Glioma Risks Associate With Genetic Polymorphisms of *XRCC1* Gene in Chinese Population

Xingjun Feng,* Guozhuan Miao, Yipeng Han, Yi Xu, and Huayun Wu

Department of Neurosurgery, General Hospital of Chinese People's Armed Police Forces, Beijing 100039, People's Republic of China

ABSTRACT

Glioma is the most common type of primary brain tumors in adults. Previous evidence indicates that the X-ray repair cross-complementing group 1 gene (*XRCC1*) is an important candidate gene which influencing the pathogenesis of glioma. This study aims to assess the potential associations between glioma risks and genetic polymorphisms of *XRCC1* gene. A total of 1,286 Chinese Han ethnic subjects consisting of 638 glioma patients and 648 controls were recruited in this case-control study. The genotyping of *XRCC1* genetic polymorphisms (c.482C>T, c.1161G>A, and c.1804C>A) were conducted using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), created restriction site-PCR (CRS-PCR) and DNA sequencing methods. Our data indicated that the allelic and genotypic frequencies of these genetic polymorphisms in glioma patients were significantly different from those of controls. We detected that the alleles/genotypes were statistically associated with the increased risks of glioma (for c.482C>T, TT versus (vs.) CC: OR = 2.24, 95% CI = 1.48–3.39, $P < 0.001$; T vs. C: OR = 1.30, 95% CI = 1.09–1.53, $P = 0.003$; for c.1161G>A, AA vs. GG: OR = 1.62, 95% CI = 1.11–2.35, $P = 0.012$; A vs. G: OR = 1.19, 95% CI = 1.01–1.41, $P = 0.040$; for c.1804C>A, AA vs. CC: OR = 2.12, 95% CI = 1.45–3.11, $P < 0.001$; A vs. C: OR = 1.32, 95% CI = 1.12–1.56, $P = 0.001$). Our findings suggest that these genetic polymorphisms of *XRCC1* gene may influence glioma risks in Chinese Han ethnic subjects, and might be potential molecular markers for evaluating glioma risks. J. Cell. Biochem. 115: 1122–1127, 2014.   © 2013 Wiley Periodicals, Inc.

KEY WORDS: GLIOMA; *XRCC1* GENE; GENETIC POLYMORPHISMS; MOLECULAR MARKERS; RISK FACTORS

Glioma is the most common type of primary brain tumors in adults, and accounts for approximately 70% of adult malignant brain tumors [Wen and Kesari, 2008; Ricard et al., 2012; Wei et al., 2013]. To date, the exact mechanism of the etiology of glioma still remains unclear and the cause of glioma has been poorly understood [Ohgaki and Kleihues, 2005; Schwartzbaum et al., 2006; Wen and Kesari, 2008; Ricard et al., 2012; Tewari et al., 2012]. It has been accepted that genetic factors may play key roles in the development of glioma [Liu et al., 2009; Melin, 2011; Zhang et al., 2012; Jin et al., 2013]. The X-ray repair cross-complementing group 1 gene (*XRCC1*), which locates at chromosome 19q13.2-13.3, comprising 17 exons and expressing a 70-kDa protein, encodes an enzyme involved in the base excision repair (BER) pathway [Tudek, 2007; Mutamba et al., 2011; Zhang et al., 2012]. *XRCC1* is considered as one of the most important candidate genes for influencing the pathogenesis 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; Jin

et al., 2013; Li et al., 2013; Luo et al., 2013; Wei et al., 2013]. Genetic polymorphisms of *XRCC1* gene have been shown to alter the efficiency of DNA repair process and to modify the risks of glioma [Wang et al., 2004, 2012; Felini et al., 2007; Kiuru et al., 2008; Liu et al., 2009, 2012; Rajaraman et al., 2010; Yosunkaya et al., 2010; Hu et al., 2011; Melin, 2011; Zhou et al., 2011; Jacobs and Bracken, 2012; Sun et al., 2012; Zhang et al., 2012; Jiang et al., 2013; Li et al., 2013; Luo et al., 2013; Pan et al., 2013; Wei et al., 2013]. Previous studies reported several single nucleotide polymorphisms (SNPs) in *XRCC1* gene, such as arginine (Arg)194 tryptophan (Trp), Arg280 histidine (His), threonine (Thr) 304 alanine (Ala), Arg399 glutanine (Gln), and serine (Ser) 593Arg, were associated with the risks of glioma [Kiuru et al., 2008; Liu et al., 2009, 2012; Rajaraman et al., 2010; Yosunkaya et al., 2010; Hu et al., 2011; Zhou et al., 2011; Sun et al., 2012; Wang et al., 2012; Jiang et al., 2013; Li et al., 2013; Luo et al., 2013; Pan et al., 2013; Wei et al., 2013]. However, up to date, no similar studies have been done to investigate the potential associations between c.482C>T, c.1161G>A, and c.1804C>A genetic

