288	45.79	259	40.41		
No	341	54.21	382	59.59		
IR exposure history (n)					4.304	0.0380
Never	587	93.32	615	95.94		
Ever	42	6.68	26	4.06		
Family history of cancer (n)					6.288	0.0122
Yes	93	14.79	65	10.14		
No	536	85.21	576	89.86		
Histology typesa (n)						
High-grade glioma	306	48.65	-			
Low-grade glioma	323	51.35	-			

SD, standard deviation; IR, ionizing radiation; High-grade glioma (glioblastoma), low-grade glioma (astrocytoma, oligodendroglioma, mixed glioma asnd other low-grade

and histologically confirmed with glioma by doctors. The 641 cancer-free subjects (males = 398, females = 243) were recruited as controls, which free from any cancer and matched with age and gender. All subjects were genetically unrelated Chinese of Han ethnicity. Table 1 shows the general characteristics on age, gender, tobacco smoking, alcohol drinking, histology types, ionizing radiation (IR) exposure history and family history of cancer. Approval to conduct this study was granted by the Ethics Committees of the First Affiliated Hospital of Dalian Medical University. The written informed consent form was obtained from all recruited subjects.

Genotyping

Genomic DNA was extracted by using the DNA Blood Mini kit (QIAGEN, Valencia, CA). The specific polymerase chain reaction (PCR) primers were designed through Primer Premier 5.0 software. Table 2 shows the detailed information of primers sequences, Annealing temperature, PCR amplification region and fragment sizes. The PCR amplifications was carried out in a reaction volume of 20 μL containing 50 ng of genomic DNA, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The condition of PCR reactions began with 95°C for 5 minutes, 35 cycles of 94°C for 30 seconds, followed by annealing temperature to 63.2°C for 30 seconds, and 72°C for 30 seconds, and a final extension at 72°C for 8 minutes. The genotypes of c.1471G>A genetic polymorphism of XRCC1 gene was determined by the created restriction site-polymerase chain reaction (CRS-PCR) method, with one of the primers containing a nucleotide mismatch, which enables the use of restriction enzymes for discriminating sequence variations (Haliassos et al., 1989; Zhao et al., 2003; Yuan et al., 2012; Yuan et al., 2013; Yuan et al., 2013). The PCR amplified products (5 μL) were digested with 2U selected restriction enzymes (Table 2) at 37°C for 10 hours, following the manufacturer's instructions. The digested products were separated by electrophoresis in 2.0% agarose gel, and observed different genotypes on the UV light. In order to verify the genotype results from CRS-PCR method, 15% of random samples were re-analyzed by DNA sequencing method (ABI3730xl DNA Analyzer, Applied Biosystems, Foster City, CA).

Statistical analyses

All statistical analyses were performed by the SPSS software (Windows version release 16.0; SPSS Inc.; Chicago, IL, USA). The differences between cases and controls in the frequencies of alleles/genotypes, general characteristics, and risk factors were assessed by the chi-square (χ^2) test. The Hardy-Weinberg equilibrium (HWE) was determined for compatibility between cases and controls using the chi-square (χ^2) test. The association between the XRCC1 genetic polymorphism and the risk of glioma was analyzed by calculating the odds ratios (ORs) and their 95% confidence intervals (CIs) using unconditional logistic regression. P-values less than 0.05 were regarded as statistically significant.

Results

General characteristics

In totally, 1270 Chinese Han subjects were enrolled in this case-control study, containing of 629 glioma subjects and 641 cancer-free controls. The general characteristics of cases and controls subjects are summarized in Table 1. The mean ages were 53.22 years old (standard deviation (SD) ± 13.66) and 54.75 years old (SD ± 14.11) for the cases and controls, respectively. The frequency-matching on age and gender between the cases and controls appeared to be adequate (*P* = 0.2842 and 0.3982, respectively). There were no statistically significant differences among the cases and controls in terms of tobacco smoking and alcohol drinking (*P* = 0.0617 and 0.0528, respectively). Those subjects who have more family history of cancer and higher IR exposure, were more likely to have higher risk of glioma (*P* = 0.0122 and 0.0380, respectively).

