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Original Article

Analysis of difference of association between polymorphisms in the *XRCC5, RPA3* and *RTEL1* genes and glioma, astrocytoma and glioblastoma

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Abstract: Background: Gliomas are the most common aggressive brain tumors and have many complex pathological types. Previous reports have discovered that genetic mutations are associated with the risk of glioma. However, it is unclear whether uniform genetic mutations exist difference between glioma and its two pathological types in the Han Chinese population. Materials and methods: We evaluated 20 SNPs of 703 glioma cases (338 astrocytoma cases, 122 glioblastoma cases) and 635 controls in a Han Chinese population using χ^2 test and genetic model analysis. Results: In three case-control studies, we found rs9288516 in *XRCC5* gene showed a decreased risk of glioma (OR, 0.85; 95% CI, 0.73-0.99; $P = 0.042$) and glioblastoma (OR, 0.70; 95% CI, 0.52-0.92; $P = 0.001$) in the allele model. We identified rs414805 in *RPA3* gene showed an increased risk of glioblastoma in allele model (OR, 1.38; 95% CI, 1.00-1.89; $P = 0.047$) and dominant model (OR, 1.57; 95% CI, 1.05-2.35; $P = 0.027$), analysis respectively. Meanwhile, rs2297440 in *RTEL1* gene showed an increased risk of glioma (OR, 1.30; 95% CI, 1.10-1.54; $P = 0.002$) and astrocytoma (OR, 1.26; 95% CI, 1.02-1.54; $P = 0.029$) in the allele model. In addition, we also observed a haplotype of "GCT" in the *RTEL1* gene with an increased risk of astrocytoma ($P = 0.005$). Conclusions: Polymorphisms in the *XRCC5, RPA3* and *RTEL1* genes, combinating with previous reaserches, are associated with glioma developing. However, those genes mutations may play different roles in the glioma, astrocytoma and glioblastoma, respectively.

Keywords: XRCC5, RPA3, RTEL1, glioma, astrocytoma, glioblastoma, case-control study

Introduction

Glial cell is regard as the origin of glioma [1]. Complex subtypes of glioma exist including World Health Organization classification astrocytoma grades I, II (astrocytoma), III (anaplastic astrocytoma), and IV (glioblastoma), oligodendrogliomas, ependy momas, and mixed gliomas [2]. Glioma is the most common and aggressive type of brain tumor and its morbidity rate is approximate six per 100,000 each year [3, 4].

Affecting glioma risk has been only found a few factors, such as family history, genetic syndromes and exposure to ionizing radiation [5, 6]. Although the etiology of gliomas have not been illuminated clearly so far, increasing data indicated that genetic variants have momentous effect on the kind of tumor [7]. Some genetic mutations in glioma have been known for years, such as *EGFR*, *CCDC26*, *TREH*, *XRCC1* and *GSTP1* gene [8-10]. It is previously reported that *TEL1*, *TERT* gene were associated with susceptibility to astrocytoma [11]. Recent studies demonstrated that mutations in *FLT3*, *EGFR*, *NEIL3* and *ALOX5* genes were associated with glioblastoma survival [12]. However, we found genes are alone with a separate analysis of glioma, glioblastoma, astrocytoma.

Table 1. Characteristics of patients with cases and controls

Pathology	Parameters	Cases	Healthy controls	*P* value
Gliomas	Male/female	385 (54.8%)/318 (45.2%)	311 (49.0%)/324 (51.0%)	0.34
	Age (years)	42.2 ± 17.4	38.0 ± 16.5	
	Total (N)	703	635	
Astrocytoma	Male/female	191 (56.5%)/147 (43.5%)	311 (49.0%)/324 (51.0%)	0.25
	Age (years)	42.5 ± 16.7	38.0 ± 16.5	
	Total (N)	338	635	
Glioblastoma	Male/female	70 (57.4%)/52 (42.6%)	311 (49.0%)/324 (51.1%)	0.89
	Age (years)	46.9 ± 15.1	38.0 ± 16.6	
	Total (N)	122	636	

P value is based on the age and sex versus healthy controls in the study.