polymorphisms of XRCC1 gene and the risks of glioma. Recently, Deng et al., reported that the c.1161G>A genetic polymorphism was statistically associated with the increased risk of hepatocellular carcinoma in Chinese Han population (AA versus (vs.) GG: OR = 2.36, 95% CI = 1.63–3.40, $P < 0.001$; A vs. G: OR = 1.48, 95% CI = 1.26–1.75, $P < 0.001$) [Deng et al., 2013]. Qiao et al., demonstrated that the c.1804C>A genetic variants was potentially related to gastric cancer susceptibility in Chinese Han population [Qiao et al., 2013]. Considering the important role of XRCC1 on the development of glioma, we suspect that the presence of these genetic variants of XRCC1 gene might increase the risks of glioma. Therefore, we conduct a case-control study to evaluate the potential associations of c.482C>T, c.1161G>A, and c.1804C>A genetic polymorphisms of XRCC1 gene with the risks of glioma.

MATERIALS AND METHODS

STUDY SUBJECTS

Patients with glioma (males = 362, females = 276) diagnosed and histologically confirmed by the doctors were recruited from the General Hospital of Chinese People's Armed Police Forces. Controls (males = 398, females = 250) were randomly selected from health volunteers who requested general examinations during the same period at the same hospital. Controls were frequency-matched with patients in gender and age. All of subjects enrolled in this case-control study were genetically unrelated Chinese Han ethnic, and lived in Beijing. The general characteristics are summarized from structured questionnaires through face-to-face interviewing by doctors, including age, gender, alcohol drinking, tobacco smoking, ionizing radiation (IR) exposure history, family history of cancer, and histology types (Table I). Approval to conduct the study was granted by the Ethics Committees of the General Hospital of Chinese People's Armed Police Forces. The written informed consent forms were obtained from all the participants.

PROCESSING OF BLOOD SAMPLES AND GENOTYPING

The whole blood samples (2–5 ml) were collected from all the participants. The genomic DNA was extracted using the Qiagen Blood Kit (Qiagen, Chatsworth, CA). The specific polymerase chain reaction (PCR) primers were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA). The primers sequences, PCR amplification region and fragment sizes, and annealing temperature are given in Table II. The PCR amplifications were carried out in a reaction volume of 20 μl consisted of 50 ng mixed DNA template, 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 PCR conditions consisted of 94°C for 6 min, then followed by 32 cycles of 94°C for 32 s, annealing at the corresponding temperature (shown in Table II) for 32 s and 72°C for 32 s, and a final extension at 72°C for 6 min. The genotyping of XRCC1 genetic polymorphisms was conducted using the PCR-restriction fragment length polymorphism (PCR-RFLP) and created restriction site-PCR (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, 2013a,b]. The PCR products (5 μl) were digested with 2 units corresponding restriction enzymes (MBI Fermentas, St. Leon-Rot, Germany, Table II) at 37°C for 10 h following the supplier's manual. The digested PCR products were electrophoresed on 2.0% agarose gel containing ethidium-bromide and observed the different genotypes under the UV light. For quality control, 10% of random amplified PCR products were genotyped by directly DNA sequencing (ABI3730xl DNA

TABLE I. The General Characteristics of Cases and Controls

Characteristics	Cases (n)		Controls (n)		χ^2-value	P-values
Number	638	49.61	648	50.39		
Gender (n)					2.9128	0.0879
Male	362	56.74	398	61.42		
Female	276	43.26	250	38.58		
Age (years)					1.2945	0.2552
Mean ± SD	52.98 ± 14.15		53.52 ± 13.73			
<45	247	38.71	231	35.65		
≥45	391	61.29	417	64.35		
Alcohol drinking					3.3110	0.0688
Ever	293	45.92	265	40.90		
Never	345	54.08	383	59.10		
Tobacco smoking					2.3239	0.1274
Ever	351	55.02	329	50.77		
Never	287	44.98	319	49.23		
Family history of cancer (n)			9.9021	0.0017		
Ever	98	15.36	62	9.57		
Never	540	84.64	586	90.43		
IR exposure history					5.3309	0.0210
Never	595	93.26	623	96.14		
Ever	43	6.74	25	3.86		
Histology types[a]						
High-grade glioma	293	45.92	—			
Low-grade glioma	345	54.08	—			

