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DNA repair gene XRCC3 variants are associated with susceptibility to glioma in a Chinese population

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ABSTRACT. The susceptibility to glioma is not well understood. It has been suggested that the X-ray cross complementing group 3 (XRCC3) gene influences the capacity to repair DNA damage, leading to increased glioma susceptibility. In this study, we evaluated the relationship between XRCC3 mutations and glioma risk. Genotypes were assessed in 389 Chinese glioma patients and 358 healthy controls. XRCC3 Thr241Met (rs861539) and 2 additional polymorphisms, rs3212112 (c.774+19T>G) and rs1799796 (c.562-14A>G), were

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directly sequenced. The frequency of the rs861539 T allele was significantly lower in the glioma group than in healthy controls [11.1 *vs* 17.7%, odds ratio = 0.62 (0.48-0.80), P < 0.001]; the frequencies of the CT or CT+TT genotypes differed between groups (18.5 *vs* 31%, 20.3 *vs* 33.2%, respectively). The frequency of the rs3212112 G allele was significantly higher in the glioma group than in healthy controls [15.8 *vs* 5.3%, odds ratio = 2.94 (2.07-4.17), P < 0.001]. The frequencies of the GT or TG+GG genotypes differed between groups (25.4 *vs* 7.8%, 28.5 *vs* 9.2%, respectively). This study demonstrates that the rs861539 and rs3212112 polymorphisms in the XRCC3 gene may influence the risk of glioma development in Chinese populations.

Key words: Glioma; Single-nucleotide polymorphism; Susceptibility; X-ray cross-complementing group 3

INTRODUCTION

Brain tumors are a group of tumors that have a high level of morbidity and mortality worldwide (Bouffet et al., 2010). Gliomas are the most common primary common central nervous system tumors in adults, accounting for nearly 80% of all primary malignant brain tumors (Schwartzbaum et al., 2006). Although numerous studies have examined the etiology of glioma, there have been no conclusive results.

Previous studies indicated that damaged DNA or lower DNA repair capacity contributes to genetic instability and the occurrence and development progress of malignant tumors (Audebert et al., 2004; Matullo et al., 2006; Abdel-Rahman and El-Zein, 2011). DNA repair genes, which play an important role in repairing damaged DNA, may prevent the activation of oncogenes or inactivation of tumor-suppressor genes (Abdel-Rahman and El-Zein, 2011). Over the past decade, an increasing number of polymorphisms in DNA repair genes have been identified and their involvement in several types of tumors was studied (Audebert et al., 2004; Matullo et al., 2006; Liu et al., 2007; Tahara et al., 2011).

Among the genes identified, the X-ray cross complementing group 3 (XRCC3) gene may influence the capacity to repair DNA damage, leading to increased cancer susceptibility. The functional single-nucleotide polymorphism (SNP) of XRCC3, Thr241Met (rs861539), is located at codon 241 in exon 7 with a C to T transition (Liu et al., 1998). Several studies have found this variant is a susceptibility factor for several cancers, including lung, breast, colorectal, and bladder cancers (Liu et al., 2007; Yan et al., 2009; Abdel-Rahman and El-Zein, 2011; Romanowicz-Makowska et al., 2012; Zhao et al., 2012). Moreover, some studies found that the XRCC3 Thr241Met polymorphism is associated with glioma risk (Wang et al., 2004; Yosunkaya et al., 2010). However, the cumulative results remain inconclusive because of the different ethnicities, research samples, ages, and subtypes of glioma included in the studies.

In this study, we sequenced the XRCC3 gene Thr241Met polymorphism to investigate the relationship between the XRCC3 Thr241Met polymorphism and glioma risk.

MATERIAL AND METHODS

Study subjects

The glioma patients and healthy controls recruited in this study were enrolled from Xiangya Hospital of Central South University during 2007-2013. All cases recruited in this study were histologically confirmed. Controls were randomly selected from people who requested general health examinations in the same hospital. Healthy controls were required to have no history of any type of cancer.

