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Original Article

LIG4 rs1805388 polymorphism involved in the NHEJ pathway modify risk of glioma in Asians

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Abstract: Polymorphisms of *LIG4* gene involved in the NHEJ pathway have been reported to influence DNA repair ability and alter an individual's susceptibility to cancer. Several recent studies have identified that the *LIG4 rs1805388* polymorphism is associated with cancer risk, but with inconsistent results. Thus, a meta-analysis was conducted to derive a more precise estimation of the effects of this enzyme. By a comprehensive literature search using PubMed and EMBASE databases, a total of 6 case-control studies were identified for inclusion in the meta-analysis. Overall, current evidence suggests that *LIG4 rs1805388* polymorphism potentially predisposes their carriers to gliomas, specifically in Asians (OR = 2.00, 95% CI: 1.43-2.80). When stratified by the genotyping method, significant association of *LIG4 rs1805388* polymorphism with cancer risk was found in using PCR-RFLP method studies (OR = 1.86, 95% CI: 1.08-3.19).

Keywords: Cancer, meta-analysis, polymorphism, LIG4

Introduction

Glioma is the most common and aggressive malignant primary brain tumor worldwide, accounting for approximately 80% of central nervous system (CNS) tumors [1-3]. Like other cancers, the genetic and environmental factors play an essential roles in the development and progression of gliomas [4]. Currently, high dose exposure to ionizing radiation is the only identified environmental risk factor which is unequivocally associated with the glioma risk [5]. However, only a minority of those exposed to ionizing radiation eventually develop glioma, suggesting that genetic factors, such as single nucleotide polymorphisms (SNPs) may play a critical role in the process of glioma carcinogenesis [6].

In eukaryotes, ionizing radiation can result in DNA double-strand breaks (DSBs) [6]. There are two major pathways for DSB repair: homologous recombination (HR) and nonhomologous end-joining (NHEJ). In the NHEJ pathway, DNA-PK complex (consisting of Ku70/Ku80 and DNAPKcs) initially recognize the broken ends of DNA. As a major protein involved in NHEJ, LIG4 forms a heterodimer with XRCC4 to execute the final rejoining step of NHEJ. DNA ligase IV gene, located on human chromosomes 13q33-34, plays a prominent role in maintenance of genomic integrity and prevention of tumorigenesis [7]. The *LIG4 rs1805388* polymorphism results in a nonsynonymous amino acid change from threonine to isoleucine at the N-terminal of the LIG4 protein that is essential for its activity [8]. Recent epidemiological studies have been performed to elucidate the effect of *LIG4 rs1805388* polymorphism on cancer risk [9-14]. However, the results are to some extent divergent, but nevertheless intriguing. Hence, we performed a meta-analysis of all eligible studies to derive more precise estimation.

Materials and methods

Publication search

To identify all previously published studies that examined the association of polymorphisms with gliomas, we performed a systematic search from PubMed and Embase databases (last

search was updated on 10 Oct 2018). The following search terms were used in isolation and combination with one another: 'LIG4', 'rs18-05388', 'polymorphism' and 'glioma' using the limits, English, Cancer. In addition, the related reference articles were then individually searched to find other potentially eligible publications. All studies matching the eligible criteria were included in our meta-analysis.

Inclusion criteria

Eligible studies included in the meta-analysis were selected according to the following explicit inclusion criteria: (a) articles about *LIG4 rs-1805388* polymorphism and cancer risk; (b) using a case-control design; (c) containing sufficient published data for estimating odds ratios (ORs) with their 95% confidence interval (CI); (d) malignant tumors were histologically confirmed.

Data extraction and quality assessment

The following data were abstracted independently in duplicate by two investigators using a standard protocol and data-collection form according to the inclusion criteria listed above: the name of first author, year of publication, country origin, ethnicity, source of controls (population- or hospital-based controls), total number of cases and controls, and numbers of cases and controls with *LIG4 rs1805388* genotypes, respectively.