XRCC1 genetic polymorphism identification

In this study, the genotypes of c.1471G>A genetic polymorphism of XRCC1 gene were determined by CRS-PCR and DNA sequencing methods in a Chinese Han population. Based on the human XRCC1 gene DNA sequences (GenBank ID: NC_000019.9), mRNA sequences (GenBank ID: NM_006297.2), and protein sequences (GenBank ID: NP_006288.2), the results from our DNA sequence analyses suggested that the c.1471G>A genetic polymorphism is a non-synonymous mutation, which corresponding to the G→A mutations and Glutamic (Glu) to Lysine (Lys) amino acid replacement (p.Glu491Lys) in the exon13 of XRCC1 gene. The AlwNI restriction enzyme has been selected and utilized to digest the PCR products of c.1471G>A genetic polymorphism.

Table 2. The Primer Sequences, PCR and CRS-PCR Analysis for c.1471 G>A Genetic Polymorphism of XRCC1 Gene

Primer sequences	Annealing temperature (°C)	PCR amplification fragment (bp)	Region	Restriction enzyme	Genotype (bp)
5'-AAGATTCTGGGGACACAGAGGCT-3' 5'-TCATCCGTGGAGCCTGCATAC-3'	63.2	209	Exon13	AlwNI	GG:188,21 GA:209,188,21 AA:209

PCR, polymerase chain reaction; CRS-PCR, created restriction site-polymerase chain reaction; Underlined nucleotides mark nucleotide mismatches enabling the use of the selected restriction enzymes for discriminating sequence variations at CRS-PCR analysis

Table 3. The Genotype and Allele Frequencies of c.1471G>A Genetic Polymorphism of XRCC1 Gene in Glioma Cases and Controls

Groups	Genotype frequencies (%)			Allele frequencies (%)			
	GG	GA	AA	G	A	χ^2	*P*
Cases (n = 629)	289 (45.95)	270 (42.93)	70 (11.13)	848 (67.41)	410 (32.59)	0.3347	0.8459
Controls (n = 641)	320 (49.92)	280 (43.68)	41 (6.40)	920 (71.76)	362 (28.24)	3.8827	0.1435
Total (n = 1270)	609 (47.95)	550 (43.31)	111 (8.74)	1768 (69.61)	772 (30.39)	0.7026	0.7038
	$\chi^2 = 9.2238$, $P = 0.0099$			$\chi^2 = 5.6903$, $P = 0.0171$			

Table 4. The Association Between c.1471 G>A Genetic Polymorphism of XRCC1 Gene and Glioma Cancer Risk

Comparisons	Test of association		
	OR (95% CI)	χ^2-value	*P*-value
Homozygote comparison (AA vs. GG)	1.89(1.25-2.87)	9.14	0.003
Heterozygote comparison (GA vs. GG)	1.07(0.85-1.35)	0.31	0.578
Dominant model (AA/GA vs. GG)	1.17(0.94-1.46)	2.01	0.156
Recessive model (AA vs. GA/GG)	1.83(1.23-2.74)	8.91	0.003
Allele contrast (A vs. G)	1.23(1.04-1.46)	5.69	0.017

SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval; vs., versus

Three distinct genotypes have been detected, consisting of genotype GG (188 and 21 bp), genotype GA (209,188 and 21 bp) and genotype AA (209 bp).

Genotype and allele frequencies of XRCC1 genetic polymorphism

Table 3 shows the genotype and allele frequencies of c.1471G>A genetic polymorphism in XRCC1 gene in glioma patients and cancer-free controls. The allele-G and genotype-GG were predominant in the studied subjects. The allele frequencies of glioma patients (G, 67.41%; A, 32.59%) were statistically significantly different from those of cancer-free controls (G, 71.76%; A, 28.24%, $\chi^2 = 5.6903$, $P = 0.0171$). The genotype distributions of glioma patients (GG, 45.95%; GA, 42.93%; AA, 11.13%) were statistically significantly different from those of cancer-free controls (GG, 49.92%; GA, 43.68%; AA, 6.40%; $\chi^2 = 9.2238$, $P = 0.0099$). The distributions of these genotype frequencies were in agreement with those expected from the HWE model for controls (P = 0.8459, 0.1435, and 0.7038, respectively).

