RESEARCH ARTICLE

Polymorphisms in DNA Repair Genes and Risk of Glioma and Meningioma

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Abstract

Polymorphisms in DNA repair genes have been shown to influence DNA repair processes and to modify cancer susceptibility. Here we conducted a case-control study to assess the role of potential SNPs of DNA repair genes on the risk of glioma and meningioma. We included 297 cases and 458 cancer-free controls. Genotyping of XRCC1 Gln399Arg, XRCC1 Arg194Trp, XRCC2 Arg188His, XRCC3 Thr241Met, XRCC4 Ala247Ser, ERCC1 Asn118Asp, ERCC2 Lys751Gln and ERCC5 Asp1558His were performed in a 384-well plate format on the Sequenom MassARRAY platform. XRCC1 Arg194Trp (rs1799782) and ERCC2 Asp312Asn rs1799793 did not follow the HWE in control group, and genotype distributions of XRCC1 Gln399Arg rs25487, XRCC2 Arg188His rs3218536 and ERCC2 Asp312Asn rs1799793 were significantly different between cases and controls (P<0.05). We found XRCC1 399G/G, XRCC1 194 T/T and XRCC3 241T/T were associated with a higher risk when compared with the wild-type genotype. For ERCC5 Asp1558His, we found G/G genotype was associated with elevated susceptibility. In conclusion, our study has shown that XRCC1 Gln399Arg, XRCC1 Arg194Trp, XRCC3 Thr241Met and ERCC5 Asp1558His are associated with risk of gliomas and meningiomas. This finding could be useful in identifying the susceptibility genes for these cancers.

Keywords: DNA repair gene - glioma - meningiomas - polymorphism

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Introduction

Gliomas and meningiomas are common central nervous system tumors, and account for about 80% of all the central nervous system tumors (Parkin et al., 2005). Although the morbidity of glioma is relatively low compared with other cancers, it is the most serious cancer in humans and influences the function of all the organs, and its prognosis is poor relative to other tumors (Bondy et al., 2008). Although many studies have been performed to explore the etiology of gliomas, the etiology is still not completely understood. The only confirmed environmental risk factors are ionizing radiation and ultraviolet rays, and the low exposure of them would protect the occurrence of this cancer (Sadetzki et al., 2007; Davis et al., 2008). The ionizing radiation and ultraviolet rays induce DNA damage, such as oxidative DNA damage, single- and double-strand breaks in DNA chains, and DNA-protein cross-links (Vogelstein and Kinzler, 2004). These damages would cause the development of cancer. Three main complex systems of DNA repairs pathways prevent the DNA damage and prevent mutagenesis, including base-excision repair (BER), nucleotide excision repair (NER), and homologous recombination repair (HRR) (National Academies of Science, 2006).

Several common and putative functional single nucleotide polymorphisms (SNPs) of DNA repaired gene have been identified, of which XRCC1 Gln399Arg, XRCC3 Thr241Met, XRCC4 Ala247Ser, ERCC1 Asn118Asp, ERCC2 Lys751Gln, ERCC5 Asp1558His may modify the risk of glioma (Shete et al., 2009; Wrensch et al., 2009; Liu et al., 2010). However, few studies examine the role of these gene polymorphisms on meningioma risk. Therefore, we conducted a case-control study to assess the role of potential eight SNPs of five DNA repaired gene on the risk of glioma and meningioma.

Materials and Methods

This case-control study was conducted at the Sun Yat-sen memorial Hospital between 2007 to 2011 years. A total of 326 cases were asked to participate, of whom 297 were successfully interviewed and provided 5 ml blood samples, for a participation rate of 91.06%. 458 cancer-free controls were selected from inpatients who admitted to our hospital for orthopedic injuries, digestive disorders and musculoskeletal disorders, and 415 controls were agreed to participate into our study, with a participation rate of 90.7%. Controls with known central nervous system-related diseases, a history of any types of cancer, and chemotherapy for unknown disease conditions were excluded. All the cases and controls were investigated by

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face to face interviewing with a structured questionnaire by doctors or nurses. The questionnaires included sex, age, family history of cancer and IR exposure history. All the informed consent was obtained from all recruited subjects.