In our study, we selected 20 SNPs in fifteen genes which have previously been reported to be associated with glioma, astrocytoma or glioblastoma onset in European. We conducted three case-controls from 703 cases and 635 controls: 1) glioma case-control (703 cases and 635 controls); 2) astrocytoma case-control (338 cases and 635 controls); 3) glioblastoma case-control (122 cases and 635 controls). A denotative association analysis was performed in a Han Chinese population by three case-control studies. The aim of the study was to investigate the different influence between mutations of *XRCC5, RPA3* and *RTEL1* genes and glioma, astrocytoma and glioblastoma, respectively.

Materials and methods

Ethics statement

The protocol in this study was cautiously affirmed to the principles of the Declaration of Helsiinki and was ratified by the Ethical Committee of Tangdu Hospital. The paticipants all had signed informed consents.

Study population

A total of 703 patients with glioma, includes astrocytoma 338 patients and glioblastoma 122 patients, between December 2010 and November 2014 were recruited from the department of Neurosurgery at Tangdu Hospital, all of the study participants are Han Chinese living in the area of Xi'an, China. Confirmed cases who were newly diagnosed and histologically ensured. All glioma cases never undergone radiotherapy, chemotherapy and cancer. According to WHO classifications [2], all the pathologies of glioma tissues were reevaluated. The clinical pathology and characteristics of all the patients were indicated in **Table 1**.

According to the recruitment and exclusion standards, the controls were 635 healthy individuals who be selected from June 2011 to October 2014 from the medical examination center, Tangdu Hospital. The controls were all Han Chinese living in Xi'an city and around area. Meanwhile, we also excluded subjucts with chronic diseases of kidney, heart, liver and brain by detailed exclusion criteria. The factors, environmental and therapeutic, may contribute to mutate, and we maximized the data in the study to be more persuative. Finally, we selected 635 unrelated healthy controls in this study.

Genotyping

Combinating genome-wide association studies (GWAS), a powerful research strategy, used to identify susceptibility genes which previously reported to be associated with glioma, astrocytoma and glioblastoma risk [8-12], we genotyped twenty tSNPs with minor allele frequency (MAF) > 5% in HapMap Asian population in fifteen genes. Genomic DNA was stored at -20°C and was extracted from whole blood by the phenol-chloroform extraction method. Using an extraction kit (GoldMag, China), we ioslated DNA from the samples. DNA concentration was measured by spectrometry (DU530 UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA). We designed Multiplexed SNP Mass EXTEND assay by Sequenom MassARRAY Assay Design 4.0 Software [13]. Using the Sequenom Mass ARRAY RS1000, genotyped SNP was recommended by the manufacturer with a standard protocol [14]. Data

Table 2. Basic information of candidate SNPs in this study

SNP ID	Chromosome	Position	Gene	HWE *P* value	Alleles A/B	MAF control	MAF case		
							Glioma	Astrocytoma	Glioblastoma
rs12022378	1	114448389	DCLRE1B	0.546	C/T	0.358	0.391	0.370	0.402
rs1800871	1	206946634	IL10	0.111	C/T	0.342	0.349	0.356	0.316
rs3770502	2	217045059	XRCC5	0.754	A/G	0.149	0.164	0.169	0.172
rs9288516	2	217053264	XRCC5	0.633	A/T	0.479	0.440	0.438	0.389
rs12645561	4	178260872	NEIL3	0.111	T/C	0.275	0.268	0.254	0.287
rs2853676	5	1288547	TERT	0.03	A/G	0.165	0.223	0.232	0.209
rs2243248	5	132008644	IL4	1	G/T	0.061	0.056	0.047	0.057
rs2070874	5	132009710	IL4	0.47	C/T	0.208	0.220	0.217	0.209
rs1801270	6	36651971	CDKN1A	0.686	A/C	0.427	0.458	0.436	0.471
rs4140805	7	7727101	RPA3	0.622	G/T	0.202	0.195	0.189	0.258
rs6947203	7	7737048	RPA3	0.719	T/C	0.126	0.120	0.126	0.164
rs7003908	8	48770702	PRKDC	0.098	C/A	0.236	0.213	0.223	0.230
rs12917	10	131506283	MGMT	0.208	T/C	0.106	0.087	0.090	0.102
rs1695	11	67352689	GSTP1	0.349	G/A	0.217	0.204	0.222	0.189
rs1042522	17	7579472	TP53	0.333	C/G	0.431	0.435	0.428	0.467
rs2952155	17	37861718	ERBB2	0.749	C/T	0.460	0.476	0.476	0.492
rs2992	19	4443046	UBXN6	0.376	A/G	0.438	0.435	0.442	0.430
rs6010620	20	62309839	RTEL1	0.189	G/A	0.269	0.321	0.314	0.320
rs2297440	20	62312299	RTEL1	0.264	C/T	0.265	0.320	0.312	0.316
rs4809324	20	62318220	RTEL1	0.699	C/T	0.115	0.116	0.111	0.090

