TOX3 rs3803662 C > T polymorphism contributes to breast cancer susceptibility in the Chinese population: evidence from 12,800 cases and 11,550 controls

Jianzhou Tang1,5*, Hui Li2*, Jiashun Luo3, Hua Mei4, Liang Peng1, Xiaojie Li5

1Department of Biological and Environmental Engineering, Changsha University, Changsha 410003, Hunan, China; 2Department of Microbiology and Immunology, 3Institute of Medical Sciences, Medical School of Jishou University, Jishou 416000, Hunan, China; 4Hunan Guangxiu Hospital, Changsha 410002, Hunan, China; 5College of Animal Science and Technology of Hunan Agriculture University, Changsha 410128, Hunan, China. *Equal contributors and co-first authors.

Abstract: The association between the TOX high mobility group box family member 3 (TOX3) gene rs3803662 C > T polymorphism and breast cancer risk have been investigated in multiple ethnic groups. However, studies conducted in the Chinese population have yielded contradictory results. Therefore, we performed the current meta-analysis to drive a more precise evaluation of the association between TOX3 rs3803662 C > T polymorphism and breast cancer risk in the Chinese population. A total of seven eligible studies with 12,800 cases and 11,550 controls were involved in this meta-analysis. Overall, the SNP was shown to significantly increase the risk of developing breast cancer in the Chinese population under all genetic models (homozygous model: OR = 1.28, 95% CI = 1.17-1.39, \( P < 0.001 \); heterozygous model: OR = 1.12, 95% CI = 1.03-1.22, \( P = 0.009 \); recessive model: OR = 1.17, 95% CI = 1.11-1.23, \( P < 0.001 \); dominant model: OR = 1.20, 95% CI = 1.10-1.30, \( P < 0.001 \); as well as allele comparison: OR = 1.13, 95% CI = 1.09-1.18, \( P < 0.001 \)). Meanwhile, no between-study heterogeneity and publication bias was observed, indicating the reliability of the findings. In conclusion, our meta-analysis results suggested that TOX3 rs3803662 C > T polymorphism might confer increased susceptibility to breast cancer in the Chinese population.

Keywords: TOX3, polymorphism, breast cancer, risk, meta-analysis

Introduction

Cancer remains an enormous burden on public health with about 14.1 million new cancer cases and 8.2 million deaths having occurred all over the world in 2012 [1]. In women, breast cancer is the most common invasive cancer and the leading cause of cancer deaths worldwide, accounting for 25% of all female cancer cases and 15% of female cancer deaths in 2012 [1]. In China, the incidence rate of breast cancer has sharply increased since 2000 [2], which constituted approximately 16.2% of all cancer cases and 7.9% of cancer deaths in women in 2010 [3]. It is well known that breast cancer is a multifactorial disease caused by interactions between genetic and environmental factors [4-6]. Several high-penetrance mutations in genes (e.g., BRCA1, BRCA2) were considered to be associated with the increase in breast cancer risk [7]. However, such mutations can be contributable to only a small part of breast cancer, approximately 25% of the familial risk and 5% of the total breast cancer incidence [8, 9]. Numerous studies have suggested that some low-penetrance genes may also play a role in the etiology of breast cancer.

TOC high mobility group box family member 3 (TOX3), also known as trinucleotide repeat containing 9 (TNRC9), is located on chromosome 16q12 [10]. TOX3 protein contains a putative high-mobility-group box motif, suggesting that it may act as a transcription factor. Abnormal activity of TOX3 protein was related involved in bone metastasis of breast cancer [11]. Many polymorphisms have been identified in the TOX3 gene [10, 12, 13]. Interestingly, genome-
wide association studies (GWASs) in 2007 indicated that genetic variants in the TOX3 gene was showed to be significantly associated with breast cancer risk [10, 13]. Moreover, TOX3 rs3803662 C > T, a common single nucleotide polymorphism (SNP), has been investigated for its association with breast cancer susceptibility [14-16]. Recently, a meta-analysis conducted by Zhang et al. suggested that TOX3 rs3803662 C > T polymorphism was associated with increased risk of breast cancer, especially in Asians [17]. However, several studies have reported opposite findings that TOX3 rs3803662 C > T polymorphism did not contribute to the risk of breast cancer in the Chinese population [18-21]. As a result, the genetic effect of this polymorphism on breast cancer susceptibility remains contradictory in the Chinese populations. Therefore, we conducted this meta-analysis to achieve high-quality estimation of the association of TOX3 rs3803662 C > T polymorphism with breast cancer risk in the Chinese population.

