Original Article

Association of XRCC3 gene rs861539 polymorphism with gastric cancer risk: evidence from a case-control study and a meta-analysis

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Abstract: The association between the X-ray repair cross-complementing group 3 (XRCC3) gene Thr241Met polymorphism (rs861539) and gastric cancer has been widely evaluated, but a definitive answer is so far lacking. We first conducted a case-control study to assess this association in a large Han Chinese population, and then performed a meta-analysis to further address this issue. Although our case-control association study and the following meta analysis involving 6,520 subjects indicated null association of XRCC3 gene rs861539 polymorphism between gastric cancer patients and controls under both allelic (odds ratio (OR) = 1.02; 95% confidence interval (CI): 0.91-1.14; \( P = 0.739 \)) and dominant (OR = 0.97; 95% CI: 0.78-1.21; \( P = 0.803 \)) models. Stratified analysis by ethnicity demonstrated a significant association in Asians. We conclude that the XRCC3 gene rs861539 polymorphism was associated with the risk for gastric cancer in Asian populations.

Keywords: Gastric cancer, XRCC3, polymorphism, association, meta-analysis

Introduction

According to a recent survey, gastric cancer (GC) is the most frequently occurring cancer in the world with 989,600 new cases diagnosed annually [1]. It is widely accepted that GC is a complex multifactorial disease with masses of genetic and environmental factors contributing to its occurrence [2]. Studies have indicated that different individuals bear different GC risk even under similar environmental conditions [3]. Although great effort has been devoted to uncover the genetic underpinnings of GC, there is no consensus on how many genes and which genetic determinants are actually involved in its development.

The gene encoding X-ray repair cross-complementing group 3 (XRCC3) on chromosome 14q32.3, which functions in the homologous recombination repair of DNA cross-links [4], double-strand break [5] caused by normal metabolic processes and/or exposure to ionizing radiation, has been regarded as one of the most important DNA-repair genes. Inactivation of XRCC3 in human cells leads to two-fold greater sensitivity to DNA cross-linking agents, elevated chromosome aberrations and five to seven-fold increased endo-reduplication [6]. Moreover, a common polymorphism in XRCC3 gene at nucleotide 18607C/T (amino acid Thr241Met, rs861539) has drawn wide attention as its mutation is linked with an increased number of micronuclei in lymphocytes and relatively low DNA repair capacity [7]. Therefore, XRCC3 has been of considerable interest as a candidate susceptibility gene for various cancers. Many studies have attempted to associate XRCC3 gene rs861539 (Thr241Met) polymorphism with GC occurrence, the results, however, are not frequently repeatable. In retrospect, individual studies with small sample sizes that are known to have low statistical power limited the identification of genetic variability and yielded poor replication record [8, 9]. Moreover, this lack of reproducibility might also stem from discrepant genetic linings or lifestyle backgrounds.

To address this issue and derive a more precise estimation, in this study, we first decided to assess the association of rs861539 (Thr-
241Met) polymorphism of XRCC3 gene with GC risk in a large Han Chinese population. Then, given the accumulating data and to shed some light on current uncertain claims, we sought to conduct a comprehensive meta-analysis of this association from all English literature.

Methods

Study population

The study population included 448 unrelated GC patients and 602 cancer-free controls of Chinese Han population had been previously studied [10]. This study was approved by the Ethics Committee of Shanghai Jiaotong University School of Medicine, and was conducted according to the Declaration of Helsinki Principles. All subjects signed the written informed consent.

Genotyping

Blood samples (1 mL) were collected, and genomic DNA was extracted from white blood cells using the TIANamp Blood DNA Kit (Tiangen Biotect [Beijing] Co., LTD). Genotyping was conducted using the PCR-LDR (ligase detection reactions) method by ABI 9600 system (Applied Biosystems, USA) [11]. Cycling parameters were as the following: 94°C for 2 min; 35 cycles of 94°C for 15 s; 60°C for 15 s; 72°C for 30 s; and a final extension step at 72°C for 5 min. Two specific probes to discriminate the specific bases and one common probe were synthesized (available upon request). The common probe was labeled at the 3' end with 6-carboxy-fluorescein and phosphorylated at the 5' end. The reacting conditions of LDR were: 94°C for 2 min, 20 cycles of 94°C for 30 s and 60°C for 3 min. After reaction, 1 mL LDR reaction products were mixed with 1 mL ROX passive reference and then denatured at 95°C for 3 min, and chilled rapidly in ice water. The fluorescent products of LDR were differentiated using ABI sequencer 377 (Applied Biosystems, USA).

