Original Article
Association of LIG4 and XRCC4 gene polymorphisms with the risk of human glioma in a Chinese population

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Abstract: We conducted a case-control study to assess the LIG4 and XRCC4 genes polymorphisms and development of glioma. A case-control study including 162 glioma cases and 324 controls was conducted in a Chinese population. Genotypes of rs10131 and rs1805388 in LIG4 and rs2075685 and rs1805377 in XRCC4 were conducted by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. Conditional logistic regression analysis showed that subjects carrying AA genotype of LIG4 rs10131 was associated with increased risk of glioma when compared with GG genotype, and the OR (95% CI) was 3.26 (1.50-7.23). We found that GA+AA of LIG4 rs10131 was associated with increased risk of glioma in those without family history of cancer, and the OR (95% CI) was 1.78 (1.12-2.83). However, no association was found between variants of LIG4 rs1805388, XRCC4 rs2075685 and XRCC4 rs1805377 and development of glioma. In conclusion, our results suggest that LIG4 rs10131 polymorphism in the DNA repair pathways plays an important role in the risk of glioma in a Chinese population.

Keywords: LIG4, XRCC4, polymorphism, glioma

Introduction

Gliomas are the most frequently occurring type of brain tumors worldwide, accounting for approximately 80% of all malignant brain tumors [1]. In 2000, the annual incidence of cerebral tumors was about 3.9 per 100,000 in men and 2.8 per 100,000 in women among Chinese population, also 2 or 3 per 100,000 in Europe and North America [2, 3]. Despite the development of therapeutics for gliomas and the improvement of diagnosis in recent years, the glioma patients showed a poor survival time of approximately 14 months after diagnosis [4]. Currently, only two relative factors have been reported to be associated with development of glioma, including exposure to high doses of ionizing radiation and inherited mutations of genes, but most patients are without ionizing radiation [5]. Therefore, understanding the genetic etiology of gliomas may help to reveal the mechanism of gliomas and provide new insight for the diagnosis and treatment.

Single strand breaks (SSBs) and double-strand breaks (DSBs) are important DNA damages.

DSBs may lead to chromosomal breakage or rearrangement and are the most detrimental form. Moreover, defects in DSBs repair can induce disastrous consequences including genomic instability, cell death and carcinogenesis [6, 7]. Homologous recombination (HR) and non-homologous end-joining (NHEJ) are two major pathways for DSBs repair [8]. In the NHEJ process, five proteins, namely X-ray repair cross-complementing group 6 (XRCC6, Ku70), XRCC5 (Ku80), DNA-dependent protein kinase catalytic subunit (DNA-PKcs), DNA ligase IV (LIG4), and XRCC4 play an important role in NHEJ [9]. The LIG4 and XRCC4 complex have a key role in performing the end-joining reaction. As a DNA ligase, LIG4 can execute the final rejoining step by forming a heterodimer with XRCC4, and XRCC4 is a nuclear phosphoprotein that links LIG4 to DNA-PK complex, stabilizes and stimulates the LIG4 activity [10, 11].

Previous studies have investigated the roles of the LIG4 gene in the development of various cancers [12, 13], however, few studies reported the effects of LIG and XRCC4 complex gene polymorphisms on the glioma risk [14, 15].

Therefore, we conducted a case-control study to assess the LIG4 and XRCC4 genes polymorphisms and development of glioma.

Materials and methods

Study population

A case-control study including 162 cases and 324 controls was conducted in a Chinese population. 162 patients histopathologically diagnosed with gliomas were selected from the Affiliated Hospital of Inner Mongolia Medical University between May 2011 and December 2013. Demographic and clinical data of included cases and controls were collected from medical records and a self-designed questionnaire.

324 control subjects were randomly selected from annual check-up visitors at the same hospital during the similar time period and frequency matched to cases by sex and age (±5 years). The controls that had a history of cancer or central nervous system-related diseases and previously receiving radiotherapy or chemotherapy for certain diseases were excluded from our study.

Written informed consent was obtained from all patients and this study was approved by ethics committee of the Affiliated Hospital of Inner Mongolia Medical University.

Blood samples and genotyping

Each patient was asked to provide 5 ml peripheral blood and kept in -70°C until use. Genomic DNA was isolated from peripheral blood using the TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. Genotypes of rs10131 and rs1805388 in LIG4 and rs2075685 and rs1805377 in XRCC4 were conducted by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. Probes and primers for rs10131 and rs1805388 in LIG4 and rs2075685 and rs1805377 in XRCC4 were designed using Sequenom Assay Design 3.1 software (Sequenom®) according to the manufacturer instructions. Briefly PCR was carried out in a final volume of 25 μL containing 50 ng genomic DNA templates, 1X PCR buffer with 2 mM $MgCl_2$, 0.5 μM of each primer, 50 μM dNTPs and 0.5 U DNA polymerase. For PCR amplification, the standard program was used as follows: one initial denaturation step at 94°C for 7 min, followed by 35 denaturation cycles of 1 min at 94°C, 1 min of annealing at 60°C, and 1 min of extension at 72°C, followed by a final elongation cycle at 72°C for 10 min. For quality control, 5% of subjects were randomly selected, and the results of repeated samples showed 100% concordance.