The clinical study admission (registration number: ChiCTR-RO-12002853) was approved by Chinese Clinical Trail Register. The study protocol was approved by the Ethics Committee of Xiangya School of Medicine and Ethics Committee of Institute of Clinical Pharmacology of Central South University. All patients and healthy controls were investigated by face-to-face interviewing with a questionnaire by doctors or nurses. All patients signed consent forms before participating in the study.

Genotyping

DNA was isolated from 3 mL whole blood samples using the phenol-chloroform extraction method and then stored at 4°C until use. The primer pairs used for amplification of the XRCC3 Thr241Met (rs861539) locus were as follows: sense primer: 5'-GGTCGAGTGACA GTCCAAAC-3', antisense primer: 5'-CTACCCGCAGGAGCCGGAGG-3'. Polymerase chain reaction (PCR) amplifications were carried out in a total volume of 25 µL containing 2.5 µL 10X PCR buffer (containing MgCl2) (Takara, Shiga, Japan), 0.2 mM of each dNTP (Takara), 0.4 mM of each primer, 200 ng genomic DNA as a template, and 1 U Taq polymerase (Takara). The PCR conditions were as follows: predenaturation at 94°C for 5 min; 30 cycles each of denaturing at 94°C for 30 s, annealing 58°C for 30 s, and extension at 72°C for 30 s; and extension at 72°C for 8 min. The PCR products were directly sequenced. PCR fragments were purified with PCR Clean-Up System (Promega, Southampton, UK), sequenced using the BigDye Terminator v. 1.1 Cycle Sequencing Kit [Applied Biosystems (ABI), Weiterstadt, Germany], and the sequencing products were sequenced by ABI 3130 Genetic Analyzer.

Statistical analysis

The SPSS software package version 13.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Hardy-Weinberg equilibrium was tested with χ^2 test or Fisher's exact test as applicable in the case-control samples. Data pertaining to age and gender as well as other characteristics were compared between glioma patients and healthy controls using the Student t-test or chi-square analysis. The relationship between various genotypes or alleles and the susceptibility to glioma was examined using the χ^2 test. Statistical significance was accepted when $P < 0.05$.

RESULTS

A total of 389 patients (199 males, 190 female, age: 48.6 ± 9.8 years) and 358 healthy controls (174 males, 184 female, age: 47.6 ± 10.4 years) were enrolled in our study. The demographic and clinical characteristics of patients are shown in Table 1. There were no differences between the 2 groups regarding gender and age distribution. The 389 glioma cases consisted of 185 (47.6%) glioblastoma, 95 (24.4%) anaplastic astrocytoma or diffuse astrocytoma or other astrocytoma, and 109 (28%) other gliomas.

Table 1. Demographic and clinical characteristics of patients.

Parameters	Patients cases (N = 389)	Healthy controls (N = 358)	P value
Male/Female	199 (51.2%)/190 (48.8%)	174 (48.6%)/184 (51.4%)	0.76
Age (years)	48.6 ± 9.8	47.6 ± 10.4	0.65
Smoking status	Yes (N = 141)/No (N = 248)	Yes (N = 112)/No (N = 246)	0.43
Gliomas types (%)			
Glioblastoma	185 (47.6%)	-	
Astrocytomas except for glioblastoma	95 (24.4%)	-	
Other gliomas	109 (28%)	-	

We sequenced the XRCC3 gene Thr241Met (rs861539) polymorphism in 389 glioma patients and 358 healthy controls and identified 2 additional polymorphisms, rs3212112 (c.774+19T>G) and rs1799796 (c.562-14A>G). The position of the SNPs and their relationship with the exon-intron boundaries of XRCC3 are shown in Table 2.

Table 2. Position of SNPs and their relation to exon-intron boundaries of XRCC3.