The quality of the included studies was evaluated through a checklist originated from Strengthening the Reporting of Genetic Association (STREGA) recommendations for reports on genetic association studies.

Statistical methods

Odds ratios (ORs) corresponding to 95% confidence intervals (CIs) were employed to assess the strength of the associations between the *LIG4 rs1805388* polymorphism and cancer risks. The significance of the pooled OR was determined by Z test, and $P < 0.05$ was considered statistically significant. The pooled ORs were calculated for homozygote comparison, heterozygote comparison, and dominant model, respectively. Subgroup analyses were also performed by cancer type, source of controls and genotyping methods. Eligible studies were classified into population-based and hospital-based, according to control source. Heterogeneity in our meta-analysis refers to the variation in study outcomes between different studies. Heterogeneity assumption was estimated using the chi-square-based Q-test. If the result of this heterogeneity test was $P < 0.05$, the pooled ORs were analyzed using the random effects model (the DerSimonian and Laird method). Otherwise, If the Q-test revealed a *P* value of more than 0.05, the fixed-effects model was selected (the Mantel-Haenszel method) [15, 16]. The I^2 ($I^2 = 100\% \times (Q\text{-}df)/Q$) statistic was then used to quantitatively estimate heterogeneity, with $I^2 < 25\%$, 25-75% and > 75% to represent low, moderate and high degrees of inconsistency, respectively [17]. We assessed Hardy-Weinberg equilibrium for the controls in each study and a *P*-value < 0.01 was considered significant. Additionally, one-way sensitivity analyses were performed by omitting each study to reflect the influence of the individual data-set to the pooled OR. Finally, publication bias of literatures was estimated using the Begg's funnel plot and Egger's test [18, 19]. The *P*-value less than 0.05 in Egger's linear regression indicated the presence of potential publication bias. Log OR was plotted versus standard error of Log OR for each included study in Begg's funnel plot. All statistical analyses were performed with the software Stata (version 11; Stata Corporation, College Station, Texas), using two-sided *P*-values.

Results

Literature search and study selection

A total of 6 articles were achieved by literature search, from the PubMed and EMBASE, using different combination of key terms and met our inclusion criteria (**Figure 1**). Characteristics of enrolled studies were assigned to the structured form (**Table 1**). A total of 6 studies involving 1869 cases and 2212 controls were ultimately analyzed for *LIG4 rs1805388* polymorphism. There were 6 studies of Asians; controls in 1 study were hospital-based and controls of the other studies were population-based. The respective studies focused on the following tumor types: 3 glioma studies, 1 breast cancer, 1 lung Cancer and 1 Head and Neck cancer. Three genotyping methods were used: PCR-RFLP (2 studies), MassARRAY (1 stu-

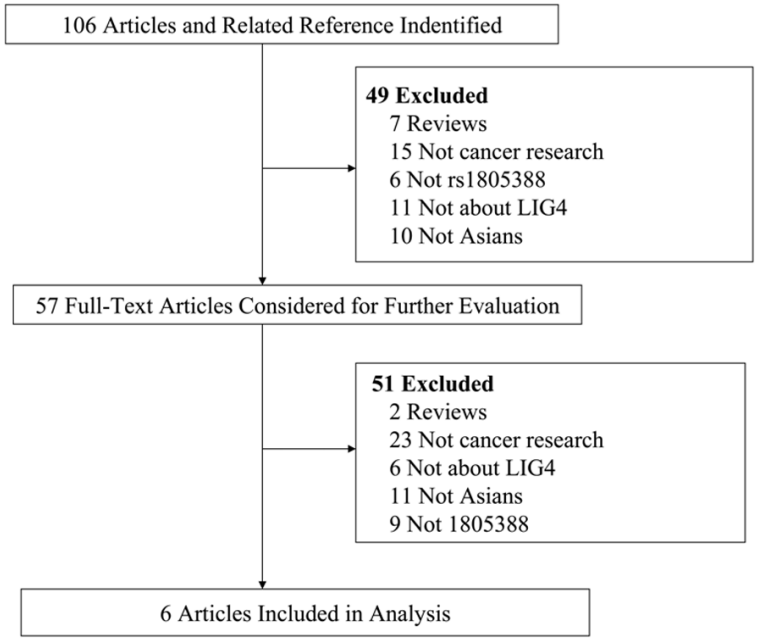

Figure 1. Flow chart showing study selection procedure.