Association between XRCC1 genetic polymorphism and risk of glioma

Table 4 shows the potential association between the c.1471G>A genetic polymorphism of XRCC1 gene and glioma risk. Our data indicated that the c.1471G>A genetic polymorphism was significantly associated with the increased risk of glioma in the homozygote comparison (AA versus (vs.) GG: OR = 1.89, 95% CI 1.25-2.87, $\chi^2 = 9.14$, $P = 0.003$), recessive model (AA vs. GA/GG: OR = 1.83, 95% CI 1.23-2.74, $\chi^2 = 8.91$, $P = 0.003$) and allele contrast (A vs. G: OR = 1.23, 95% CI 1.04-1.46, $\chi^2 = 5.69$, $P = 0.017$, Table 4).

Discussion

Recently, previous studies indicate that the XRCC1 gene is one of the most important candidate genes for influecning the risk of glioma, and analysis of genetic polymorphisms in XRCC1 gene allows to effectively screen for the susceptibility to glioma (Wang et al., 2004; Kiuru et al., 2008; Liu et al., 2009; Yosunkaya et al., 2010; Zhou et al., 2011; Zhang et al., 2012; Jiang et al., 2013; Luo et al., 2013; Wei et al., 2013). In the present study, we firstly investigated the distribution of the c.1471G>A genetic polymorphism of XRCC1 gene using CRS-PCR and verified by DNA sequencing methods and evaluated the relationship of this genetic polymorphism with respect to glioma risk in a Chinese Han population by an association analysis on the basis of analysis of 629 glioma patients and 641 cancer-free controls. Our data indicated that the allele and genotype frequencies of this genetic variant in glioma patients were significantly different from those of cancer-free controls (All P-values < 0.05, Table 3). The genotype-AA was statistically associated with the increased risk of glioma compared to wild genotype-GG and GA/GG carriers (OR = 1.89, 95% CI 1.25-2.87, P = 0.003 and OR = 1.83, 95% CI 1.23-2.74, $P = 0.003$, Table 4). The A allele might be an increased risk factor for glioma susceptibility (A vs. G: OR = 1.23, 95% CI 1.04-1.46, P = 0.017, Table 4). Results from this study suggested that this genetic polymorphism has a statistically significant association with glioma risk and may affect the subjects susceptibility toward glioma in Chinese Han population. It could be used as molecular markers for evaluating glioma risk. Up to now, several similar studies concerned the influence of other genetic polymorphisms in the XRCC1 gene on glioma risk (Wang et al., 2004; Felini et al., 2007; Kiuru et al., 2008; Liu et al., 2009; Rajaraman et al., 2010; Yosunkaya et al., 2010; Hu et al., 2011; Melin, 2011; Zhou et al., 2011; Jacobs et al., 2012; Liu et al., 2012; Sun et

al., 2012; Wang et al., 2012; Zhang et al., 2012; Jiang et al., 2013; Luo et al., 2013; Pan et al., 2013; Wei et al., 2013). Results from these observations are in accordance with our conclusion that the genetic polymorphisms of XRCC1 gene may contribute to influences on glioma risk (Kiuru et al., 2008; Liu et al., 2009; Rajaraman et al., 2010; Yosunkaya et al., 2010; Hu et al., 2011; Zhou et al., 2011; Liu et al., 2012; Sun et al., 2012; Wang et al., 2012; Jiang et al., 2013; Luo et al., 2013; Pan et al., 2013; Wei et al., 2013). Previous studies demonstrated that many of other non-synonymous genetic polymorphisms (for example, Arg194Trp, Arg280His and Arg399Gln) have been approved significantly associated with the risk of glioma and influenced the function of XRCC1 protein (Kiuru et al., 2008; Liu et al., 2009; Rajaraman et al., 2010; Yosunkaya et al., 2010; Hu et al., 2011; Zhou et al., 2011; Liu et al., 2012; Wang et al., 2012; Luo et al., 2013; Pan et al., 2013). In our study, DNA sequence analyses suggested that the c.1471G>A genetic polymorphism is also a non-synonymous mutation and causes Glu to Lys amino acid replacement (p.Glu491Lys). This c.1471G>A genetic polymorphism might be linked to those other non-synonymous genetic polymorphisms and play the similar function on the development of glioma. Our findings could provide more evidence to explain the role of XRCC1 gene in the development of glioma. Further functional studies on larger different populations analyzing the c.1471G>A and other genetic polymorphisms spanning the whole XRCC1 gene region are still necessary to elucidate the underlying biological mechanisms underlying XRCC1-mediated autism susceptibility.

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The author(s) declare that they have no competing interests.

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