Statistical analysis

Comparisons between GC patients and controls were conducted by unpaired t-test for continuous variables and by $\chi^2$ test for categorical variables. To avoid gross genotyping error, Thr241Met polymorphism was checked for consistency with Hardy-Weinberg equilibrium by $\chi^2$ test. Genotypes were compared by conditional logistic regression analysis under assumptions of additive, dominant and recessive models of inheritance, respectively. Statistical significance was accepted as $P < 0.05$.

Meta analysis

Search strategy for identification of studies

PubMed and EMBASE databases were screened for articles published before July 20, 2014 using the Boolean combinations of subjects terms (XRCC3 OR rs861539) AND (gastric cancer OR carcinoma OR neoplasm) AND (polymorphism OR allele OR genotype OR variant OR variation). Articles were restricted to English or Chinese-language and human studies. The full text of the retrieved articles was scrutinized to decide whether information on the topic of interest was included. Reference lists of these retrieved articles and systematic reviews were also checked to determine whether citations of articles that were not initially identified. For these articles involving more than one geographic or ethnic heterogeneous groups, each was treated separately.

Articles were included in this meta-analysis if they examined the hypothesis that XRCC3 gene rs861539 polymorphism was associated with GC, if they followed a case-control or cross-sectional study design, and if they provided sufficient information on rs861539 (Thr241Met) genotype counts between GC patients and controls for determining an estimate of odds ratio (OR) and its corresponding 95% confidence interval (95% CI). Where there were multiple articles from the same study population, the most complete and recent results were extracted.

In this meta-analysis, we assessed the association between rs861539 241 Thr allele and GC risk with respect to 241Met (241Thr versus 241Met: allelic model), as well as the homozygous comparison (241Thr/Thr versus 241Met/Met), the dominant genetic model (241Thr/Thr + 241Thr/Met versus 241Met/Met), and the recessive genetic model (241Thr/Thr versus 241Thr/Met + 241Met/Met). Irrespective of between-study heterogeneity, the random-effects model using the DerSimonian & Laird method was implemented to bring the individual effect-size estimates together, and the estimate of heterogeneity was taken from the Mantel-Haenszel model [12]. Unadjusted OR and 95% CI were used to compare genetic contrasts between patients and controls. Satis-
Gastric cancer and XRCC3 gene rs861539 polymorphism

<table>
<thead>
<tr>
<th>Status</th>
<th>Thr241Met genotypes (number)</th>
<th>Thr241Met alleles (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>Thr/Thr 389</td>
<td>Thr/Met 50</td>
</tr>
<tr>
<td>Controls</td>
<td>Thr/Thr 549</td>
<td>Thr/Met 52</td>
</tr>
</tbody>
</table>

$\chi^2 = 2.1983; P = 0.2987$

Additive model (a) 0.74; 0.50-1.10; 0.142
Dominant model (a) 0.73; 0.05-11.71; 0.824
Recessive model (a) 0.74; 0.49-1.10; 0.139

$P$-values were calculated using $\chi^2$-test from a series of 3 × 2 contingency tables for genotype data and 2 × 2 contingency tables for allele data. (a) Data are expressed as odds ratio; 95% confidence interval; $P$-values for genetic modes of inheritance.

Results

Single-locus analysis

The success rates of genotyping for Thr241Met polymorphism were 98.21% and 100% in patients and controls, respectively. Genotype distributions of examined polymorphism respected Hardy-Weinberg equilibrium in controls ($P > 0.05$). There was no significant difference in the genotype and allele distributions of Thr241Met polymorphism between GC and controls, and this non-significance was also mirrored under assumptions of the additive (OR = 0.74; 95% CI: 0.50-1.10; $P = 0.142$), dominant (OR = 0.73; 95% CI: 0.05-11.71; $P = 0.824$) and recessive (OR = 0.74; 95% CI: 0.49-1.10; $P = 0.139$) models (Table 1).