**Materials and methods**

**Identification of eligible relevant studies**

We systematically searched the PubMed and Embase databases for studies that assessed the association between TOX3 rs3803662 C > T polymorphism and the risk of breast cancer in the Chinese population. The following search terms were used: “TOX3 or TNRC9”, “polymorphism or variant or variation or rs3803662”, and “cancer or tumor or carcinoma”. Furthermore, reference lists of important studies and reviews were screened and reviewed manually for additional eligible publications. We performed latest literature search on December 31, 2015. There was no language limitation.

**Inclusion and exclusion criteria**

Eligible studies included into our meta-analysis were required to meet all of the following inclusion criteria: (1) human studies, (2) case-control studies, (3) investigation of the association between TOX3 rs3803662 C > T polymorphism and the risk of breast cancer in the Chinese population, (5) sufficient data for estimating odds ratio (OR) and their 95% confidence interval (CI), (6) genotype frequency distributions in the control group being in Hardy-Weinberg equilibrium (HWE). If the studies involved partly overlapped subjects, only the one with largest sample size or the latest study was chosen.

**Data extraction**

Information was extracted by two investigators independently. Disagreements between the two investigators were resolved by discussion. The following data were extracted: first author’s surname, year of publication, country of origin, ethnicity, genotyping method, numbers of cases and controls and the genotype counts of cases and controls for TOX3 rs3803662 C > T polymorphism.

**Statistical analysis**

HWE calculation for genotype frequency distributions was conducted in the control group for each selected study, using chi-squared goodness-of-fit test. A P value > 0.05 was applied for HWE. The strength of the association of TOX3 rs3803662 C > T polymorphism with the risk of breast cancer in the Chinese population was measured by crude OR with corresponding 95% CI. The pooled ORs (95% CIs) were estimated for TOX3 rs3803662 C > T polymorphism genotypes under the homozygous (TT vs. CC), heterozygous (CT vs. CC), recessive (TT vs. CT + CC), and dominant models (CT + TT vs. CC). Moreover, comparison of allele frequency was also carried out (T vs. C). The heterogeneity between studies was quantified with Chi square-based Q-test. A P value > 0.10 was considered the absence of significant heterogeneity. In the case of no heterogeneity we chose the fixed-effects model (the Mantel-Haenszel method) [22]. Otherwise, the random-effects model (the DerSimonian and Laird method) was used [23]. Furthermore, the heterogeneity was also assessed by I² statistics. Values of I² range from 0 to 100%, with higher score suggesting a greater degree of heterogeneity [24]. Potential publication bias was evaluated using both Begg's funnel plots and Egger's linear regression test [25, 26]. A P value < 0.05 was considered a significant publication bias. We also conducted sensitivity test by recalculating the ORs (95% CIs) after consecutively excluding individual studies. All statistical analyses were performed using the STATA software (version 11.0; Stata Corporation, College Station, TX).
Results

Study characteristics

As shown in Figure 1, a total of 79 potentially relevant articles were retrieved from PubMed and EMBASE databases. After title and abstract screening, 41 studies were removed because they did not investigate the association between TOX3 gene polymorphisms and breast cancer risk. We assessed full texts of the remaining 38 studies. Among them, 27 publications were ruled out for not analyzing the Chinese population. In addition, one publication was excluded for not focusing on TOX3 rs3803662 C > T polymorphism; two articles were excluded because genotyping data were not reported. Finally, one study was removed because of deviation from HWE [27]. Thus, seven eligible studies with 12,800 cases and 11,550 controls [18-21, 28-30] were ultimately involved in the meta-analysis (Table 1). All the selected studies were in agreement with HWE.

Meta-analysis results

The association between TOX3 rs3803662 C > T polymorphism and breast cancer risk in the Chinese population was summarized in Table 2. Overall, a significantly increased risk of developing breast cancer in the Chinese population was identified under all genetic models (homozygous model: OR = 1.28, 95% CI = 1.17-1.39, P < 0.001; heterozygous model: OR = 1.12, 95% CI = 1.03-1.22, P = 0.009; recessive model: OR = 1.17, 95% CI = 1.11-1.23, P < 0.001; dominant model: OR = 1.20, 95% CI = 1.10-1.30, P < 0.001, Figure 2; and comparison of allele frequency: OR = 1.13, 95% CI = 1.09-1.18, P < 0.001).

Publication bias

Begg's funnel plots test and Egger's linear regression test were used to evaluate the potential publication bias. As shown in Table 2 and Figure 3, there was no evidence of publication bias under all genetic models (homozygous model: P = 0.167; heterozygous model: P = 0.659; recessive model: P = 0.070; and dominant model: P = 0.352; as well as allele comparison: P = 0.079). The absence of publication bias further proved the reliability and accuracy of the present assessment of the association between TOX3 rs3803662 C > T polymorphism and breast cancer risk in the Chinese population.