Statistical analysis

Continuous variables were shown as the mean ± SD and analyzed by student t test. Categorical variables were expressed as n (%) of study subjects and analyzed by χ^2-test. The χ^2 test was used to compare the differences in demographic characteristics and genotypes of LIG4 and XRCC4 genes. The Hardy-Weinberg equilibrium (HWE) was tested by Fisher's exact test for each SNP in controls. The association between gene polymorphisms of LIG4 and XRCC4 and development of glioma was assessed by conditional logistic regression models adjusted for gender and age for genotype analysis, and the results was expressed by OR and 95% confidence interval (CI) were calculated. The SPSS software (SPSS, Chicago, IL) was used for statistical analyses. All *P*-values were two sided, and a *P*-value was regarded as statistically significant when it less than 0.05.

Results

Characteristics of included subjects

The demographic and clinical characteristics of 162 glioma cases and 324 health controls were shown in **Table 1**. Cases and controls were matched on age and sex, and no significant difference between them (*P*=0.80 and 1.00, respectively). Moreover, there was no significant in tobacco smoking between cases and controls (*P*=0.42). Glioma patients were more likely to have a family history of cancer (*P*=0.006).

Genotype distributions of LIG4 and XRCC4

The allele and genotype distributions of rs1805388, rs2075685 and rs1805377 were found to be in Hardy-Weinberg equilibrium in the control group, while rs10131 was not (**Table 2**). We found the Minor allele frequencies in controls were similar to them in NCBI (http://www.ncbi.nlm.nih.gov/pubmed/).

Table 1. Demographic and clinical characteristics in cases and controls

Variables	Cases N=162	%	Controls N=324	%	t or χ^2	P value
Age						
Mean ± SD	45.2±11.3		46.5±10.5		1.25	0.11
<50	91	56.17	178	54.94		
≥50	71	43.83	146	45.06	0.00	1.00
Sex						
Male	92	56.79	184	56.79		
Female	70	43.21	140	43.21	0.11	0.75
Tobacco smoking						
Never	85	52.47	190	58.64		
Ever	36	22.22	65	20.06		
Current	41	25.31	69	21.30	1.74	0.42
Family history of cancer						
No	143	88.27	308	95.06		
Yes	19	11.73	16	4.94	7.45	0.006
Histological types						
Glioblastoma	61	37.65				
Other gliomas	101	62.35				

Table 2. Allele frequencies of LIG4 and XRCC4 in glioma and control subjects

SNP	Minor allele frequency in NCBI	Minor allele frequency in controls	P value for Hardy-Weinberg equilibrium
LIG4			
rs10131	0.1298	0.1343	<0.05
rs1805388	0.1458	0.1605	0.16
XRCC4			
rs2075685	0.4449	0.4228	0.06
rs1805377	0.3752	0.3704	0.19

Association between LIG4 and XRCC4 gene polymorphisms and risk of glioma

Conditional logistic regression analysis showed that subjects carrying AA genotype of LIG4 rs10131 was associated with increased risk of glioma when compared with GG genotype, and the OR (95% CI) was 3.26 (1.50-7.23) (**Table 3**). However, no association was found between variants of LIG4 rs1805388, XRCC4 rs2075685 and XRCC4 rs1805377 and development of glioma.

Interaction between LIG4 and XRCC4 gene polymorphisms and demographic characteristics of glioma

To determine whether the demographic characteristics can influence the genetic susceptibility, we investigated the association between

LIG4 rs10131 polymorphism and glioma risk stratified by demographic characteristics (**Table 4**). We found that GA+AA of LIG4 rs10131 was associated with increased risk of glioma in those without family history of cancer, and the OR (95% CI) was 1.78 (1.12-2.83).

Discussion

In the present study, we investigated the relationship between LIG4 and XRCC4 gene polymorphisms and development of glioma. We found that AA genotype of LIG4 rs10131 significantly increased the risk of glioma, but we found no significant association between LIG4 rs1805388, XRCC4 rs2075685 and XRCC4 rs1805377 polymorphisms and development of glioma. Moreover, we found that LIG4 rs-10131 had interaction with family history of cancer in the risk of glioma.