Genotyping

Extraction of DNA was performed by buffy-coat fractions with TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). Genotyping of XRCC1 Gln399Arg(rs25487), XRCC1 Arg194Trp(rs1799782), XRCC2 Arg188His (rs3218536), XRCC3 Thr241Met (rs861539), XRCC4 Ala247Ser (rs3734091), ERCC1 Asn118Asp (rs11615), ERCC2 Lys751Gln (rs1799793), ERCC5 Asp1558His (rs17655) was conducted in a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). Sequenom Assay Design 3.1 software was used to design the primers for polymerase chain reaction (PCR) amplification and single base extension (SBE) assays(Sequenom, San Diego, CA, USA) according to the manufacturer's instructions. PCR was carried out in a reaction volume of 20 μl containing 50ng of genomic DNA, 200μM dNTP, 2.5 units of Taq DNA polymerase (Promega Corporation, WI, USA), and 200μM primers. The condition of PCR reaction began with 95°C for 120 seconds, 35 cycles of 94°C for 30 seconds, followed by annealing temperature to 64°C for 30 seconds, and 72°C for 60 seconds. PCR product was electrophoresis on 1.0% agrose gel to testify PCR products. For quality control, a random sample of 10% of cases and controls was genotyped again by different researchers. The reproducibility was 100%.

Statistical analyses

All statistical analyses were performed by SPSS for Windows software (version 16.0 SPSS, Chicago, IL). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were shown as frequencies and percentages. Difference of demographic characteristics and frequencies of genotypes between cases and controls were calculated by means of a chi-square test and Student's t test. Hardy-Weinberg equilibrium (HWE) was checked for controls with the χ^2 test. Unconditional logistic regression was used to calculate the adjusted odds ratios (ORs) and 95% confidence intervals (CIs) by potential sex, age, ionizing radiation and family history of cancer. The effect of these DNA repaired gene polymorphisms on the glioma and meningioma risk were assessed after adjusting potential risk factors. All comparisons were two-sides, and $P < 0.05$ was regarded as statistically significant.

Results

Among 297 patients, 202 patients were gliomas and 95 were meningiomas. The mean ages of the cases and controls were 48.7±7.9 and 50.2±8.1 years old. There was no significant differences in the sex and age distribution between the two groups ($P>0.05$). However, individuals who have higher IR exposure and more family history of cancer were more likely to have higher risk of glioma and meningiomas ($P<0.05$). Similarly, cases were had a more family history of cancer than controls ($P<0.05$). Most of the patients were low grade cancer (58.7%).

The minor allele frequencies among selected controls of XRCC1 Gln399Arg, XRCC1 Arg194Trp, XRCC2 Arg188His, XRCC3 Thr241Met, XRCC4 Ala247Ser, ERCC1 Asn118Asp, ERCC2 Lys751Gln and ERCC5 Asp1558His were consistent with the MAF from NCBI SNP database (Table 1). Six tested tSNPs were in line with the Hardy-Weinberg equilibrium (HWE) in control population ($P>0.05$), which suggested no population stratification and sample bias. However, XRCC1 Arg194Trp

Table 1. Frequencies of Glioma and Meningioma Patients and Controls with Respect to Selected Characteristics

Characteristics	Case N=297	%	Control N=415	%	χ^2	*P* value
Age	48.7±7.9		50.2±8.1			
Age (years)						
<40	45	15.2	66	15.9	0.11	0.95
40~55	153	51.5	206	49.7		
>55	99	33.3	143	34.4		
Sex						
Male	170	57.3	250	60.3	0.001	0.99
Female	127	42.7	165	39.7		
IR exposure history						
Never	277	93.3	410	98.8	4.65	0.03
Ever	20	6.8	5	1.2		
History of cancer						
No	272	91.6	406	97.9	4.29	0.038
Yes	25	8.4	9	2.1		
Type of tumor						
Glioma	202	68				
Meningioma	95	32				
Histological types						
High grade glioma	123	41.4				
Low grade glioma	174	58.6				

Table 2. Genotype Characteristics of the Selected Single Nucleotide Polymorphisms

Genes	Genotype frequency of cases			Genotype frequency of controls			*P* value	MAF		*P* for HWE
	A/A[1]	A/a[2]	a/a[3]	A/A	A/a	a/a		From dbSNP	Control	in controls
XRCC1 rs Gln399Arg 25487	111	134	51	189	181	45	<0.05	0.2633	0.301	0.22
XRCC1 Arg194Trp rs1799782	204	63	30	297	96	22	0.41	0.1296	0.146	<0.05
XRCC2 Arg188His rs3218536	242	52	4	364	41	10	<0.05	0.0426	0.064	0.06
XRCC3 Thr241Met rs861539	145	131	21	229	168	17	0.16	0.25	0.251	0.07
XRCC4 Ala247Ser rs3734091	262	31	4	369	35	11	0.38	0.0371	0.053	0.09
ERCC1 Asn118Asp rs11615	138	114	45	188	158	69	0.83	0.3439	0.336	0.06
ERCC2 Asp312Asn rs1799793	230	58	9	343	62	10	<0.05	0.1937	0.218	<0.05
ERCC5 Asp1558His rs17655	138	123	36	213	181	21	0.4	0.3768	0.367	0.1