dy), Direct sequencing (1 study) and TaqMan (2 study). The quality assessment of these included studies was provided in **Table 2**.

Quantitative synthesis

As shown in **Table 3**, a significant association between the *LIG4 rs1805388* polymorphism and cancer susceptibility was revealed by the results of the meta-analysis (TC vs. CC: OR = 1.18, 95% CI = 1.03-1.35, $P_{heterogeneity}$ = 0.135) (**Figure 2**). Moreover, there was a significant association between *LIG4 rs1805388* polymorphism and glioma risks (TT vs. CC: OR = 2.00, 95% CI = 1.43-2.80, $P_{heterogeneity}$ = 0.064) (**Figure 3**).

Subgroup analyses

For *LIG4 rs1805388* polymorphism, with consideration of control source, studies with population based controls showed elevated risks (TC vs. CC: OR = 1.28, 95% CI = 1.08-1.53, $P_{heterogeneity}$ = 0.198). Meanwhile, as the genotyping method may influence the results, we also performed a subgroup analysis according to genotyping method used in studies. Significant associations were found in studies using PCR-RFLP method (TC vs. CC: OR = 1.86, 95% CI = 1.08-3.19, $P_{heterogeneity}$ = 0.642; dominant model-TT +TC vs. CC: OR = 1.37, 95% CI = 1.01-1.85, $P_{heterogeneity}$ = 0.453).

Test of heterogeneity

Significant heterogeneity existed in two genetic models (TT versus CC and Dominant model) of the *LIG4 rs1805388* polymorphism (**Table 3**). However, stratification based on the cancer type reduced the heterogeneity in the gliomas subgroups (TT versus CC: $P_{heterogeneity}$ = 0.064, I = 63.5) and other cancer subgroups (TT versus CC: $P_{heterogeneity}$ = 0.088, I = 58.8; Dominant model: $P_{heterogeneity}$ = 0.211, I = 35.7).

Sensitivity analysis

Sensitivity analysis was carried out by investigating the influence of each study on the overall OR, and the result showed that no individual study affected the overall OR dominantly.

Assessment of bias

Funnel plot and Egger's test were done to assess the publication bias. The results did not show any evidence of publication bias (t = -0.89, P = 0.425 for TT versus CC), and the 95% confidence interval (95% CI: -7.12-3.67) included zero, indicating no publication bias (**Figure 4**).

Discussion

The pathogenesis of the development and progression of glioma is far from being clear at present. Recently, extensive case-control studies in different populations reported that the *LIG4 rs1805388* polymorphism have been implicated in glioma risks. However, by the limitation of inadequate publications, they did not calculate pooled ORs of gliomas comprehensively. Hence, we performed a meta-analysis of all eligible studies to derive more precise estimation. In this study, our data show that *LIG4 rs1805388* polymorphism was associated with the presence of gliomas.