Gene	rs No.	SNP	Position	Location
XRCC1	rs861539	C/T	103,699,416	Exon
	rs1799796	A/G	103,699,590	Intron
	rs3212112	T/G	103,699,345	Intron

The SNPs investigated were all in Hardy-Weinberg equilibrium. The allele and genotype frequencies of the 3 SNPs in glioma patients (N = 389) and healthy controls (N = 358) are shown in Table 3. We found that the XRCC3 gene rs1799796 and rs861539 alleles and genotypes were associated with glioma in our Chinese population. The frequency of the rs861539 T allele was significantly lower in the glioma group than in healthy controls [11.1 *vs* 17.7%, odds ratio (95% confidence interval) = 0.62 (0.48-0.80), P < 0.001]; the frequencies of the CT or CT+TT genotypes differed between groups (18.5 *vs* 31%, 20.3 *vs* 33.2%, respectively). The frequency of the rs3212112 G allele was significantly higher in the glioma group than in healthy controls [15.8 *vs* 5.3%, odds ratio (95% confidence interval) = 2.94 (2.07-4.17), P < 0.001]; the frequencies of the GT or TG+GG genotypes differed between the 2 groups (25.4 *vs* 7.8%, 28.5 *vs* 9.2%, respectively). We observed no difference in allele or genotypes frequencies for rs1799796 between the glioma patients and healthy controls after Bonferroni correction.

Table 3. Allele and genotype frequencies of 3 SNPs in gliomas patients (N = 389) and healthy controls (N = 358).

SNP	Genotype	Case (N = 389)	Control (N = 358)	χ^2	P value	Odds ratio (95%CI)
rs3212112	T	655 (84.2%)	678 (94.7%)			Reference
	G	123 (15.8%)	38 (5.3%)	41.7	<0.001	2.94 (2.07-4.17)
	TT	278 (71.5%)	325 (90.8%)			Reference
	TG	99 (25.4%)	28 (7.8%)	46.6	<0.001	3.31 (2.23-4.91)
	GG	12 (3.1%)	5 (1.4%)	3.98	0.046	2.23 (0.97-7.66)
	TG + GG	111 (28.5%)	33 (9.2%)	44.7	<0.001	3.10 (2.16-4.44)
rs861539	C	692 (88.9%)	589 (82.3%)			Reference
	T	86 (11.1%)	127 (17.7%)	13.6	<0.001	0.62 (0.48-0.80)
	CC	310 (79.7%)	239 (66.8%)			Reference
	CT	72 (18.5%)	111 (31%)	16.1	<0.001	0.59 (0.46-0.77)
	TT	7 (1.8%)	8 (2.2%)	0.57	0.45	0.68 (0.25-1.85)
	CT + TT	79 (20.3%)	119 (33.2%)	16.0	<0.001	0.61 (0.48-0.78)
rs1799796	A	525 (67.5%)	506 (70.7%)			Reference
	G	253 (32.5%)	210 (39.3%)	5.32	0.02	1.19 (1.03-1.37)
	AA	161 (41.4%)	162 (45.3%)			Reference
	AG	203 (52.2%)	182 (50.8%)	0.17	0.68	1.03 (0.90-1.18)
	GG	25 (6.4%)	14 (3.9%)	0.18	0.67	1.13 (0.66-1.93)
	AG + GG	228 (58.6%)	214 (54.7%)	0.23	0.63	1.03 (0.91-1.17)

DISCUSSION

Numerous studies have demonstrated that DNA injury and repair play an important role in the occurrence and development of various types of tumors. Moreover, there is evidence that some SNPs in the DNA repair pathway genes can modify the DNA repair capability and increase cancer risk (Takanami et al., 2005). XRCC3 belongs to the DNA double-strand break repair pathway. Previous studies found that the XRCC3 Thr241Met (rs861539) polymorphism may influence DNA repair capacity and also showed that the XRCC3 Thr241Met polymorphism may be associated with cancer risk (Matullo et al., 2006; Liu et al., 2007; López-Cima et al., 2007; Romanowicz-Makowska et al., 2012). Numerous studies have examined the relationship between the XRCC3 Thr241Met polymorphism and glioma risk, and meta-analysis studies showed positive results (Liang et al., 2013; Zhao et al., 2013; Wang et al., 2014) and also some negative results (Kiuru et al., 2008; Feng et al., 2014). The association strength between XRCC3 Thr241Met and glioma susceptibility remains unclear and requires further analysis.