Note: SD, standard deviation; IR, ionizing radiation.
[a]High-grade glioma (glioblastoma), low-grade glioma (astrocytoma, oligodendroglioma, mixed glioma, and other low-grade glioma).

TABLE II. The PCR Analysis for SNPs of *XRCC1* Gene

SNPs	Primer sequences	Annealing temperature (°C)	PCR amplification fragment (bp)	Region	Genotyping methods	Restriction enzyme	Genotype (bp)
c.482C>T	5′-CAAAGATGAGGCAGAGGCC<u>G</u>-3′ 5′-CTGAAGAAGAGAGCCCCCGG-3′	63.5	213	Exon5	CRS-PCR	*Aci*I	CC: 195, 18 CT: 213, 195, 18 TT: 213
c.1161G>A	5′-CCGCATCGTGCGTAAGGAGT-3 5′-CTGCCCCGCTCCTCTCAGTAG-3′	63.7	210	Exon10	PCR-RFLP	*Mae*I	GG: 210 GA: 210, 184, 26 AA: 184, 26
c.1804C>A	5′-GACAATATGAGTGACCGGGTTCAG-3′ 5′-CGAACGAATGCCAGGGA<u>C</u>G-3′	64.3	207	Exon17	CRS-PCR	*Mae*II	CC: 207 CA: 207, 190, 17 AA: 190, 17

Note: SNPs, single nucleotide polymorphisms; PCR, polymerase chain reaction; PCR-RFLP, PCR-restriction fragment length polymorphism; CRS-PCR, created restriction site-PCR.
Underlined nucleotides mark nucleotide mismatches enabling the use of the selected restriction enzymes for discriminating sequence variations at CRS-PCR analysis.

Analyzer, Applied Biosystems, Foster City, CA) to confirm the genotyping results from PCR-RFLP and CRS-PCR methods.

STATISTICAL ANALYSIS

Statistical analyses were analyzed using the Statistical Package for Social Sciences statistical software release 16.0 (SPSS Windows version16.0; SPSS, Inc., Chicago, IL). Categorical variables were shown as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation (SD). Differences in demographic characteristics, risk factors and frequencies of alleles and genotypes were compared between cases and controls by the chi-square (χ^2) test. The Hardy–Weinberg equilibrium (HWE) was determined for compatibility between glioma patients and controls using the chi-square (χ^2) test. Unconditional logistic regression was used to evaluate the odds ratios (ORs) and 95% confidence intervals (CIs) for each genetic polymorphism. The potential associations of *XRCC1* genetic polymorphisms with glioma were estimated by calculating the ORs and their 95% CIs using the chi-square (χ^2) test. All comparisons were two-sided, and *P*-values less than 0.05 were regarded as statistically significant level.

RESULTS

CHARACTERISTICS OF STUDY SUBJECTS

In this case-control study, a total of 1,286 Chinese Han ethnic subjects consisted of 638 glioma patients and 648 controls were enrolled. Table I shows the distributions of general characteristics and potential risk factors among glioma patients and controls. The mean age was 52.98 ± 14.15 years among glioma patients and 53.52 ± 13.73 years among controls. There were no statistically significant differences in the gender, age, alcohol drinking, and tobacco smoking distribution between glioma patients and controls (*P* = 0.0879, 0.2552, 0.0688, and 0.1274, respectively). Individuals who have higher IR exposure were more likely to have higher risks of glioma (*P* = 0.0210). Similarly, individuals who have more family history of cancer were more likely to have higher risks of glioma than controls (15.36% vs. 9.57%, *P* = 0.0017). Of all the glioma patients, 45.92% were high-grade glioma.

