Original Article XRCC4 rs1805377 polymorphism increases glioma risk in Asian populations

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Received January 8, 2016; Accepted March 23, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: X-ray cross-complementing group 4 (XRCC4) is crucial for cells to maintain genetic stability thereby inflicting carcinogenesis. To date, epidemiologic findings have reached conflicting and ambiguous conclusions on the role of XRCC4 rs1805377 polymorphism in cancer risks. We made a comprehensive quantitative evaluation by performing a meta-analysis. Eligible publications assessing the association between XRCC4 rs1805377 polymorphism and cancer risks from PubMed, Embase and China national knowledge infrastructure (CNKI) databases were indentified. We used odds ratios (ORs) with 95% confidence intervals (Cls) to assess association strengths with the fixed-effect model or the random-effects model dependent on the heterogeneity. At the same time, subgroup analysis and sensitivity analysis were conducted. A total of 8 studies including 1911 cases and 2688 controls were included based on the search criteria. It was revealed by this meta-analysis that, in the Asian population, there was significant correlation between XRCC4 rs1805377 polymorphism and the risk of cancers (GG vs. AA: OR = 1.28, 95% CI = 1.05-1.57, Pheterogeneity = 0.392). In further stratified analyses, XRCC4 rs1805377 polymorphism was associated with increased glioma risk among Asians in homozygote comparison (GG vs. AA: OR = 1.59, 95% CI = 1.12-2.25, Pheterogeneity = 0.261). Significantly elevated cancers risk were also observed in population-based studies (GG versus AA: OR = 1.38, 95% CI = 1.11-1.72, Pheterogeneity = 0.571) and using other method studies (GG versus AA: OR = 1.63, 95% CI = 1.11-2.39, Pheterogeneity = 0.275). This meta-analysis indicated that XRCC4 rs1805377 polymorphism probably was associated with gliomas susceptibility in Asians.

Keywords: Cancer, meta-analysis, polymorphism, susceptibility, XRCC4

Introduction

DNA double strand breaks (DSBs), the principle genotoxic form of DNA damage, arise naturally by various exogenous exposures such as ionizing radiation (IR) and may occur intrinsically during physiological DNA rearrangement events such as the V(D)J recombination in lymphocytes or class-switch recombination at the immunoglobulin heavy chain (IgH) locus [1-3]. In mammalian cells, the ability to maintain genomic stability by DNA DSB repair mechanisms are of particular etiological importance in preventing tumor formation. There are two major pathways for the repair of DSBs: homologous recombination (HR) and nonhomologous end-joining (NHEJ) [4]. NHEJ pathway, a wellorchestrated multistep process involving numerous proteins, is the predominant pathway of DNA DSB repair in mammalian cells and can function at any time during the cell cycle in higher eukaryotes [4, 5]. The core NHEJ machinery involves Ku70/Ku80 heterodimer, DNAdependent protein kinase (DNA-PKCS), Artemis, Cernunnos-XLF and XRCC4/ligase DNA IV complex [5, 6]. Human XRCC4 gene, located on chromosomal 5q14.2, restores DNA doublestrand break repair and supports V(D)J recombination [7]. XRCC4 and DNA ligase IV form a complex that plays an important role in the repair of DSB by the NHEJ pathway. It has recently been demonstrated that DNA ligase IV interacts with and is catalytically stimulated by the XRCC4 protein [8]. Inability to faithfully repair DSBs can induce disastrous consequences, including genomic instability, cell death, immunodeficiency and carcinogenesis [9-12]. Some experiments have demonstrated that XRCC4-deficiency embryonic fibroblasts and human cells exhibited marked severe DSB repair defect [13, 14]. It is therefore logical to speculate that the inter-individual variability in XRCC4 gene may contribute to cancer predisposition.