Discussion

The etiology of breast cancer has not been fully clarified. The association of the high- and moderate-penetrance susceptibility genes with breast cancer risk has been well documented.
### Table 1. Characteristics of eligible studies in the meta-analysis

<table>
<thead>
<tr>
<th>Surname</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Genotyping method</th>
<th>Cases All</th>
<th>Cases CC</th>
<th>Cases CT</th>
<th>Cases TT</th>
<th>Controls All</th>
<th>Controls CC</th>
<th>Controls CT</th>
<th>Controls TT</th>
<th>MAF</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li</td>
<td>2009</td>
<td>China</td>
<td>Chinese</td>
<td>PCR-ligation detection reaction</td>
<td>291</td>
<td>32</td>
<td>141</td>
<td>118</td>
<td>291</td>
<td>40</td>
<td>128</td>
<td>123</td>
<td>0.64</td>
<td>0.470</td>
</tr>
<tr>
<td>Liang</td>
<td>2010</td>
<td>China</td>
<td>Chinese</td>
<td>SNP stream high-throughput 12-plex</td>
<td>1025</td>
<td>126</td>
<td>413</td>
<td>486</td>
<td>1046</td>
<td>127</td>
<td>464</td>
<td>455</td>
<td>0.66</td>
<td>0.603</td>
</tr>
<tr>
<td>Long</td>
<td>2010</td>
<td>USA</td>
<td>Chinese</td>
<td>Affymetrix</td>
<td>6345</td>
<td>650</td>
<td>2761</td>
<td>2934</td>
<td>3795</td>
<td>465</td>
<td>1727</td>
<td>1603</td>
<td>0.65</td>
<td>0.996</td>
</tr>
<tr>
<td>Zheng</td>
<td>2010</td>
<td>USA</td>
<td>Chinese</td>
<td>Affymetrix</td>
<td>3039</td>
<td>313</td>
<td>1325</td>
<td>1401</td>
<td>1401</td>
<td>386</td>
<td>1410</td>
<td>1286</td>
<td>0.65</td>
<td>0.987</td>
</tr>
<tr>
<td>Jiang</td>
<td>2011</td>
<td>China</td>
<td>Chinese</td>
<td>SNaPshot</td>
<td>493</td>
<td>48</td>
<td>212</td>
<td>233</td>
<td>510</td>
<td>54</td>
<td>224</td>
<td>232</td>
<td>0.67</td>
<td>0.995</td>
</tr>
<tr>
<td>Barzan</td>
<td>2013</td>
<td>Germany</td>
<td>Chinese</td>
<td>Sequenom MassArray</td>
<td>984</td>
<td>89</td>
<td>413</td>
<td>482</td>
<td>2206</td>
<td>255</td>
<td>990</td>
<td>961</td>
<td>0.66</td>
<td>0.999</td>
</tr>
<tr>
<td>He</td>
<td>2014</td>
<td>China</td>
<td>Chinese</td>
<td>Sequenom MassArray</td>
<td>623</td>
<td>72</td>
<td>280</td>
<td>271</td>
<td>620</td>
<td>72</td>
<td>278</td>
<td>270</td>
<td>0.66</td>
<td>0.973</td>
</tr>
</tbody>
</table>

MAF, Minor Allele Frequency; HWE, Hardy-Weinberg equilibrium.
TOX3 rs3803662 C > T polymorphism and breast cancer susceptibility

However, these susceptibility genes account for only a small proportion of individuals who are at high risk of breast cancer [7-9]. TOX3, a low-penetrance gene, had been found to be implicated in breast cancer in GWASs since 2007 [10, 13]. But, the role of TOX3 in the development of breast cancer is unclear. TOX3 is located on chromosome 16q12 and its protein product contains a putative high-mobility-group box motif. Such motif suggests that TOX3 may act as a transcription factor, which has been reported to be involved in bone metastasis of breast cancer [11]. TOX3 rs3803662 C > T polymorphism has been widely studied in breast cancer. However, association studies on its association with breast cancer susceptibility yielded conflicting result.

In this meta-analysis, we evaluated the association between TOX3 rs3803662 C > T polymorphism and breast cancer risk in the Chinese population by pooling together seven studies comprising 12,800 cases and 11,550 controls [18-21, 28-30]. Overall, our risk estimates indicated that TOX3 rs3803662 C > T polymorphism was associated with 1.12- to 1.28-fold increased risk of breast cancer in the Chinese population under all genetic models. Meanwhile, no heterogeneity and publication bias was observed, indicating the reliability and accuracy of the current meta-analysis. In summary, these results suggested that TOX3 rs3803662 C > T polymorphism was linked to the risk of breast cancer in the Chinese population.