In this case-control study, we analyzed 3 SNPs in XRCC3 in 389 glioma patients and 358 controls. We found that rs861539 and rs3212112 were significantly associated with glioma risk. These findings suggest that XRCC3 SNPs can be used as markers for testing the genetic susceptibility to glioma. Our results were consistent with those of several studies reporting that rs861539 Thr241Met is a risk allele for glioma. The DNA repair protein XRCC3 contains 346 amino acids and is involved in the repair of DNA strand breaks via the homologous recombination repair pathway (Lee et al., 2007). The XRCC3 Thr241Met polymorphism (rs861539), located in exon 7, shows substitution of thymine (T) to cytosine (C) at codon 241, changing threonine (Thr) to methionine (Met), which may affect enzyme function and/or its interaction with other proteins involved in DNA damage repair (Matullo et al., 2001; Jiao et al., 2008; Williams and Sobol, 2013). Previous meta-analysis studies have examined the XRCC3 Thr241Met polymorphism and glioma risk. Most of the results were consistent with our results showing that the XRCC3 Thr241Met polymorphism is a risk factor for glioma. However, Feng et al. (2014), who analyzed 8 case-control studies including a total of 3455 glioma cases

and 4435 controls, found no significant association between the XRCC3 T241M polymorphism and glioma (Feng et al., 2014). In subgroup analysis, this polymorphism appeared to be associated with an increased glioma risk in Asians in their analysis (Feng et al., 2014). Only 4 case-control studies have examined Asian glioma patients (Zhou et al., 2009; Liu et al., 2012; Luo et al., 2013; Pan et al., 2013), and more sample sizes are needed to confirm whether an association exists between the XRCC3 T241M polymorphism and glioma risk.

In this study, we found that the XRCC3 rs3212112 polymorphism was significantly associated with glioma risk. The frequency of the XRCC3 rs3212112 G allele was 15.8% in glioma patients and 5.3% in healthy controls. The frequencies of the TG or GG and TG+GG genotypes were significantly higher in glioma patients. The XRCC3 rs3212112 polymorphism is located in an intron of the XRCC3 gene, and the function of this polymorphism is unknown. In our study, we found that the heterozygotes TG and TG+GG variants of XRCC3 increased the risk of glioma by 3.3- and 3.1-fold, respectively, compared to the homozygous wild-type genotype.

In conclusion, our case-control analysis of 3 SNPs in the XRCC3 gene suggests that rs861539 and rs3212112 in the XRCC3 gene are associated with glioma risk. Our study provides important information regarding the etiology of glioma. Furthermore, large-sample studies are needed to validate our results.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Abdel-Rahman SZ and El-Zein RA (2011). Evaluating the effects of genetic variants of DNA repair genes using cytogenetic mutagen sensitivity approaches. *Biomarkers* 16: 393-404.

Audebert M, Salles B and Calsou P (2004). Involvement of poly (ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double-strand breaks rejoining. *J. Biol. Chem.* 279: 55117-55126.

Bouffet E, Tabori U, Huang A and Bartels U (2010). Possibilities of new therapeutic strategies in brain tumors. *Cancer Treat. Rev.* 36: 335-341.

Feng Y, Zeng M and Xu Q (2014). Association between XRCC3 T241M polymorphism and glioma risk: a meta-analysis. *Tumour Biol.* 35: 5589-5592.

Jiao L, Hassan MM, Bondy ML, Wolff RA, et al. (2008). XRCC2 and XRCC3 gene polymorphism and risk of pancreatic cancer. *Am. J. Gastroenterol.* 103: 360-367.

Kiuru A, Lindholm C, Heinävaara S, Ilus T, et al. (2008). XRCC1 and XRCC3 variants and risk of glioma and meningioma. *J. Neurooncol.* 88: 135-142.

Lee SA, Lee KM, Park SK, Choi JY, et al. (2007). Genetic polymorphism of XRCC3 Thr241Met and breast cancer risk: case-control study in Korean women and meta-analysis of 12 studies. *Breast Cancer Res. Treat.* 103: 71-76.