Single nucleotide polymorphisms (SNPs) are currently being identified for application to association studies of complex genetic diseases [15]. SNPs have provided some valuable insight into the etiology of differences genetic susceptibility to cancers by modifying the functions of the candidate genes or alleles at different loci through linkage disequilibrium (LD) [16]. The XRCC4 (rs1805377) A > G polymorphism has been found to be associated with the A to G substitution at position intron 7/exon 8 junction region of XRCC4, which abolishes an acceptor splice site at exon 8 [17]. The XRCC4 rs1805377 polymorphism is thought to alter XRCC4 expression or protein function and consequently may be involved in the etiology of various cancers. However, the results of XRCC4 rs1805377 polymorphism studies are inconsistent, even contradictory [18-25]. Hence, there is a need to reconcile this inconsistency and to derive more precise estimation of the associations. In this paper, we extensively reviewed literatures and conducted a metaanalysis to investigate the association of XRCC4 rs1805377 polymorphism and cancer risks.

Materials and methods

Publication search

Literature search was performed from PubMed, EMBASE as well as CNKI database using key words '*XRCC4'*, '*rs1805377'*, 'polymorphism' and 'cancer'. The last search was updated on 01 December 2015. In addition, the reference lists of reviews and retrieved articles were also screened by hand. All published studies matching the inclusion criteria were included in this meta-analysis.

Inclusion criteria

All human-associated studies, regardless of sample size, were included if they fulfilled all of the following entry criteria: (1) using case-control study method to assess the relationship of *XRCC4 rs180*-5377 polymorphism and cancer risks; (2) containing genotype and allele distributions of *XRCC4* polymorphism; (3) malignant tumors were histologically confirmed.

Data extraction

The following data was extracted from each study: the last name of first author, year of publication, country origin, cancer type, source of controls (population- or hospital-based controls), genotyping methods, total number of cases and controls, *P* value for Hardy-Weinberg equilibrium, genotype counts of cases and controls, respectively.

Statistical methods

All of the calculations were performed using STATA software. The strength of the association between XRCC4 rs1805377 polymorphism and cancer risk was estimated by Odds ratios (OR) with 95% confidence intervals (CI). The Z test was performed to estimate the significance of the pooled OR, and P < 0.05 was considered statistically significant. The pooled OR was estimated for XRCC4 rs1805377 polymorphism from the extracted dataset by homozygote comparison (GG vs. AA), heterozygote comparison (GA vs. AA), and dominant model (GA+GG vs. AA), respectively. Heterogeneity assumption in our meta-analysis refers to the variation in study outcomes between different studies. We used χ^2 -based Q statistic text and Chi-squarebased l^2 index to detect the heterogeneity between the studies [26, 27]. DerSimonian and Laird (D-L) random-effects model was used to analyze datasets showing significant heterogeneity, otherwise the Mantel-Haenszel (M-H) fixed-effects model was used [28, 29], in which P < 0.05 indicated significant heterogeneity.

Int J Clin Exp Med 2016;9(6):10211-10219

| First author | Year | Country | Cancer type | Source of controls | Genotyping method | Cases | Controls | Case | | Control | | | HWE | |
|-----------------|------|---------|-------------------|--------------------|----------------------|-------|----------|------|-----|---------|-----|-----|-----|------|
| | | | | | | | | AA | AG | GG | AA | AG | GG | |
| Fu | 2003 | China | Breast cancer | PB | Mass ARRAY | 251 | 379 | 14 | 102 | 135 | 24 | 159 | 196 | 0.27 |
| Tseng | 2008 | China | Oral Cancer | HB | PCR-RFLP | 318 | 318 | 173 | 127 | 18 | 167 | 130 | 21 | 0.52 |
| Tseng | 2009 | China | Lung Cancer | PB | PCR-RFLP | 150 | 151 | 83 | 48 | 19 | 83 | 59 | 9 | 0.73 |
| Mandal | 2011 | India | Prostate cancer | HB | PCR-RFLP | 192 | 224 | 131 | 55 | 6 | 149 | 65 | 10 | 0.40 |
| Zhao | 2013 | China | Glioma | PB | TaqMan OpenArray | 384 | 384 | 179 | 143 | 62 | 195 | 153 | 36 | 0.45 |
| Su | 2015 | China | Glioma | PB | PCR-RFLP | 162 | 324 | 62 | 70 | 30 | 137 | 134 | 53 | 0.04 |
| Shen | 2015 | China | Pancreatic cancer | PB | PCR-RFLP | 248 | 496 | 92 | 112 | 44 | 201 | 216 | 79 | 0.10 |
| Ding | 2015 | China | Pancreatic cancer | PB | PCR-RFLP | 206 | 412 | 74 | 95 | 37 | 159 | 184 | 69 | 0.21 |

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

PB, Population Based; HB, Hospital Based; PCR-RFLP: Polymerase Chain Reaction-restriction Fragment Length Polymorphism; HWE: P values for Hardy-Weinberg equilibrium (HWE) for each study's control group.