IDENTIFICATION OF *XRCC1* GENETIC POLYMORPHISMS

Through the CRS-PCR and DNA sequencing methods, the genotyping of c.482C>T and c.1804C>A genetic polymorphisms of *XRCC1* gene were identified. The c.1161G>A genetic polymorphism of *XRCC1* gene were determined by the PCR-RFLP and DNA sequencing methods. According to the results from DNA sequence analyses which based on the human *XRCC1* gene DNA, mRNA and protein sequences (GenBank IDs: NC_000019.9, NM_006297.2, and NP_006288.2), the c.482C>T genetic variant is a non-synonymous mutation which corresponding to the C → T mutations and proline (Pro) to leucine (Leu) amino acid replacement (p.Pro161Leu) in the exon5 of *XRCC1* gene. The c.1161G>A genetic variant is a synonymous mutation which caused by G → A mutations (p. Leu387Leu) in exon10 of human *XRCC1* gene. As for the c.1804C>A genetic variant, it is a non-synonymous mutation which caused by C → A mutations and led to the Pro to threonine (Thr) amino acid replacement (p.Pro602Thr) in the exon17 of human *XRCC1* gene. The amplified PCR products of c.482C>T were digested with *Aci*I restriction enzyme and divided into three genotypes, CC (195 and 18 bp), CT (213, 195, and 18 bp) and TT (213 bp). As for c.1161G>A, the amplified PCR products were digested with *Mae*I restriction enzyme, and three genotypes were found, GG (210 bp), GA (210, 184, and 26 bp), and AA (184 and 26 bp). As for c.1804C>A, the amplified PCR products were digested with *Mae*II restriction enzyme, and three genotypes were detected, CC (207 bp), CA (207, 190, and 17 bp), and AA (190 and 17 bp).

ALLELIC AND GENOTYPIC FREQUENCIES OF *XRCC1* GENETIC POLYMORPHISMS

Table III shows the allelic and genotypic frequencies of *XRCC1* genetic polymorphisms in glioma patients and controls. Our data indicated that the allelic and genotypic frequencies of c.482C>T, c.1161G>A, and c.1804C>A genetic polymorphisms of *XRCC1* gene in glioma patients were significantly different from those of controls (All *P*-values < 0.05, Table III). The distributions of these three genetic polymorphisms were consistent with the HWE among the glioma patients and controls (all *P*-values > 0.05, Table III).

TABLE III. The Genotypic and Allelic Frequencies of *XRCC1* Genetic Polymorphisms in Glioma Cases and Controls

SNPs		Genotypic frequencies (%)			Allelic frequencies (%)		χ^2-value	*P*-value
c.482C>T	Groups	CC	CT	TT	C	T		
	Cases (n = 638)	295 (46.24)	265 (41.54)	78 (12.23)	855 (67.01)	421 (32.99)	2.3433	0.3098
	Controls (n = 648)	330 (50.92)	279 (43.06)	39 (6.02)	939 (72.45)	357 (27.55)	4.0071	0.1349
	Total (n = 1286)	625 (48.60)	544 (42.30)	117 (9.10)	1794 (69.75)	778 (30.25)	0.0078	0.9961
		$\chi^2 = 15.2435$, $P = 0.0005$			$\chi^2 = 9.0429$, $P = 0.0026$			

SNPs		Genotypic frequencies (%)			Allelic frequencies (%)		χ^2-value	*P*-value
c.1161G>A	Groups	GG	GA	AA	G	A		
	Cases (n = 638)	301 (47.18)	255 (39.97)	82 (12.85)	857 (67.16)	419 (32.84)	5.6204	0.0602
	Controls (n = 648)	326 (50.31)	267 (41.20)	55 (8.49)	919 (70.91)	377 (29.09)	0.0010	0.9995
	Total (n = 1286)	627 (48.76)	522 (40.59)	137 (10.65)	1776 (69.05)	796 (30.95)	3.2540	0.1965
		$\chi^2 = 6.5165$, $P = 0.0385$			$\chi^2 = 4.2252$, $P = 0.0398$			

SNPs		Genotypic frequencies (%)			Allelic frequencies (%)		χ^2-value	*P*-value
c.1804C>A	Groups	CC	CA	AA	C	A		
	Cases (n = 638)	277 (43.35)	271 (42.41)	91 (14.24)	825 (64.55)	453 (35.45)	3.4315	0.1798
	Controls (n = 648)	316 (48.77)	283 (43.67)	49 (7.56)	915 (70.60)	381 (29.40)	1.7570	0.4154
	Total (n = 1286)	593 (48.77)	554 (43.04)	140 (10.88)	1740 (67.60)	834 (32.40)	0.3870	0.8241
		$\chi^2 = 15.3627$, $P = 0.000$			$\chi^2 = 10.7457$, $P = 0.0010$			