There is biologic plausibility for the associations observed in our study. DNA ligase IV (LIG4) is a nonhomologous end-joining (NHEJ) prote-

Table 1. Main characterics of all studies included in the meta-analysis

First author	Year	Country	Cancer type	Source of controls	Genotyping method	Cases	Controls	Case			Control		
								CC	CT	TT	CC	CT	TT
Fu	2003	China	breast cancer	PB	MassARRAY	253	376	137	100	16	198	150	28
Liu	2008	China	Glioma	HB	TaqMan	766	725	460	269	37	446	253	26
Tseng	2009	China	Lung Cancer	PB	PCR-RFLP	149	152	67	62	20	85	55	12
Al-Hadyan	2012	Saudi Arabia	Head and Neck Cancer	PB	Direct sequencing	156	251	132	24	0	216	31	4
Zhao	2013	China	Glioma	PB	TaqMan	384	384	163	172	49	222	142	20
Su	2015	China	Glioma	PB	PCR-RFLP	161	324	111	37	13	237	70	17

PB, Population Based; HB, Hospital Based; PCR-RFLP: Polymerase Chain Reaction-restriction Fragment Length Polymorphism

Table 2. Table Quality assessment of included studies

Last name of first author	Year	Clear description of background, objectives and study design	Clear eligibility criteria	Clear Definition of variables	Credible genotyping methods	Hardy-Weinberg equilibrium assessment	Clear Description of statistical methods	Summary of characteristics of participants	Publicly available genotype data	Comprehensive discussion
Fu	2003	+	+	+	+	+	+	+	+	+
Liu	2008	+	+	+	+	+	+	+	+	+
Tseng	2009	+	+	+	+	+	+	+	+	+
Al-Hadyan	2012	+	+	+	+	+	+	+	+	+
Zhao	2013	+	+	+	+	+	+	+	+	+
Su	2015	+	+	+	+	+	+	+	+	+

"+": detailed description; "±": incomplete description; "-": no description.

Table 3. Stratified analyses of the LIG4 rs1805388 polymorphism on cancer risk

Variables	n[a]	Cases/controls	TT versus CC			TC versus CC			Dominant model		
			OR (95% CI)	P[b]	I^2 (%)	OR (95% CI)	P[b]	I^2 (%)	OR (95% CI)	P[b]	I^2 (%)
Total	6	1869/2212	1.58 (0.96-2.60)	0.019	62.9	1.18 (1.03-1.35)	0.135	40.5	1.26 (0.99-1.58)	0.018	63.4
Cancer type											
Glioma	3	1311/1433	2.00 (1.43-2.80)	0.064	63.5	1.24 (0.90-1.71)	0.043	68.2	1.34 (0.92-1.94)	0.008	79.2
Other cancer	3	558/779	1.11 (0.69-1.78)	0.088	58.8	1.12 (0.88-1.44)	0.380	0.00	1.11 (0.88-1.41)	0.211	35.7
Genotyping method											
PCR-RFLP	2	310/476	1.86 (1.08-3.19)	0.642	0.00	1.26 (0.91-1.76)	0.486	0.00	1.37 (1.01-1.85)	0.453	0.00
TaqMan	2	1150/1109	2.13 (0.90-5.07)	0.023	80.7	1.29 (0.81-2.04)	0.013	83.9	1.39 (0.81-2.41)	0.002	89.5
Other method	2	409/627	0.73 (0.39-1.37)	0.317	0.00	1.03 (0.77-1.38)	0.420	0.00	0.98 (0.74-1.30)	0.596	0.00
Source of controls											
PB	5	1103/1487	1.60 (0.83-3.09)	0.012	68.7	1.28 (1.08-1.53)	0.198	33.5	1.32 (0.99-1.75)	0.031	62.4

[a]Number of comparisons. [b]*P* value of Q-test for heterogeneity test.