Among various DNA damage lesions caused by the ionizing radiation, the DSBs are the principle genotoxic to pose major threats to genomic instability, integrity and carcinogenesis [7]. Previous studies showed that NHEJ repair pathway had an important role in repairing DSBs in mammalian cells, and LIG4 and XRCC4 genes play an important role in performing the end-

Table 3. Association between LIG4 and XRCC4 gene polymorphisms and development of glioma

Variables	Cases	%	Controls	%	OR (95% CI)[1]	P value
LIG4 rs10131						
GG	110	67.90	251	77.47	1.0 (Ref.)	-
GA	32	19.75	59	18.21	1.24 (0.73-2.06)	0.39
AA	20	12.35	14	4.32	3.26 (1.50-7.23)	<0.05
LIG4 rs1805388						
CC	111	68.52	237	73.15	1.0 (Ref.)	-
CT	37	22.84	70	21.60	1.13 (0.69-1.82)	0.61
TT	13	8.02	17	5.25	1.63 (0.70-3.70)	0.20
XRCC4 rs2075685						
GG	47	29.01	122	37.65	1.0 (Ref.)	-
GT	69	42.59	130	40.12	1.38 (0.86-2.21)	0.16
TT	46	28.40	72	22.22	1.66 (0.95-2.82)	0.06
XRCC4 rs1805377						
AA	62	38.27	137	42.28	1.0 (Ref.)	-
AG	70	43.21	134	41.36	1.15 (0.74-1.79)	0.50
GG	30	18.52	53	16.36	1.25 (0.70-2.21)	0.42

[1]Adjusted for sex, age, tobacco smoking and family history of cancer.

Table 4. Interaction between LIG4 rs10131 and demographic characteristics in the risk of glioma

| Variables | LIG4 rs10131 | | | | OR (95% CI)[1] | P value |
| | GG | | GA+AA | | | |
	Cases	Controls	Cases	Controls		
Age, years						
<50	63	141	28	37	1.69 (0.91-3.12)	0.07
≥50	47	110	24	36	1.56 (0.80-3.02)	0.16
Sex						
Male	61	149	31	40	1.89 (1.04-3.42)	0.02
Female	49	102	21	33	1.32 (0.66-2.64)	0.39
Tobacco smoking						
Never	58	148	27	42	1.64 (0.88-3.01)	0.09
Ever	24	50	12	15	1.67 (0.61-4.49)	0.26
Current	28	53	13	16	1.54 (0.58-3.96)	0.33
Family history of cancer						
No	95	240	48	68	1.78 (1.12-2.83)	0.01
Yes	15	11	4	5	0.59 (0.09-3.51)	0.49

[1]Adjusted for sex, age, tobacco smoking and family history of cancer.

ing-joining reaction and promoting various cancer tumorigenesis [6, 8]. Our study found that LIG4 rs10131 polymorphisms significantly increased the risk of glioma in Chinese population, which indicated that LIG4 gene polymorphisms had a key role in development of glioma.

Previous studies reported the association between LIG4 gene polymorphisms and risk of several kinds of cancers, including ovarian cancer, cervical cancer, colorectal cancer and breast cancer, etc. [16-20]. Schildkraut et al. conducted a case-control study with 364 ovarian cancer cases and 761 controls, and found that LIG4 rs10131 was associated with risk of ovarian cancer [16]. However, three studies did not find significant association between LIG gene polymorphisms and risk of cervical cancer, colorectal cancer and breast cancer [17-

19]. In a recent meta-analysis, it assessed the association between LIG4 rs1805388 and rs1805386 polymorphisms and cancer risk, and it found that LIG4 rs1805388 was associated with a decreased risk of cancer in Caucasians [20]. The discrepancies of these results may be caused by differences in ethnicities, study design, tumor types, and sample size as well as by chance.

For the association between LIG4 gene polymorphisms and risk of glioma, only two previous studies reported their association [14, 15]. Liu et al. conducted a case-control study of 771 glioma patients and 752 cancer-free controls, and found that LIG4 rs1805388 and XRCC4 rs7734849 polymorphisms contributed to glioma susceptibility [14]. Zhao et al. conducted a study to investigate the association between 10 SNPs in DNA repaired genes and risk of glioma, and found that LIG4 rs1805388 and XRCC4 rs1805377 polymorphisms were associated with an increased glioma risk, even in smokers [15]. In our study, we only found that AA genotype of LIG4 rs10131 significantly increased the risk of glioma, but we found no significant association between LIG4 rs1805-388, XRCC4 rs2075685 and XRCC4 rs1805-377 polymorphisms and development of glioma. Therefore, further large sample studies are with more ethnicities are greatly needed to confirm our results.

Several limitations should be considered in our study. First, cases and controls were selected from one hospital, and the rs10131 was not in Hardy-Weinberg equilibrium in the control group. The sample of our study would not be representative of other populations. However, the controls were a random sample from a pool of individuals who came to receive a health check-up, which may well represent the general population. Second, since the rarity of glioma, the sample size of glioma patients is relatively small. The small sample size could limit the statistical power to find the association between groups. Third, the risk of glioma could be modified by many other genetic factors in DNA repaired pathway except for LIG4 and XRCC4 genes. Therefore, further studies with more subjects are greatly needed to confirm the association between LIG4 and XRCC4 genes polymorphisms and risk of glioma.

In conclusion, our results suggest that LIG4 rs10131 polymorphism in the DNA repair path-

ways plays an important role in the risk of glioma in a Chinese population. Further multicenter studies involving various populations are greatly needed to confirm our results.

Disclosure of conflict of interest

None.

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