GLIOMA RISKS ASSOCIATE WITH GENETIC POLYMORPHISMS OF *XRCC1* GENE

Table IV summarizes the association between the risks of glioma and *XRCC1* genetic polymorphisms. Our data indicated that the c.482C>T genetic polymorphism was significantly associated with the increased risks of glioma in the homozygote comparison (TT vs. CC: OR = 2.24, 95% CI = 1.48–3.39, $\chi^2 = 14.92$, $P < 0.001$), recessive model (TT vs. CT/CC: OR = 2.18, 95% CI = 1.46–3.25, $\chi^2 = 14.97$, $P < 0.001$) and allele contrast (T vs. C: OR = 1.30, 95% CI = 1.09–1.53, $\chi^2 = 9.04$, $P = 0.003$, Table IV). As for c.1161G>A genetic polymorphism, there were significantly increased susceptibility to glioma in the homozygote comparison (AA vs. GG: OR = 1.62, 95% CI 1.11–2.35, $\chi^2 = 6.30$, $P = 0.012$), recessive model (AA vs. GA/GG: OR = 1.59, 95% CI = 1.11–2.28, $\chi^2 = 6.43$, $P = 0.011$) and allele contrast (A vs. G: OR = 1.19, 95% CI = 1.01–1.41, $\chi^2 = 4.22$, $P = 0.040$, Table IV). As for c.1804C>A genetic polymorphism, significantly increased risks of glioma were detected in the homozygote comparison (AA vs. CC: OR = 2.12, 95% CI = 1.45–3.11, $\chi^2 = 15.13$, $P < 0.001$), recessive model (AA vs. CA/CC: OR = 2.03, 95% CI = 1.41–2.93, $\chi^2 = 14.79$, $P < 0.001$) and allele contrast (A vs. C: OR = 1.32, 95% CI = 1.12–1.56, $\chi^2 = 10.74$, $P = 0.001$, Table IV).

TABLE IV. The Association of Glioma Risks With SNPs of *XRCC1* Gene

SNPs	Comparisons	Test of association		
		OR (95% CI)	χ^2-value	*P*-value
c.482C>T	Homozygote comparison (TT vs. CC)	2.24 (1.48–3.39)	14.92	<0.001
	Heterozygote comparison (CT vs. CC)	1.06 (0.84–1.34)	0.27	0.606
	Dominant model (TT/CT vs. CC)	1.21 (0.97–1.50)	2.83	0.093
	Recessive model (TT vs. CT/CC)	2.18 (1.46–3.25)	14.97	<0.001
	Allele contrast (T vs. C)	1.30 (1.09–1.53)	9.04	0.003
c.1161G>A	Homozygote comparison (AA vs. GG)	1.62 (1.11–2.35)	6.30	0.012
	Heterozygote comparison (GA vs. GG)	1.03 (0.82–1.31)	0.08	0.776
	Dominant model (AA/GA vs. GG)	1.13 (0.91–1.41)	1.26	0.262
	Recessive model (AA vs. GA/GG)	1.59 (1.11–2.28)	6.43	0.011
	Allele contrast (A vs. G)	1.19 (1.01–1.41)	4.22	0.040
c.1804C>A	Homozygote comparison(AA vs. CC)	2.12 (1.45–3.11)	15.13	<0.001
	Heterozygote comparison (CA vs. CC)	1.09 (0.87–1.38)	0.56	0.455
	Dominant model (AA/CA vs. CC)	1.24 (1.00–1.55)	3.80	0.051
	Recessive model (AA vs. CA/CC)	2.03 (1.41–2.93)	14.79	<0.001
	Allele contrast (A vs. C)	1.32 (1.12–1.56)	10.74	0.001