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|-------------|-----------------|----------------|----------------|---------|----------------|-------------|
| Table 2. Su | auneu anan | ses of the ARC | JU4 IST80221 I | polymor | phism on | cancer risk |

| Variables | nª | Cases/ controls | GG versus AA | | | AG versus AA | | | Dominant model | | | |
|--------------------|----|--------------------|------------------|----------------|--------|------------------|-------|--------|------------------|---------|--------|--|
| | | | OR (95% CI) | P ^b | l² (%) | OR (95% CI) | Pb | l² (%) | OR (95% CI) | P^{b} | l² (%) | |
| Total | 8 | 1911/2688 | 1.28 (1.05-1.57) | 0.392 | 4.9 | 1.03 (0.90-1.18) | 0.956 | 0.00 | 1.08 (0.95-1.23) | 0.929 | 0.00 | |
| Cancer type | | | | | | | | | | | | |
| Glioma | 2 | 546/708 | 1.59 (1.12-2.25) | 0.261 | 20.8 | 1.06 (0.83-1.36) | 0.634 | 0.00 | 1.18 (0.94-1.48) | 1.000 | 0.00 | |
| Pancreatic cancer | 2 | 454/908 | 1.19 (0.86-1.65) | 0.871 | 0.00 | 1.12 (0.87-1.44) | 0.935 | 0.00 | 1.14 (0.90-1.44) | 0.900 | 0.00 | |
| Other cancer | 4 | 911/1072 | 1.10 (0.75-1.61) | 0.282 | 21.5 | 0.93 (0.75-1.16) | 0.911 | 0.00 | 0.96 (0.78-1.18) | 0.951 | 0.00 | |
| Genotyping method | | | | | | | | | | | | |
| PCR-RFLP | 6 | 1276/1925 | 1.16 (0.92-1.48) | 0.546 | 0.00 | 1.03 (0.88-1.20) | 0.845 | 0.00 | 1.05 (0.91-1.22) | 0.862 | 0.00 | |
| Other method | 2 | 635/763 | 1.63 (1.11-2.39) | 0.275 | 16.0 | 1.03 (0.78-1.36) | 0.844 | 0.00 | 1.18 (0.91-1.53) | 0.932 | 0.00 | |
| Source of controls | | | | | | | | | | | | |
| PB | 6 | 1401/2146 | 1.38 (1.11-1.72) | 0.571 | 0.00 | 1.06 (0.90-1.24) | 0.904 | 0.00 | 1.14 (0.98-1.32) | 0.992 | 0.00 | |
| НВ | 2 | 510/542 | 0.78 (0.45-1.37) | 0.759 | 0.00 | 0.95 (0.73-1.23) | 0.941 | 0.00 | 0.93 (0.72-1.19) | 0.994 | 0.00 | |
| | | | | | | | | | | | | |

^aNumber of comparisons. ^bP value of Q-test for heterogeneity test.

The l^2 statistic was then used to quantitatively estimate heterogeneity and an $l^2 > 50\%$ indicates large heterogeneity [30]. Sub-group analyses were harnessed based on cancer type, source of controls and genotyping methods. Genotype frequencies of control group were assessed for Hardy-Weinberg equilibrium and a *P*-value < 0.01 was regarded a significant deviation from equilibrium. Additionally, one-way sensitivity analyses were performed by sequential removal of each study to confirm the stability of the results. Finally, the potential publication bias of literatures was assessed using the Begg's funnel plot and Egger linear regression test [31, 32].