To data, no previous meta-analysis has assessed the association between TOX3 rs3803662 C > T polymorphism and breast cancer risk in the Chinese population. There was two published meta-analyses investigating the relationship of this polymorphism and breast cancer risk [17, 31]. Chen et al. found that TOX3 rs3803662 C > T polymorphism was associated with the risk of breast cancer in all the studied populations under the homozygous and recessive models, and in comparison of allele frequency [31]. However, they did not perform the subgroup analysis by ethnicity; therefore the association was not clear in the Chinese population. In the other meta-analysis, authors carried out the stratified analysis by ethnicity and observed that TOX3 rs3803662 C > T polymorphism was associated with increased risk of breast cancer in Asians under all genetic models [17]. However, individuals harboring TOX3 rs3803662 T allele are common in Chinese, while C allele is prevalent among Koreans [32]. Due to the discrepancy in the genotype distri-

Table 2. Meta-analysis results of the association between TOX3 rs3803662 C > T polymorphism and breast cancer risk in the Chinese population

<table>
<thead>
<tr>
<th>Genetic comparison</th>
<th>OR (95% CI)</th>
<th>P (het)</th>
<th>I² (%)</th>
<th>P value</th>
<th>Model</th>
<th>Publication bias (P for Begg’s test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous model (TT vs. CC)</td>
<td>1.28 (1.17-1.39)</td>
<td>0.580</td>
<td>0.0</td>
<td>&lt; 0.001</td>
<td>Fixed-effects</td>
<td>0.167</td>
</tr>
<tr>
<td>Heterozygous model (CT vs. CC)</td>
<td>1.12 (1.03-1.22)</td>
<td>0.694</td>
<td>0.0</td>
<td>0.009</td>
<td>Fixed-effects</td>
<td>0.659</td>
</tr>
<tr>
<td>Recessive model (TT vs. CT + CC)</td>
<td>1.17 (1.11-1.23)</td>
<td>0.541</td>
<td>0.0</td>
<td>&lt; 0.001</td>
<td>Fixed-effects</td>
<td>0.070</td>
</tr>
<tr>
<td>Dominant model (CT + TT vs. CC)</td>
<td>1.20 (1.10-1.30)</td>
<td>0.649</td>
<td>0.0</td>
<td>&lt; 0.001</td>
<td>Fixed-effects</td>
<td>0.352</td>
</tr>
<tr>
<td>Allele comparison (T vs. C)</td>
<td>1.13 (1.09-1.18)</td>
<td>0.528</td>
<td>0.0</td>
<td>&lt; 0.001</td>
<td>Fixed-effects</td>
<td>0.079</td>
</tr>
</tbody>
</table>

Figure 2. Forest plot for the association between TOX3 rs3803662 C > T polymorphism and breast cancer risk under dominant model.
bution of TOX3 rs3803662 among different ethnic groups, it is indispensible to understand the association between TOX3 rs3803662 C > T polymorphism and breast cancer risk in the Chinese population. Our meta-analysis results was in accordance with the previous meta-analysis [17]. The present meta-analysis, with the larger sample size (24,350 subjects) revealed that TOX3 rs3803662 C > T polymorphism contributed to the increased risk of breast cancer in the Chinese population.

Despite the advantage of large sample size and the lack of heterogeneity and publication bias in our meta-analysis, several limitations should be addressed. First, the source of control groups was not uniformly defined. Some were population-based studies, whereas others were hospital-based studies. Second, the estimation of the association between TOX3 rs3803662 C > T polymorphism and breast cancer risk in the Chinese population was solely dependent on crude without adjustment for other risk factors, such as age, obesity, smoking and estrogen receptor status. Third, the sample sizes of some included studies were relatively small. Finally, we failed to conduct stratified analysis by some risk factors and explore gene-environment interaction because of insufficient data in some eligible studies.

In conclusion, our meta-analysis suggested that TOX3 rs3803662 C > T polymorphism was associated with increased susceptibility to breast cancer in the Chinese population. Future large sample size studies should be warranted to solidify these conclusions.

Disclosure of conflict of interest
None.

Address correspondence to:
Jianzhou Tang, Department of Biotechnology and Environmental Science, Changsha University, Changsha 410003, China. Tel: +86 7318426-1506; Fax: +86 7318426-1506; E-mail: Z20050711@ccsu.edu.cn

References