Note: SNPs, single nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

DISCUSSION

Glioma is a common brain cancer and causes a heavy health burden in adults globally. Many emergence reports demonstrate that glioma is a common complex and multi-factorial disorder, which causing by complex interactions between genetic and environmental factors [Kiuru et al., 2008], while genetic factors play crucial roles in the pathogenesis of glioma. In recently years, the human *XRCC1* gene has been considered as a candidate gene for evaluating the genetic risks of glioma. However, these observations still remain conflicting rather than conclusive. Luo et al. [2013] observed that *XRCC1* 399G/G and *XRCC1* 194 T/T were associated with a higher risk of glioma when compared with the wild-type genotype (OR = 2.02, 95% CI = 1.17–3.46 and OR = 2.15, 95% CI = 1.09–4.22). Liu et al. [2009] found that *XRCC1* Arg399Gln had significant increased risk effects on glioma when compared with wild-type homozygote carriers (adjusted OR = 1.43; 95% CI = 1.05–1.92). Liu et al. [2012] revealed that the *XRCC1* Arg194Trp genotype TT conferred elevated risk for glioma (adjusted ORs = 2.66, 95% CI = 1.48–4.88). Wang et al. [2012] suggested that the *XRCC1* Arg399Gln variant (allele A) carriers were associated with an increased glioma risk compared to the wild-type (allele G) homozygous carriers (OR = 1.40, 95% CI = 1.12–1.76) in a Han population. Pan et al. [2013] demonstrated that the *XRCC1* Arg194Trp genotype TT was associated with a lower risk of glioma when compared with the wild genotype CC (OR = 2.45, 95% CI = 1.43–4.45), and individuals carrying the allele A of *XRCC1* Arg399Gln had an increased risk of glioma (OR = 1.33, 95% CI = 1.02–1.64). Hu et al. detected that the Trp/Trp and Arg/Trp genotypes of *XRCC1* Arg194Trp were associated with a 2.12- and 1.46-fold increased risk of glioma compared to the Arg/Arg wild genotype. The Gln/Gln and Arg/Gln genotypes of *XRCC1* Arg399Gln had a 2.24- and 1.67-fold increased risk in glioma compared to the Arg/Arg wild genotype [Hu et al., 2011]. Rajaraman et al. [2010] reported that the *XRCC1* Arg399Gln were associated with decreased glioma risk, but the *XRCC1* Arg194Trp and Arg280His was not associated with decreased glioma risk. Jin et al. [2013] suggested that the genotypes/alleles of Thr304Ala and Ser593Arg genetic polymorphisms were statistically associated with the increased risk of glioma in Chinese Han populations. Zhang et al. [2012] reported that the *XRCC1* Arg194Trp was not associated with glioma risk. Felini et al. [2007] indicated that there was no evidence of association between *XRCC1* Arg399Gln genotypes and glioma. Wang et al. [2004] did not find any statistically significant differences in the distributions of *XRCC1* Arg399Gln between glioma patients and controls. Jacobs and Bracken [2012] suggested that the *XRCC1* Arg399Gln not influence the risk of glioma among Caucasians. In the present study, on the basis of analysis of 638 glioma patients and 648 healthy controls, we firstly detected that the c.482C>T, c.1161G>A, and c.1804C>A SNPs of *XRCC1* gene have statistically significant impact on the risks of glioma in Chinese Han populations through association analyses. Our data suggested that there were significant differences in the allelic/genotypic frequencies between glioma patients and healthy controls, the genotypes/alleles of these SNPs were statistically associated with the increased risk of glioma. As for c.482C>T, genotype TT had a 2.24- and 2.18-fold increased risk effects on glioma when compared with wild-type genotype CC and

AT/TT carriers. As for c.1161G>A, the genotype AA were associated with a 1.62- and 1.59-fold increased risk of glioma compared to the wild-type genotype GG and GA/GG carriers. As for c.1804C>A, the genotype AA conferred a 2.12- and 2.03-fold elevated risk for glioma compared to the wild-type genotype CC and CA/CC carriers. The allele T of c.482C>T, allele A of c.1161G>A and allele A of c.1804C>A might be increased risk alleles for glioma, and may contribute to the risks of glioma. There are many other non-synonymous genetic variants of *XRCC1* gene (such as Arg194Trp, Arg280His, Thr304Ala, Arg399Gln, and Ser593Arg) have been approved to influence the function of *XRCC1* protein and significantly associated with the risks of glioma [Kiuru et al., 2008; Liu et al., 2009, 2012; Rajaraman et al., 2010; Yosunkaya et al., 2010; Hu et al., 2011; Zhou et al., 2011; Wang et al., 2012; Luo et al., 2013; Pan et al., 2013]. In this study, DNA sequence analyses indicated that the c.482C>T and c.1804C>A genetic variants are also non-synonymous mutations and cause amino acid replacement. The c.482C>T, c.1161G>A, and c.1804C>A genetic variants might be linked to those other non-synonymous genetic variants of *XRCC1* gene to influence the function of *XRCC1* protein and play the similar roles on the pathogenesis of glioma. Our results suggest these SNPs influence glioma risks in Chinese Han ethnic subjects, and might be potential molecular markers for evaluating the risks of glioma. The findings could provide new evidence for further analysis of the biological function role of *XRCC1* gene on the pathogenesis of glioma. Larger prospective investigations will be needed to validate the findings from our study on different populations.