Results

Subject characteristics

The detailed screening process was shown in **Figure 1**. Finally, there are a total of 8 eligible case-control studies included in this meta-

analysis, containing 1911 cases and 2688 controls. The detailed characteristics of the eligible studies included in this meta-analysis are shown in **Table 1**. The distribution of the genotypes in the controls was consistent with the Hardy-Weinberg equilibrium. The respective studies focused on the following tumor types: 2 glioma studies, 2 pancreatic cancers, 1 prostate cancer, 1 breast cancer, 1 lung Cancer and 1 oral cancer. 6 study designs were population based (PB), 2 were hospital based (HB). Three genotyping methods were used: PCR-RFLP (6 studies), MassARRAY (1 study), and TaqMan OpenArray (1 study).

Quantitative synthesis

In the overall analysis, a significantly increased tumor risk was found for GG vs. AA (OR = 1.28, 95% CI = 1.05-1.57, $P_{heterogeneity}$ = 0.392). On the contrary, there was no significant association of this SNP with tumor risk under GA vs. AA (OR



Figure 2. Meta-analysis of the association between XRCC4 rs1805377 polymorphism and susceptibility to cancers (GG versus AA).



Figure 3. Forest plots of the subgroup analysis (cancer type) for the association between XRCC4 rs1805377 polymorphism and cancer risk (GG versus AA).

= 1.03, 95% CI = 0.90-1.18, $P_{heterogeneity}$ = 0.956) and dominant genetic model (OR = 1.08, 95% CI = 0.95-1.23, $P_{heterogeneity}$ = 0.929) (**Table 2**; **Figure 2**).

Subgroup analyses

In order to obtain the exact consequence of the relationship between *XRCC4* rs1805377 polymorphism and cancer susceptibility, stratified analyses by study type, cancer type and genotyping method were performed. 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).

In the stratification analysis of cancer type, we observed that the variant homozygote GG were consistently associated with increased risks of gliomas (GG vs. AA: OR = 1.59, 95% CI = 1.12-2.25, P_{heterogeneity} = 0.261) when compared with the wild-type AA genotype, but not in heterozygote comparison (GA versus AA, OR = 1.06, 95% CI = 0.83-1.36, P_{heterogeneity} = 0.634) and dominant model (GG/GA versus AA, OR = 1.18, 95% CI = 0.94-1.48, P_{heterogeneity} = 1.000). Nevertheless, no significant association was found in other tumor sites subgroups under all genetic models (Table 2; Figure 3).

When the subgroup analyses were carried out according to source of controls, we found a borderline significant increased risk of cancers in population-based studies (GG versus AA: OR = 1.38, 95% CI = 1.11- $1.72, P_{heterogeneity} = 0.571$) (Table 2; Figure 4).

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 other method (GG versus AA: OR = 1.63, 95% CI = 1.11-2.39, $P_{heterogeneity}$ = 0.275), whereas for studies using PCR-RFLP, no such associations were observed (**Table 2**; Figure 5).

Evaluation of heterogeneity

Heterogeneity between studies in each comparison for the overall datasets was shown in **Table 2**. No significant between-study heterogeneity was detected in all genetic models (homozygote comparison, $P_{heterogeneity} = 0.392$; heterozygote comparison, 0.956; dominant model, 0.929).



Figure 4. Forest plots of the subgroup analysis (source of controls) for the association between XRCC4 rs1805377 polymorphism and cancer risk (GG versus AA).



Figure 5. Forest plots of the subgroup analysis (genotyping method) for the association between XRCC4 rs1805377 polymorphism and cancer risk (GG versus AA).

Sensitivity analysis

We conducted one-way sensitivity analysis by excluding each single study in turn from pooled analysis. The pool OR was not altered significantly when any single study was omitted, confirming our results are reliable and robust.

Potential publication bias

We used Begg's funnel plot and Egger's test to estimate the publication bias of the available

literature. As shown in **Figure 6**, the shape of the funnel plot did not reveal any evidence of funnel plot asymmetry, which was further proven by Egger's linear regression test (P = 0.438 for GG versus AA).

Discussion

In the present study, we found that individuals with *XRCC4 rs1805377* polymorphism might have increased cancer risks. The subgroup analysis for cancer type showed that *XRCC4 rs1805377* polymorphism has marginally elevated glioma risks among Asians. Moreover, subgroup analysis revealed significantly increased risk in population-based studies.