Search results

Based on our search strategy, the primary screening produced 18 potentially relevant articles, of which 9 met the inclusion criteria in an attempt to evaluate the association of XRCC3 gene rs861539 polymorphism with GC [15-23]. Therefore, 9 separate studies plus the present study encompassing a total of 2649 patients with GC and 3871 controls were finally meta-analyzed. Of these 10 study populations, 5 populations were conducted in Caucasians, 4 in Asians and one in Brazilians.

Study characteristics

The baseline characteristics of qualified studies are presented in Table 2. The overall rs861539 241Thr allele frequency was 73.94%/84.83% (patients/controls) in Asians, which was exceedingly higher than that in Caucasians (63.42%/62.30%) and Brazilians (69.06%/64.67%). Taking into account only the controls, genotype distributions were in Hardy-Weinberg equilibrium for all qualified studies.

Overall analysis

After combining all qualified studies, we found null association of XRCC3 gene Thr241Met polymorphism with GC under both allelic (OR = 0.89; 95% CI: 0.65-1.24; $P = 0.504$) and dominant (OR = 0.89; 95% CI: 0.57-1.39; $P = 0.600$)
models, and this association suffered from significant evidence of heterogeneity between studies (allelic and dominant models: $P = 92.2\%$ and $77.9\%$). However, there was low probability of publication bias for both models ($P_{Egger} = 0.055$ and $0.184$).

**Figure 1.** Subgroup analysis of XRCC3 gene rs861539 polymorphism for gastric cancer by ethnicity in the allelic (A), homozygous (B), dominant (C) and recessive (D) models.

### Table 2. The baseline characteristics of all eligible studies

<table>
<thead>
<tr>
<th>References</th>
<th>Ethnicity</th>
<th>Country</th>
<th>Sources of Con.</th>
<th>Number of Ca.</th>
<th>Number of Con.</th>
<th>Sources of Ca./Con.</th>
<th>MAF (%) Ca/Con.</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duarte MC (2005)</td>
<td>Others</td>
<td>Brazil</td>
<td>HCC</td>
<td>160</td>
<td>150</td>
<td>84/67</td>
<td>63/60</td>
<td>23/23</td>
</tr>
<tr>
<td>Huang WY (2005)</td>
<td>Caucasian</td>
<td>Poland</td>
<td>PCC</td>
<td>281</td>
<td>390</td>
<td>128/174</td>
<td>128/163</td>
<td>25/53</td>
</tr>
<tr>
<td>Huang GP (2006)</td>
<td>Asian</td>
<td>China</td>
<td>HCC</td>
<td>309</td>
<td>188</td>
<td>149/112</td>
<td>135/66</td>
<td>25/10</td>
</tr>
<tr>
<td>Ye W (2006)</td>
<td>Asian</td>
<td>Sweden</td>
<td>PCC</td>
<td>126</td>
<td>472</td>
<td>52/203</td>
<td>63/218</td>
<td>11/51</td>
</tr>
<tr>
<td>Ruzzo A (2007)</td>
<td>Caucasian</td>
<td>Italy</td>
<td>PCC</td>
<td>90</td>
<td>121</td>
<td>35/36</td>
<td>44/66</td>
<td>11/19</td>
</tr>
<tr>
<td>Palli D (2010)</td>
<td>Caucasian</td>
<td>Italy</td>
<td>PCC</td>
<td>294</td>
<td>546</td>
<td>95/189</td>
<td>148/268</td>
<td>51/89</td>
</tr>
<tr>
<td>Canbay E (2010)</td>
<td>Caucasian</td>
<td>Turkey</td>
<td>HCC</td>
<td>40</td>
<td>247</td>
<td>16/74</td>
<td>19/146</td>
<td>5/27</td>
</tr>
<tr>
<td>Zhao L (2011)</td>
<td>Asian</td>
<td>China</td>
<td>HCC</td>
<td>721</td>
<td>989</td>
<td>257/635</td>
<td>321/274</td>
<td>143/80</td>
</tr>
<tr>
<td>Hu WS (the present study)</td>
<td>Asian</td>
<td>China</td>
<td>HCC</td>
<td>440</td>
<td>602</td>
<td>389/549</td>
<td>50/52</td>
<td>1/1</td>
</tr>
</tbody>
</table>