[1]Wide-type; [2]Heterozygous; [3]Homozygous variant

Table 3. Genotype Frequencies and OR(95% CI) for Association Between Eight SNPs and Glioma Risk

Genes	Major/Minor allele	Total cases		Glioma		Meningiomas	
		OR(95% CI)	*P* value	OR(95% CI)	*P* value	OR(95% CI)	*P* value
XRCC1 Gln399Arg	A/A			-			
rs25487	A/G	1.36(0.90-1.77)	0.16	1.20(0.81-1.75)	0.34	1.42(0.84-2.38)	0.16
	G/G	1.93(1.18-3.15)	0.005	2.02(1.17-3.46)	0.006	1.72(0.78-3.64)	0.12
XRCC1 Arg194Trp	C/C	-		-		-	
rs1799782	C/T	0.96(0.65-1.40)	0.81	1.06(0.69-1.65)	0.78	0.94(0.52-1.65)	0.83
	T/T	1.70(0.90-3.23)	0.07	2.15(1.09-4.22)	0.01	0.78(0.19-2.41)	0.66
XRCC2 Arg188His	A/A	-		-		-	
rs3218536	A/G	1.49(0.92-2.43)	0.09	1.47(0.84-2.53)	0.14	1.53(0.73-3.04)	0.2
	G/G	0.87(0.26-2.69)	0.79	1.07(0.28-3.52)	0.89	0.45(0.01-3.24)	0.44
XRCC3 Thr241Met	C/C	-		-		-	
rs861539	C/T	1.23(0.89-1.70)	0.19	1.22(0.85-1.76)	0.26	1.25(0.77-2.02)	0.34
	T/T	2.16(1.04-5.52)	0.02	2.20(1.02-4.83)	0.03	1.43(0.39-4.21)	0.5
XRCC4 Ala247Ser	A/A	-		-		-	
rs3734091	A/G	1.31(0.78-2.24)	0.39	1.25(0.67-2.28)	0.44	1.25(0.53-2.72)	0.55
	G/G	1.25(0.72-2.14)	0.59	0.75(0.17-2.61)	0.64	0.40(0.01-2.82)	0.37
ERCC1 Asn118Asp	C/C	-		-		-	
rs11615	C/T	1.12(0.86-1.65)	0.09	0.98(0.66-1.45)	0.92	0.99(0.60-1.63)	0.96
	T/T	0.89(0.56-1.40)	0.53	1.08(0.65-1.77)	0.76	0.52(0.21-1.15)	0.09
ERCC2 Lys751Gln	G/G	-		-		-	
rs13181	G/T	1.40(0.92-2.11)	0.09	1.33(0.83-2.12)	0.21	1.54(0.83-2.77)	0.13
	T/T	1.34(0.47-3.74)	0.53	1.30(0.38-4.03)	0.61	1.43(0.25-5.73)	0.59
ERCC5 Asp1558His	C/C	-		-		-	
rs17655	C/G	1.05(0.75-1.45)	0.77	1.05(0.73-1.52)	0.77	1.04(0.62-1.73)	0.88
	G/G	2.64(1.43-4.97)	<0.001	2.11(1.03-4.30)	0.02	3.86(1.72-8.48)	<0.001

and ERCC2 Asp312Asn did not follow the HWE in control group (Table 2). We found genotype distributions of XRCC1 Gln399Arg, XRCC2 Arg188His and ERCC2 Asp312Asn were significant difference between cases and controls ($P<0.05$).

Association between these SNPs and the risk of glioma and meningiomas were analyzed using unconditional logistical regression analysis (Table 3). We found XRCC1 399G/G was associated with 2.02-fold increased risk of cancer when compared with A/A genotype, while no risk was found in risk of meningiomas. The XRCC1 Arg194Trp was found an enhanced risk of glioma, and the OR (95% CI) for cases with T/T genotype was 2.15 (1.09-4.22) when compared with those carrying C/C genotype. For XRCC3 Thr241Met, the variant genotype T/T was strongly significantly associated with a higher risk of glioma when compared with the wide-type C/C, with an adjusted OR (95% CI) of 2.20 (1.02-4.83). For ERCC5 Asp1558His, we found G/G genotype was associated with elevated susceptibility to glioma and meningiomas, with the OR (95% CI) of 2.11 (1.03-4.30) and 3.86 (1.72-8.48), respectively.