If left unrepaired, DSBs pose major threats to genomic instability, lymphocyte development and carcinogenesis. Nonhomologous end joining (NHEJ) is a pathway that repairs DSBs to maintain genomic stability. The first step involves recognition and signaling of DSB by the Ku70/80 heterodimer, which can slide onto DNA ends that have diverse configurations. In the second step, the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) interacts with Ku to form a fully functional DNA-PK holoenzyme that functions in the

synapsis of two broken DNA ends. Finally, the processed double-stranded DNA molecules are ligated by XRCC4 and LIG4 [33, 34]. The coiledcoil region of human XRCC4 interacts with LIG4 via the carboxy-terminal tandem BRCT repeat of DNA ligase IV [35]. XRCC4 forms higher-order complexes with the LIG4 protein, facilitating LIG4 stability and stimulating LIG4 adenylation in cells [36, 37]. In addition, XRCC4 serves as a flexible join that links LIG4 to other components of the NHEJ apparatus [38].



Figure 6. Begg's funnel plot of XRCC4 rs1805377 polymorphism and cancer risk (GG versus AA).

The role of XRCC4 in tumorigenesis is wellestablished. In the gene-targeting mutation mice model, XRCC4 deficient primary murine cells exhibited marked sensitivity to ionizing radiation, late embryonic lethality, defective neurogenesis and defective lymphogenesis [39]. XRCC4/p53 double-null mice routinely succumbed to RAG-dependent pro-B lymphomas which had chromosomal translocations [13]. In a p53-deficient background, absence of XRCC4 in nestin-expressing neuronal progenitor cells can lead to early onset of neuronally differentiated medulloblastomas [40]. Wang et al. found that CD21-cre-mediated deletion of the Xrcc4 in p53-deficient peripheral mouse B cells resulted in surface Ig-negative B-cell lymphomas [41]. In particular, the expression of the XRCC4 gene were significantly down-regulated in grade II, III, IV of astrocytoma and decreased expression of XRCC4 was intimately correlated with a poor prognosis (P < 0.05) [42]. It was biologically plausible that SNP of rs1805377 might increase the susceptibility of cancers. Biologically, the XRCC4 rs1805377 polymorphism in intron 7 involves a substitution of $G \rightarrow A$ in the intron 7/exon 8 junction region and may have functional significance since the nucleotide change from G to A potentially abolishes an acceptor splice site at exon 8 [17, 43]. Rs1805377 polymorphism in XRCC4 is linked significantly with chromosome instability, which is the pathogenic basis of tumorigenesis [12, 23, 44]. Disruption of genomic integrity contributes to malignant transformation and subsequent cancer development [45, 46]. The patients who had a homozygous variant GG genotype of the *XRCC4* gene had a poorer prognosis compared with other patients (P = 0.015; log-rank test) [23].

After subgroup analyses according to types of cancer, we found that there was an increased cancer risk for gliomas in the homozygote comparison. This suggests that the XRCC4 *rs1805377* polymorphism might have diverse mechanism of carcinogenesis in different cancer sites. Interestingly, when stratifying by source of controls, a significantly elevated risk was found

among population-based studies. It is well recognized that the population-based studies may be a representative of the general population in genetic association studies.

The main advantage of meta-analysis is maximization of power to analyze the accumulated data from varied investigations in which individual sample sizes are small [47]. However, certain potential limitations in our meta-analysis should be mentioned. First, only the published studies were included in our meta-analysis, which may not provide sufficient power. Second, due to lack of original data, our metaanalysis was based on single-factor estimates without adjustment for other potential interactions. Third, we observed that gene-typing method may influence our results and these aspects are worthy of consideration.

In conclusion, this meta-analysis showed some evidence of the *XRCC4* rs1805377 polymorphism and altered cancer risks among Asian populations. Moreover, larger scale case-control studies will be warranted in diverse populations to verify these findings.

Acknowledgements

This work is supported by Shandong Province Natural Science Foundation (ZR2014HL070).

Disclosure of conflict of interest

None.

Int J Clin Exp Med 2016;9(6):10211-10219

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