**Abbreviations:** HCC = Hospital-based case-control study; PCC = Population-based case-control study; Ca = Case; Con = Control; HWE = Hardy-Weinberg equilibrium in the control group; MAF = minor allele frequency.

**Subgroup analysis**

To evaluate the possible effect of study design on the variability of overall estimates, the studies were divided into population-based and hospital-based studies. The magnitude of asso-
### Table 3. Overall and subgroup analysis of XRCC3 gene rs861539 (Thr241Met) polymorphism and gastric cancer

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Study number</th>
<th>Thr vs Met</th>
<th>Thr/Thr vs Met/Met</th>
<th>Dominant</th>
<th>Recessive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P (I² (%)</td>
<td>OR (95% CI)</td>
<td>P (I² (%)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>10</td>
<td>0.89 (0.65, 1.24)</td>
<td>0.504 (92.2)</td>
<td>0.08 (0.46, 1.58)</td>
<td>0.86 (0.624)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asians</td>
<td>4</td>
<td>0.62 (0.40, 0.96)</td>
<td>0.033 (86.5)</td>
<td>&lt; 0.001 (35.1)</td>
<td>0.32 (0.39)</td>
</tr>
<tr>
<td>Caucasians</td>
<td>5</td>
<td>1.05 (0.93, 1.19)</td>
<td>0.432 (0.523)</td>
<td>0.281 (0.467)</td>
<td>1.16 (1.15)</td>
</tr>
<tr>
<td>Others</td>
<td>1</td>
<td>1.22 (0.87, 1.71)</td>
<td>0.245 (0.253)</td>
<td>0.503 (0.503)</td>
<td>1.26 (1.08)</td>
</tr>
<tr>
<td><strong>Study design</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCC</td>
<td>5</td>
<td>0.77 (0.46, 1.28)</td>
<td>0.307 (93.0)</td>
<td>&lt; 0.001 (85.7)</td>
<td>0.309 (0.64)</td>
</tr>
<tr>
<td>PCC</td>
<td>5</td>
<td>1.04 (0.91, 1.18)</td>
<td>0.578 (0.542)</td>
<td>1.15 (0.324)</td>
<td>1.1 (0.400)</td>
</tr>
</tbody>
</table>

**Abbreviations:** HCC = Hospital-based case-control study; PCC = Population-based case-control study; OR = odds ratio; CI = confidence interval.
Gastric cancer and XRCC3 gene rs861539 polymorphism

Association in population-based studies was higher than the overall estimate for rs861539 polymorphism across all genetic models. Contrarily, the magnitude of association in hospital-based studies was consistently weakened across all genetic models (Table 3). However, no significant association was detected in the comparison between hospital-based group and population-based group.

Further stratification by ethnicity supported the protective profiles of rs861539 241Thr allele and Thr/Thr genotype on GC in Asians in all genetic models, but a weak and nonsignificant risk tendency was identified in Caucasians (Figure 1).

Publication bias

As reflected by the funnel plots (Figure 2) and the corresponding Egger’s test, there was a low probability of publication bias for the polymorphism examined.