sSNP, single-nucleotide polymorphism, A/B stands for minor/major alleles on the control sample frequencies. HWE, Hardy-Weinberg equilibrium, The SNPs are excluded at 5% HWE *P* level.

analyses and management were conducted by Sequenom Typer 4.0 Software [15].

Statistical analysis

The data analysis was used by SPSS 16.0 statistical package (SPSS, Chicago, IL) and microsoft Excel. We excluded the *P* value which $P \geq 0.05$ was considered the deviation value of statistical significance. Each SNP of the genotype frequencies in control subjects were checked by using Hardy-Weinberg equilibrium (HWE). The genotype frequencies of cases and controls were calculated by using χ^2 test [16, 17]. Odds ratios (ORs) and 95% confidence intervals (CIs) were tested by using unconditional logistic regression analysis with adjustment for age and gender [18]. The three genetic models (allele, dominant and Log-additive) were applied by PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) to assess the association of SNP with the risk of glioma, astrocytoma and glioblastoma. Finally, we analysed haplotype construction, and genetic association at polymorphism loci by the SHEsis software platform (www.nhgg.org/analysis/) [19].

Results

In this study, 703 glioma patients contained 385 males and 318 females which those cases diagnosed mean age was 42.2 ± 17.4 years. The 703 cases included 338 cases of astrocytoma, 122 cases of glioblastoma, 35 cases of ependymoma, 24 cases of oligodendrogliomas, and 98 cases of other glioma types. The 635 healthy controls included 311 males and 324 females which those controls diagnosed mean age was 38.0 ± 16.5 years. We found no differences between gender and age distribution by *p* value. The Characteristics of patients with cases and controls are shown in **Table 1**. 20 SNPs in the fifteen genes were analyzed in this study. Chromosomal position, gene, Allele, HWE test results and MAF of cases and controls of all the SNPs were appeared in **Table 2**. The minor allele of each SNP, a risk factor, was compared to the wild-type allele. A total of 20 SNPs were conducted in patients and controls, and rs2853676 were cut off at 3% HWE *P* level.

Further model association analyses used logistic tests were presented in **Table 3**. rs9288516 was observed to be associated with the

Table 3. Association of tSNPs with glioma, astrocytoma and glioblastoma risk based on logistic tests (adjusted for sex + age)

SNP ID	Model	Genetype	Glioma		Astrocytoma		Glioblastoma	
			OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* Value
rs9288516	Allele model	A/T	0.85 (0.73-0.99)	0.042*	0.85 (0.70-1.02)	0.08	0.70 (0.52-0.92)	0.001*
	Dominant model	A/T-A/A	0.85 (0.67-1.08)	0.2	0.90 (0.67-1.21)	0.48	0.70 (0.46-1.06)	0.096
	Log-additive model	---	0.87 (0.74-1.01)	0.07	0.86 (0.71-1.04)	0.13	0.72 (0.55-0.96)	0.026*
rs4140805	Allele model	G/T	0.96 (0.79-1.16)	0.657	0.92 (0.73-1.17)	0.507	1.38 (1.00-1.89)	0.047*
	Dominant model	G/T-G/G	0.94 (0.75-1.18)	0.62	0.92 (0.69-1.21)	0.54	1.57 (1.05-2.35)	0.027*
	Log-additive model	---	0.94 (0.78-1.14)	0.56	0.92 (0.73-1.17)	0.52	1.36 (0.98-1.88)	0.07
rs2297440	Allele model	C/T	1.30 (1.10-1.54)	0.002*	1.26 (1.02-1.54)	0.029*	1.28 (0.95-1.72)	0.107
	Dominant model	T/C-C/C	1.25 (1.01-1.56)	0.042*	1.28 (0.98-1.67)	0.073	1.12 (0.75-1.67)	0.57
	Log-additive model	---	1.31 (1.10-1.55)	0.002*	1.28 (1.04-1.59)	0.022*	1.29 (0.95-1.77)	0.11