Discussion

Previous studies of brain tumors, mainly glioma, have demonstrated that common genetic variation in DNA repair genes might affect the risk of cancer (Shete et al., 2009; Wrensch et al., 2009; Liu et al., 2010). Although few previous studies have examined the risk of meningioma with variation of DNA repaired genes, and there are some indications that some variation of DNA repaired genes might be susceptible to meningioma than the glioma (Bethke et al., 2008). This study has examined the risk of glioma and meningioma in 1127 polymorphisms of DNA repair genes, and has indicated BR1P1 rs4968541 is associated with the risk of meningioma (OR=1.6, 95%CI=1.3-1.9) and CHAF1A rs243356 is related to a moderate higher risk of glioma (OR=1.3, 95%CI=1.1-1.5). In our study, we firstly attempt to investigate the potential association of eight SNPs of seven DNA repaired genes with glioma and meningioma. We have demonstrated that variants of XRCC1 Gln399Arg, XRCC1 Arg194Trp, XRCC3 Thr241Met and ERCC5 Asp1558His are associated with risk of glioma, and ERCC5 1558G/G genotype is associated with 3.86-fold risk of meningioma.

We found a strong association of polymorphisms in XRCC1 Gln399Arg, XRCC1 Arg194Trp, XRCC3 Thr241Met and ERCC5 Asp1558His with glioma risk, with ORs ranged from 2.02 to 2.20. The association of XRCC1 polymorphisms with glioma has been discussed in various studies (Liu et al., 2009; Sterpone et al., 2010; Yosunkaya et al., 2010; Zipprich et al., 2010). A previous study conducted in the United State has shown polymorphisms of XRCC1 Gln399Arg is associated with increased risk of glioma in 373 glioma patients and 365 controls (Liu et al., 2009). Another study conducted in Turkey has indicated the XRCC1 399G allele carries a 3.5 times greater risk for glioma (Yosunkaya et al., 2010). The rs25487 located in the region of the BRCT1 binding domain, and mutations in the BRCT1 domain of BRCA1 have been implicated in the altered function of this tumor suppressor gene (Sterpone et al., 2010). Previous several studies have shown that XRCC1 399 variant allele was associated with increased gene expression of various cancers, such as head and neck cancer, ovarian cancer and hepatocellular carcinoma (Cheng et al., 2012; Kumar et al., 2012; Yuan et al., 2012). The results of our study are in line with previous studies, suggesting a strong increased risk of glioma among individuals carrying with XRCC1 399G/G or XRCC1 194T/T genotypes.

In addition, the results of our study have showed XRCC3 241T/T genotype is associated with glioma risk. Although previous studies explored the association between XRCC3 and risk of glioma have shown the polymorphisms in rs3212092, rs861530 and rs861539 of XRCC3 are associated with glioma (Kiuru et al., 2008; Custódio et al., 2012; Liu et al., 2012; Zhou et al., 2012). A recent study conducted in China has indicated the rs861539 is associated with glioma risk (Liu et al., 2012). Another study conducted in Brasil has shown that patients with Met of the XRCC3 Thr241Met polymorphism have a significantly 3.13-fold risk of tumor development, which suggests that XRCC3 Thr241Met polymorphism is involved in susceptibility for developing astrocytomas and glioblastomas. However, Rajaraman reported that no association was found in adult meningioma, glioma and acoustic neuroma in an American population (Rajaraman et al., 2010). The difference of the reported results might be explained by differences in ethnicities, source of selected subjects, study design, sample size, and also by change. The confirmation of existing finding is strongly needed in future studies.

Moreover, we found ERCC5 Asp1558His polymorphisms were associated with both glioma and meningiomas risk, and the cancer risk was even higher in meningiomas. However, no previous evidence indicated the variation of ERCC5 Asp1558His was associated with risk of glioma and meningiomas in Chinese population. Only one study conducted in the United States has shown that ERCC5 Asp1558His rs17655 increased the risk of acoustic neuroma (Rajaraman et al., 2010). Further studies in different ethnicities are strongly warranted to confirm the association between ERCC5 Asp1558His polymorphisms and risk of glioma and meningiomas.

There are already plenty of approaches which aim at the genetic risk factors of glioma and meningiomas, and the polymorphisms of DNA repair system have been thought to be related with various cancers (Chiu et al., 2008; Kiuru et al., 2008; Mandal et al., 2011; Zhou et al., 2011; Mittal et al., 2012). All these findings strengthen the linkage of DNA repair systems, genome instability and carcinogenesis. Our study has shown that XRCC1 Gln399Arg, XRCC1 Arg194Trp, XRCC3 Thr241Met and ERCC5 Asp1558His are associated with risk of glioma and meningiomas. This finding could be useful in identifying the susceptible genet of this cancer.

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