Discussion

XRCC3, coding the key protein of DNA double-strand break/recombination repair pathway, is the crucial gene involved in the homologous recombination repair mechanism. With respect to the important roles of XRCC3 in the DNA repair, it is biologically plausible that XRCC3 genetic polymorphism may modulate the risk of various cancers. As for the XRCC3 rs861539 polymorphism with cancer risk, Yu et al. [24] conducted a meta-analysis to confirm that XRCC3 Thr241Met polymorphism contributes to the risk of thyroid cancer, A meta-analysis of nine case-control studies suggested that the XRCC3 Thr241Met polymorphism was significantly associated with the risk of gliomas [25]. A meta-analysis of five case-control studies suggested h XRCC3 Thr241Met polymorphism is associated with cervical cancer risk among East Asians [26]. Although numerous studies have regarded XRCC3 gene rs861539 polymorphism as a promising candidate for GC, our case-control study in Han Chinese population, along with the meta-analysis, failed to confirm this relation. This non-significance was also in accordance with previous meta result, although the previous analysis only included six studies with small study samples [27]. To our knowledge, this is the most comprehensive meta-

Figure 2. Funnel plots for studies investigating the effect of XRCC3 gene rs861539 polymorphism on gastric cancer risk across the allelic (A), homozygous (B), dominant (C) and recessive (D) models.
analysis investigating the genetic susceptibility of XRCC3 gene rs861539 polymorphism to GC.

Some aspects of the current meta-analysis need to be considered to appreciate the findings. First, this is to date the largest synthesis exploring the association of XRCC3 gene rs861539 polymorphism with GC. Second, the results of the present case-control study were in line with that of the corresponding meta-analysis. Third, our results are less prone to selection bias in view of low probability of publication bias.

In addition, ethnicity was regarded as a potential source of between-study heterogeneity by subgroup analysis. After stratifying studies into Caucasians, Asians and other population studies, we observed that polymorphism rs861539 displayed reverse association with GC across all genetic models between Asians and non-Asians. Moreover, we noticed striking differences in terms of rs861539 241Thr allele frequency between Asians (73.94%/84.83% in patients/controls) and non-Asians, with the former exceedingly higher than that in Caucasians (63.42%/62.30%) and Brazilians (69.06%/64.67%), suggesting that different genetic backgrounds may cause this discrepancy or different populations may have different linkage disequilibrium patterns. However, the situation is not rare. We have found similar phenomenon in previous studies in inflammatory disease [28] and cancerous disease [10]. A polymorphism may be in close linkage with another nearby causal variant in one ethnic population but not in another [29]. The XRCC3 gene rs861539 polymorphism may be in close linkage with different nearby causal variants in different populations. It is therefore reasonable to speculate that if involved, rs861539 polymorphism may have pleiotropic effects on the etiology of gastric carcinogenesis cross different races or ethnic groups. In view of the divergent genetic backgrounds, it is necessary to construct a database of polymorphisms related to GC in each ethnic group.

Despite the clear strengths of our study including large sample sizes, and low possibility of publication bias across all genetic models, interpretation of our current study, however, should be viewed in light of several technical limitations. First, all of the studies in this meta-analysis were case-control studies, which are susceptible to selection bias by including only nonfatal cases. Second, because only published studies were retrieved in this meta-analysis and the “grey” literature (articles in languages other than English or Chinese) was not included, publication bias might be possible, even though our funnel plots and statistical tests did not show it. Third, the single-locus-based nature of meta-analysis precluded the possibility of gene-gene and gene-environment interactions, as well as haplotype-based effects, suggesting that additional studies assessing these aspects will be necessary. Furthermore, we only centered on XRCC3 gene rs861539 polymorphism, and did not covered other candidate genes or polymorphisms. It seems likely that the polymorphism rs861539 individually makes a moderate contribution to risk prediction in GC patients, but whether this polymorphism integrated with other risk factors will enhance the prediction requires additional research. Moreover, due to the relative small number of some studies or lack of necessary information, we were unable to perform further subgroup analyses upon various confounding factors such as smoking and drinking. Thus, the jury must refrain from drawing a conclusion until large, well-performed studies confirm or refuse our results.

Taken together, we expand previous single studies on GC by suggesting that XRCC3 gene rs861539 polymorphism might contribute to the occurrence of GC in Asians but not in other populations. Also our observations leave open the question regarding the pleiotropic effects of rs861539 in different ethnic populations. Further studies should investigate XRCC3 gene adjacent markers to confirm whether the present association is causal or due to linkage disequilibrium.

Disclosure of conflict of interest

None.

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References

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