OR, odds ratio, 95% CI, 95% confidence interval. *$P < 0.05$, statistical significance.

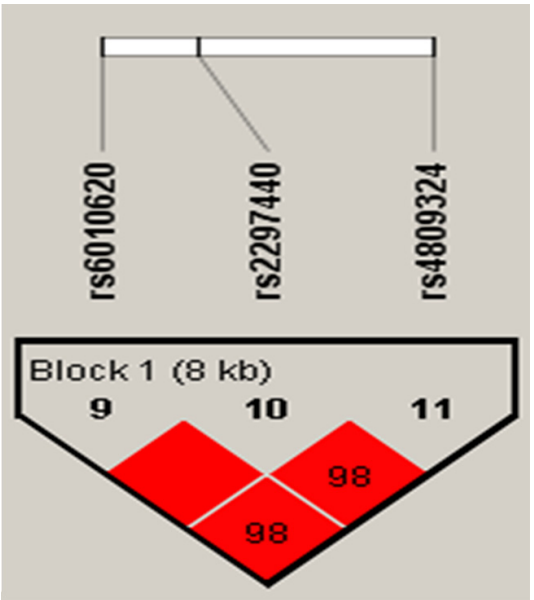

Figure 1. Haplotype-Block Map for *RTEL1* based on SNPs rs6010620, rs2297440 and rs4809324 which were included in Block 1.

decreased glioblastoma risk by both allel model analyses (OR, 0.70; 95% CI, 0.52-0.92; *P* = 0.001) and log-additive model analyses (OR, 0.72; 95% CI, 0.55-0.96; *P* = 0.026). We also found rs9288516 decreased glioma risk by allele model analyses (OR, 0.85; 95% CI, 0.73-0.99; *P* = 0.042). Otherwise, individual with the genetype "G/T-G/G" of rs4140805 only increaced glioblastoma risk in an allele model (OR, 1.38; 95% CI, 1.00-1.89; *P* = 0.047) and in a dominant model (OR, 1.57; 95% CI, 1.05-2.35; *P* = 0.027). Meanwhile, we discovered the genetype "C/T-C/C" of rs2297440 as the risk for glioma in the allele model (OR, 1.30; 95% CI, 1.10-1.54; *P* = 0.002), in the dominant model (OR, 1.25; 95% CI, 1.01-1.56; *P* = 0.042) and in the log-additive model (OR, 1.31; 95% CI, 1.10-1.55; *P* = 0.0018). We also discovered rs2297440 as the risk for astrocytoma in the allele model (OR, 1.26; 95% CI, 1.02-1.54; *P* = 0.029) and in the log-additive model (OR, 1.28; 95% CI, 1.04-1.59; *P* = 0.022), analysis respectively.

Only one block was detected in *RTEL1* gene by haplotype analysis (**Figure 1**). Global result for the block was: total case = 338, total control = 635, global haplotype association *p* value: 0.038. The results of the association between the *RTEL1* gene haplotype "GCT" and the risk of astrocytoma (OR, 1.45; 95% CI, 1.12-1.86; Pearson's *P* = 0.0046) are presented in **Table 4**.

Disscusion

In three case-control studies, we observed rs9288516 in the *XRCC5* gene was associated with a decreased risk of glioma and glioblastoma, rs4140805 in the *RPA3* gene was only associated with an increased risk of glioblastoma and rs2297440 in the *RTEL1* gene was associated with an increased risk of glioma and astrocytoma. In addition, we showed that haplotype "GCT" was associated with the risk of astrocytoma at a 5% level by haplotype association analysis.