Liang HJ, Yan YL, Liu ZM, Chen X, et al. (2013). Association of XRCC3 Thr241Met polymorphisms and gliomas risk: evidence from a meta-analysis. *Asian Pac. J. Cancer Prev.* 14: 4243-4247.

Liu HB, Peng YP, Dou CW, Su XL, et al. (2012). Comprehensive study on associations between nine SNPs and glioma risk. *Asian Pac. J. Cancer Prev.* 13: 4905-4908.

Liu N, Lamerdin JE, Tebbs RS, Schild D, et al. (1998). XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol. Cell* 1: 783-793.

Liu R, Yin LH and Pu YP (2007). Reduced expression of human DNA repair genes in esophageal squamous-cell carcinoma in china. *J. Toxicol. Environ. Health A* 70: 956-963.

López-Cima MF, González-Arriaga P, García-Castro L, Pascual T, et al. (2007). Polymorphisms in XPC, XPD, XRCC1, and XRCC3 DNA repair genes and lung cancer risk in a population of northern Spain. *BMC Cancer* 7: 162.

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

Matullo G, Palli D, Peluso M, Guarrera S, et al. (2001). XRCC1, XRCC3, XPD gene polymorphisms, smoking and (32) P-DNA adducts in a sample of healthy subjects. *Carcinogenesis* 22: 1437-1445.

Matullo G, Dunning AM, Guarrera S, Baynes C, et al. (2006). DNA repair polymorphisms and cancer risk in non-smokers in a cohort study. *Carcinogenesis* 27: 997-1007.

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

Romanowicz-Makowska H, Bryś M, Forma E, Maciejczyk R, et al. (2012). Single nucleotide polymorphism (SNP) Thr241Met in the XRCC3 gene and breast cancer risk in Polish women. *Polish J. Pathol.* 63: 121-125.

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

Tahara T, Shibata T, Nakamura M, Yamashita H, et al. (2011). Effect of genetic polymorphisms related to DNA repair and the xenobiotic pathway on the prognosis and survival of gastric cancer patients. *Anticancer Res.* 31: 705-710.

Takanami T, Nakamura J, Kubota Y and Horiuchi S (2005). The Arg280His polymorphism in X-ray repair cross-complementing gene 1 impairs DNA repair ability. *Mutat. Res.* 582: 135-145.

Wang LE, Bondy ML, Shen H, El-Zein R, et al. (2004). Polymorphisms of DNA repair genes and risk of glioma. *Cancer Res.* 64: 5560-5563.

Wang R, Li M, Gao WW, Gu Y, et al. (2014). Quantitative assessment of the association between XRCC3 C18607T polymorphism and glioma risk. *Tumour Biol.* 35: 1101-1105.

Williams K and Sobol RW (2013). Mutation research/fundamental and molecular mechanisms of mutagenesis: special issue: DNA repair and genetic instability. *Mutat. Res.* 743-744: 1-3.

Yan L, Yanan D, Donglan S, Na W, et al. (2009). Polymorphisms of XRCC1 gene and risk of gastric cardiac adenocarcinoma. *Dis. Esophagus* 22: 396-401.

Yosunkaya E, Kucukyuruk B, Onaran I, Gurel CB, et al. (2010). Glioma risk associates with polymorphisms of DNA repair genes, XRCC1 and PARP1. *Br. J. Neurosurg.* 24: 561-565.

Zhao B, Ye J, Li B, Ma Q, et al. (2013). DNA repair gene XRCC3 Thr241Met polymorphism and glioma risk: a meta-analysis. *Int. J. Clin. Exp. Med.* 6: 438-443.

Zhao Y, Deng X, Wang Z, Wang Q, et al. (2012). Genetic polymorphisms of DNA repair genes XRCC1 and XRCC3 and risk of colorectal cancer in Chinese population. *Asian Pac. J. Cancer Prev.* 13: 665-669.

Zhou K, Liu, Y, Zhang H, Liu H, et al. (2009). XRCC3 haplotypes and risk of gliomas in a Chinese population: a hospital-based case-control study. *Int. J. Cancer* 12: 2948-2953.