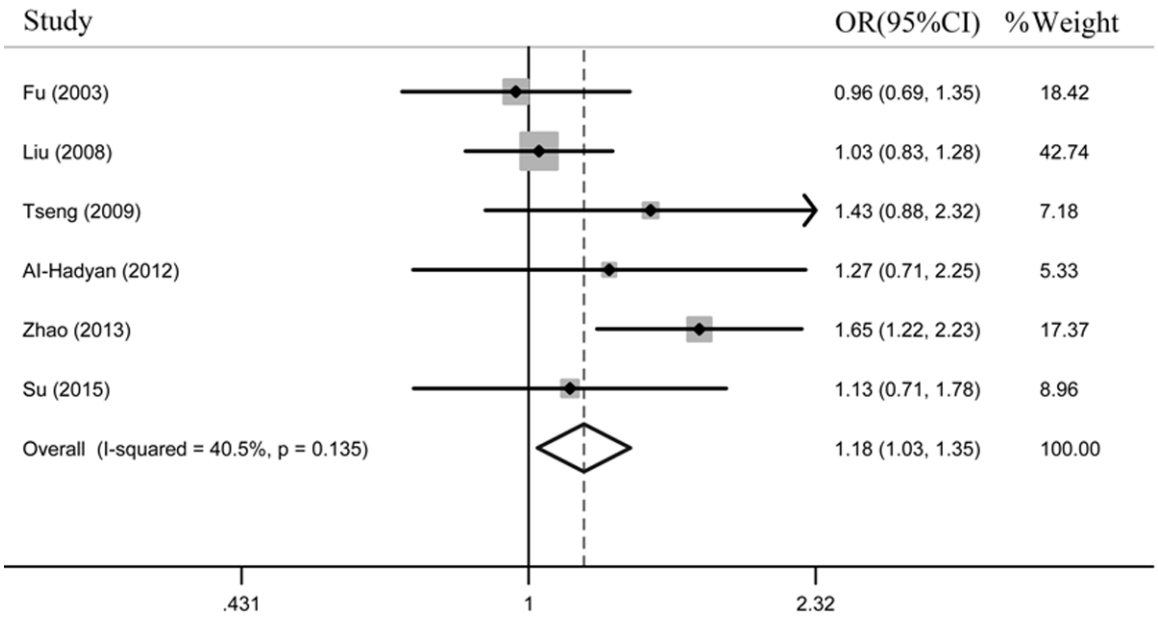

Figure 2. Meta-analysis of the association between *LIG4 rs1805388* polymorphism and susceptibility to cancer (TC versus CC).

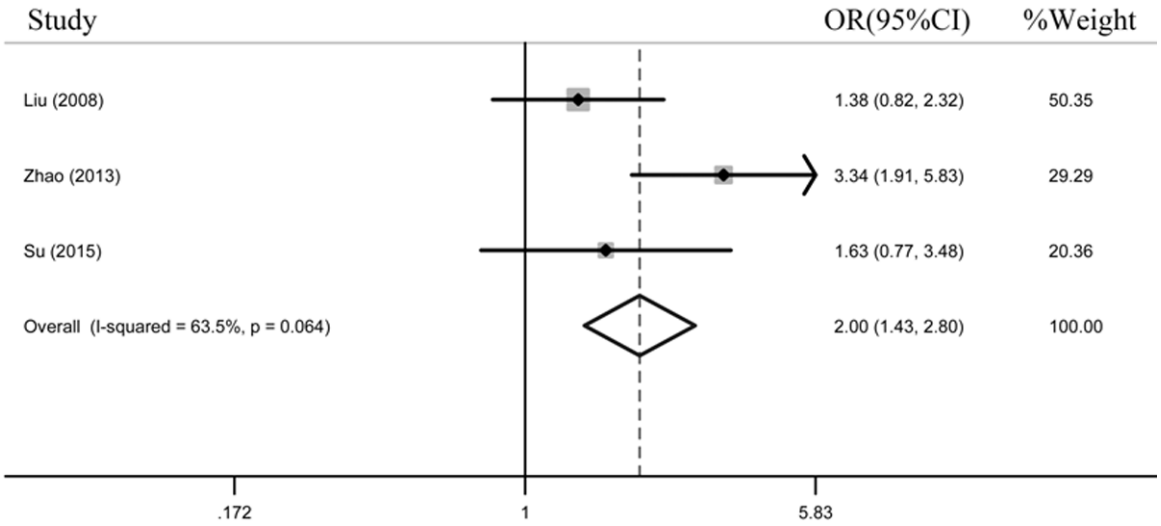

Figure 3. Meta-analysis of the association between *LIG4 rs1805388* polymorphism and susceptibility to glioma (TT versus CC).