The *XRCC5* gene, which is located in 2q35, a risk SNP has been found for hepatocellular carcinoma [20]. XRCC5 gene mutation of a single patient can not be discovered, because XRCC5 gene is a vital evolved gene for human life, indi-

Table 4. RTEL1 haplotype frequencies and the association with astrocytoma risk (n = 973, adjusted for sex + age)

Haplotype	Freq (case + control)	Chi2	OR (95% CI)	*P* value
GCT	0.1691	8.122	1.45 (1.12-1.86)	0.005
GCC	0.1125	0.074	1.05 (0.77-1.44)	0.76

Loci chosen for hap-analysis: rs6010620, rs2297440 and rs4809324 in *RTEL1* gene. OR, odd ratio; CI, confidence interval.

cating that genetic variations in non-coding regions may be the underlying basis of differing levels of gene transcription and translation [21]. It was also reported in the chronic obstructive pulmonary disease, breast cancer and digestive system cancer as a risk factor [22-24]. In our study, we found genotype "A/T" of rs9288516 as a protective factor was just associated with a decreased risk of glioma and glioblastoma in Chinese patients. Comparing with previous studies, we found that *XRCC5* gene is not a risk factor, can be used as a protective factor in glioma and glioblastoma. Our finding suggested that this gene may play a different role in complex diseases. In further studies, we should realize *XRCC5* gene different disease mechanisms in glioma, astrocytoma and glioblastoma.

The *RPA3* gene, which is located in 7p21.3, a risk SNP (genetype "G/T-G/G" of rs4140805) has been found in our study. Meanwhile, we only found rs4140805 of *RPA3* gene with an increased risk of developing glioblastoma. However, glioma and astrocytoma have no significance. It is a single stranded DNA-binding protein that functions in many aspects of DNA metabolism and has a central role in DNA replication, playing an essential function in both initiation and elongation [25]. It was reported that, as a protective risk, *RPA3* gene showed decreased risk of glioma [26]. However, we have not yet found about relationship between *RPA3* gene and risk of glioblastoma and astrocytoma in previous studies. According to World Health Organization classification astrocytoma grades IV (glioblastoma) [2], whether rs4140805 of the *RPA3* gene only for high malignant degree of glioblastoma is in danger. Combinating with previous reseaches, we discovered uniform SNP of gene in glioma and glioblastoma showed the opposite effect. Therefore, it is necessary to study biological functions of the *RPA3* gene in further reseach.

The *RTEL1* gene locates in 20q13.3, including 40 exons. It was reported that *RTEL1* kept genomic stability in suppressing homologous recombination [27]. A recent review point out that *RTEL1* was an significant helicase for telomere maintenance and the regulation of homologous recombination [28]. In this study, we found rs2297440 of *RTEL1* gene with an increased risk of developing glioma and astrocytoma. The result is consistent with the previous research [11, 29]. However, we found no significant between *RTEL1* gene and glioblastoma, this may be related to our sample is less. Previous study suggested polymorphism in the *RTEL1* gene was associated with glioblastoma survival [30]. Therefore, relationship between *RTEL1* gene and glioblastoma is of great interest and warrant further investigation.

This study was the first associated study between polymorphisms of *XRCC5, RPA3* and *RTEL1* genes and glioma, astrocytoma and glioblastoma risk respectively in a Chinese population. However, some limitations must be mentioned in our study. Firstly, we collected all the samples from the same hospital for avoiding two or more definite selection bias, and they maintained adiaphorous, did not exist difference in genotype frequencies. Together, all the samples were selected from Han Chinese population who lived in Xi'an city or aroud area. Substantial population of confounding factors, which may cause type-I error for association study. Secondly, the sample size (703 cases and 635 controls) is not large enough in our study for association studies, especially, included 338 astrocytoma cases and 122 glioblastoma cases, large sample size will be convincing. Thirdly, it was ensure that analyzing data was convince, and we select SNPs with MAF > 5% in HapMap Asian population. However, this method will ignore some significant SNPs in other studies.

In conclusion, our study may provides new evidence that *XRCC5, RPA3* and *RTEL1* genes mutations play different roles in the glioma, astrocytoma and glioblastoma in Chinese Han population.

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Disclosure of conflict of interest

None.

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