REFERENCES

Deng X, Liang J, Jiang M, Zhou X, Liu H. 2013. Association between the C. 1161G > A and C. 1779C > G genetic variants of XRCC1 gene and hepatocellular carcinoma risk in Chinese population. Int J Biol Sci 9(3):289–294.

Felini MJ, Olshan AF, Schroeder JC, North KE, Carozza SE, Kelsey KT, Liu M, Rice T, Wiencke JK, Wrensch MR. 2007. DNA repair polymorphisms XRCC1 and MGMT and risk of adult gliomas. Neuroepidemiology 29(1–2):55–58.

Haliassos A, Chomel JC, Tesson L, Baudis M, Kruh J, Kaplan JC, Kitzis A. 1989. Modification of enzymatically amplified DNA for the detection of point mutations. Nucleic Acids Res 17(9):3606.

Hu XB, Feng Z, Fan YC, Xiong ZY, Huang QW. 2011. Polymorphisms in DNA repair gene XRCC1 and increased genetic susceptibility to glioma. Asian Pac J Cancer Prev 12(11):2981–2984.

Jacobs DI, Bracken MB. 2012. Association between XRCC1 polymorphism 399 G->A and glioma among Caucasians: A systematic review and meta-analysis. BMC Med Genet 13:97.

Jiang L, Fang X, Bao Y, Zhou JY, Shen XY, Ding MH, Chen Y, Hu GH, Lu YC. 2013. Association between the XRCC1 polymorphisms and glioma risk: A meta-analysis of case-control studies. PLoS ONE 8(1):e55597.

Jin Z, Xu H, Zhang X, Zhao G. 2013. Genetic polymorphisms in XRCC1 gene and susceptibility to glioma in Chinese Han population. Tumour Biol. 34: DOI:10.1007/s13277-013-1050-2.

Kiuru A, Lindholm C, Heinavaara S, Ilus T, Jokinen P, Haapasalo H, Salminen T, Christensen HC, Feychting M, Johansen C, Lonn S, Malmer B, Schoemaker MJ, Swerdlow AJ, Auvinen A. 2008. XRCC1 and XRCC3 variants and risk of glioma and meningioma. J Neurooncol 88(2):135–142.

Li M, Zhou Q, Tu C, Jiang Y. 2013. A meta-analysis of an association between the XRCC1 polymorphisms and gliomas risk. J Neurooncol 111(3):221–228.

Liu Y, Scheurer ME, El-Zein R, Cao Y, Do KA, Gilbert M, Aldape KD, Wei Q, Etzel C, Bondy ML. 2009. Association and interactions between DNA repair gene polymorphisms and adult glioma. Cancer Epidemiol Biomarkers Prev 18(1):204–214.

Liu HB, Peng YP, Dou CW, Su XL, Gao NK, Tian FM, Bai J. 2012. Comprehensive study on associations between nine SNPs and glioma risk. Asian Pac J Cancer Prev 13(10):4905–4908.

Luo KQ, Mu SQ, Wu ZX, Shi YN, Peng JC. 2013. Polymorphisms in DNA repair genes and risk of glioma and meningioma. Asian Pac J Cancer Prev 14(1):449–452.

Melin B. 2011. Genetic causes of glioma: New leads in the labyrinth. Curr Opin Oncol 23(6):643–647.

Mutamba JT, Svilar D, Prasongtanakij S, Wang XH, Lin YC, Dedon PC, Sobol RW, Engelward BP. 2011. XRCC1 and base excision repair balance in response to nitric oxide. DNA Repair (Amst) 10(12):1282–1293.

Ohgaki H, Kleihues P. 2005. Epidemiology and etiology of gliomas. Acta Neuropathol 109(1):93–108.