in employed in DNA double strand break repair and in V(D)J recombination. Inactivation of the ligase IV gene in mice fibroblasts leads to marked sensitivity to ionizing radiation, growth defects and late embryonic lethality [20]. Furthermore, LIG4-deficiency in mice causes impaired cellular proliferation and massive apoptotic cell death of newly generated postmitotic neurons [21, 22]. The deficiency of LIG4 in animals can lead to increased rates of neoplastic transformation. A hypomorphic LIG4 mutation in mice exhibit multiple immunodeficiencies and a high incidence of lymphoid malignancies [23]. In addition to tumors of the immune system, Sharpless et al. demonstrated that LIG4 haploinsufficiency provokes soft tissue sarcomas harboring chromosomal amplifications, and translocations [24]. Inactivation of DNA

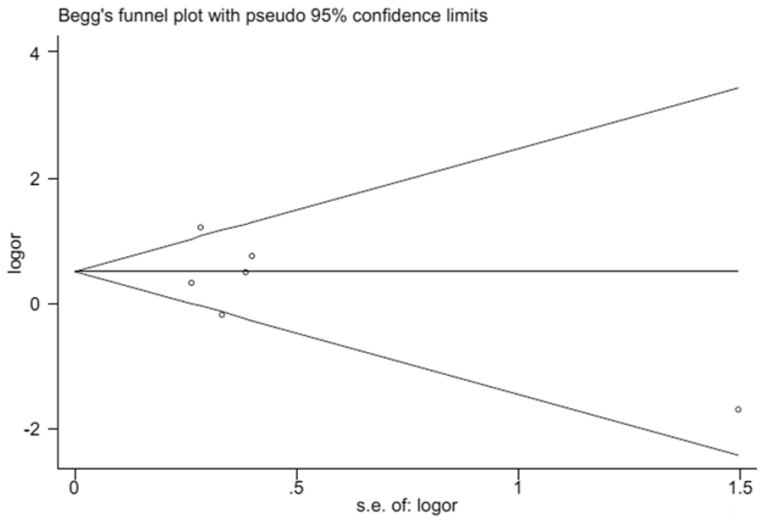

Figure 4. Begg's funnel plot for publication bias test.

ligase IV (Lig4) in the nervous system can also lead to brain tumors. Recent data from mice with inactivation of Lig4 have demonstrated that LIG4/p53 double-null mice can develop medulloblastoma [25].

The *LIG4* rs1805388 polymorphism is predicted to increase the hydrophobicity of the N-terminal of the LIG4 protein. *Rs1806389* and *rs1805388* in the N-terminal of LIG4 mildly impairs adenylation and ligation activity approximately 2-fold and increase the hydrophobic nature of LIG4 [26, 27]. SNPs in *XRCC4* and *LIG4* are linked significantly with high fractional allelic loss, an indicator of genomic instability and chromosomal abnormalities [11].

Some limitations of this meta-analysis should be considered. First, language bias might derive from the screened references of English documents only. Second, lack of individual information inhibited us from controlling the possible confounding factors such as age, sex, family history, environmental factors and lifestyle. Third, the number of studies included in the meta-analysis was too small to perform subgroup analysis. Therefore, further larger population studies should be performed

This meta-analysis provided novel insights into the biological mechanism underlying glioma formation and development. *LIG4 rs1805388* polymorphism may act considerably as a candidate biomarker for gliomas screening, diagnosis and therapy in the future. To confirm our findings, further well-designed studies with large sample size in glioma populations, more types of gliomas along with tissue-specific biochemical, functional and expressional characteristics are required.

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Disclosure of conflict of interest

None.

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