Pan WR, Li G, Guan JH. 2013. Polymorphisms in DNA repair genes and susceptibility to glioma in a chinese population. Int J Mol Sci 14(2):3314–3324.

Qiao W, Wang T, Zhang L, Tang Q, Wang D, Sun H. 2013. Association study of single nucleotide polymorphisms in XRCC1 gene with the risk of gastric cancer in Chinese population. Int J Biol Sci 9(7):753–758.

Rajaraman P, Hutchinson A, Wichner S, Black PM, Fine HA, Loeffler JS, Selker RG, Shapiro WR, Rothman N, Linet MS, Inskip PD. 2010. DNA repair gene polymorphisms and risk of adult meningioma, glioma, and acoustic neuroma. Neuro Oncol 12(1):37–48.

Ricard D, Idbaih A, Ducray F, Lahutte M, Hoang-Xuan K, Delattre JY. 2012. Primary brain tumours in adults. Lancet 379(9830):1984–1996.

Schwartzbaum JA, Fisher JL, Aldape KD, Wrensch M. 2006. Epidemiology and molecular pathology of glioma. Nat Clin Pract Neurol 2(9):494–503;quiz 491 p following 516.

Sun JY, Zhang CY, Zhang ZJ, Dong YF, Zhang AL, Wang ZW, Mei XL. 2012. Association between XRCC1 gene polymorphisms and risk of glioma development: A meta-analysis. Asian Pac J Cancer Prev 13(9):4783–4788.

Tewari R, Choudhury SR, Mehta VS, Sen E. 2012. TNFalpha regulates the localization of CD40 in lipid rafts of glioma cells. Mol Biol Rep 39(9):8695–8699.

Tudek B. 2007. Base excision repair modulation as a risk factor for human cancers. Mol Aspects Med 28(3–4):258–275.

Wang LE, Bondy ML, Shen H, Li J, Ren Y, Li G, Liu A. 2004. Polymorphisms of DNA repair genes and risk of glioma. Cancer Res 64(16):5560–5563.

Wang D, Hu Y, Gong H, El-Zein R, Aldape K, Cao Y, Pudavalli V, Levin VA, Yung WK, Wei Q. 2012. Genetic polymorphisms in the DNA repair gene XRCC1 and susceptibility to glioma in a Han population in northeastern China: A case-control study. Gene. 509(2):223–227.

Wei X, Chen D, Lv T. 2013. A functional polymorphism in XRCC1 is associated with glioma risk: Evidence from a meta-analysis. Mol Biol Rep 40(1):567–572.

Wen PY, Kesari S. 2008. Malignant gliomas in adults. N Engl J Med 359(5):492–507.

Yosunkaya E, Kucukyuruk B, Onaran I, Gurel CB, Uzan M, Kanigur-Sultuybek G. 2010. Glioma risk associates with polymorphisms of DNA repair genes, XRCC1 and PARP1. Br J Neurosurg 24(5):561–565.

Yuan ZR, Li JY, Li J, Zhang LP, Gao X, Gao HJ, Xu SZ. 2012. Investigation on BRCA1 SNPs and its effects on mastitis in Chinese commercial cattle. Gene 505(1):190–194.

Yuan ZR, Li J, Li JY, Gao X, Xu SZ. 2013a. SNPs identification and its correlation analysis with milk somatic cell score in bovine MBL1 gene. Mol Biol Rep 40(1):7–12.

Yuan ZR, Li JY, Li J, Gao X, Xu SZ. 2013b. Effects of DGAT1 gene on meat and carcass fatness quality in Chinese commercial cattle. Mol Biol Rep 40(2):1947–1954.

Zhang L, Wang Y, Qiu Z, Luo J, Zhou Z, Shu W. 2012. The XRCC1 Arg194Trp polymorphism is not a risk factor for glioma: A meta-analysis involving 1,440 cases and 2,562 controls. Exp Ther Med 4(6):1057–1062.

Zhao CJ, Li N, Deng XM. 2003. The establishment of method for identifying SNP genotype by CRS-PCR. Yi Chuan 25(3):327–329.

Zhou LQ, Ma Z, Shi XF, Yin XL, Huang KX, Jiu ZS, Kong WL. 2011. Polymorphisms of DNA repair gene XRCC1 and risk of glioma: A case-control study in Southern China. Asian Pac J Cancer Prev 12(10):2547–2550.