A meta-analysis on association of CYP2E1 rs2031920 and rs3813867 polymorphisms with breast cancer risk

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Abstract. Breast cancer remains a challenging disease globally due to its heterogeneity and complexity, with an annual increase rate of 3.1%. Cytochrome P450 2E1 (CYP2E1) rs2031920 and rs3813867 polymorphisms that reside in the 5'-flanking promoter region of the gene are in a complete linkage disequilibrium and have been associated with breast cancer risk, but the findings are inconsistent and inconclusive. Therefore, we investigated the association of the CYP2E1 rs2031920 and rs3813867 polymorphisms with the risk of breast cancer through a meta-analysis. After literature search, eight eligible studies were included in this meta-analysis with a total of 3650 breast cancer cases and 3607 controls. Our meta-analysis revealed no significant association of the CYP2E1 rs2031920 and rs3813867 polymorphisms with breast cancer risk in all comparison models including the allelic (OR = 0.968, 95% CI = 0.855-1.097; p = 0.612), heterozygous (OR = 1.003, 95% CI = 0.869-1.159; p = 0.963), homozygous (OR = 0.792, 95% CI = 0.519-1.207; p = 0.278), dominant (OR = 0.987, 95% CI = 0.858-1.134; p = 0.851), and recessive (OR = 1.265, 95% CI = 0.831-1.924; p = 0.273). In conclusion, this meta-analysis suggests that the CYP2E1 rs2031920 and rs3813867 polymorphisms are not associated with the risk of breast cancer.

Keywords: breast cancer, CYP2E1, meta-analysis, single nucleotide polymorphisms

INTRODUCTION

Breast cancer is the most common global incidence in women and it has been reported to increase from approximately of 0.64 million cases in 1980 to 1.64 million cases in 2010, with an increase rate of 3.1% per year (Forouzanfar et al., 2011). In general there are two types of breast cancer, namely non-invasive breast cancer and invasive breast cancer (Sharma et al., 2010). The susceptibility of certain genes combining with environmental factors plays an important role in developing breast cancer (Lichtenstein et al., 2000). Studies have shown that consumption of alcohol increases the level of estrogen that may subsequently stimulate the proliferation of breast cancer cells (Purohit, 2000) or the production of reactive oxygen species during alcoholic metabolism that results in breast cancer through the damage of DNA (Wright et al., 1999).

Cytochrome P450 2E1 (CYP2E1) of the cytochrome P450 gene family is involved in metabolism of more than 70 different chemicals with diverse structures (Boniza et al., 2009) and it can reduce molecular oxygen to highly reactive forms that may result in DNA damage and carcinogenesis (Danko & Chaschin, 2005). The rs2031920 and rs3813867 polymorphisms that reside in the 5'-flanking promoter region of the CYP2E1 gene were...
previously reported to be in a complete linkage disequilibrium (Watanabe et al., 1990), and these polymorphisms have been empirically demonstrated to alter the transcription activity of the CYP2E1 gene (Hayashi et al., 1991; Watanabe et al., 1994) that may modulate the progression and metastasis of breast cancer (Leung et al., 2013). To date, there are many case-control studies that assessed the association of the rs2031920 and rs3813867 polymorphisms with the risk of breast cancer, but the findings are inconsistent and inconclusive (Anderson et al., 2012; Choi et al., 2003; Chong et al., 2016; Khedhaier et al., 2008; McCarty et al., 2012; Sangrajrang et al., 2010; Wu et al., 2006; Zgheib et al., 2013). Hence, we investigated the association of the CYP2E1 rs2031920 and rs3813867 polymorphisms with the risk of breast cancer through a meta-analysis.

MATERIALS AND METHODS

**Literature search strategy and inclusion criteria.** A systematic literature search was performed in the PubMed database for studies reporting the association on CYP2E1 rs2031920 and rs3813867 polymorphisms with breast cancer risk until 31 August 2016, using the following keywords: “CYP2E1” or “CYP450 2E1” or “Cytochrome P450 2E1”, “polymorphism” or “variant”, “rs2031920” or “rs3813867” or “C1019T” or “G1259C” and “breast” or “breast cancer” or “breast carcinoma” or “breast tumor”. The following inclusion criteria were specified: (1) case-control study assessing the association on CYP2E1 rs2031920 and rs3813867 polymorphisms with breast cancer risk; (2) sufficient data on genotype distributions of the CYP2E1 rs2031920 and rs3813867 polymorphisms were provided. To avoid duplication, only studies with the most recent data were included when the study populations were overlapped.

**Data extraction.** Data including first author’s name, year of publication, studied population, total sample size for both cases and controls, and genotype distribution in both cases and controls were extracted from all the included studies. Data extraction from included studies were individually conducted by two investigators and disagreements were solved through discussion.

**Statistical analyses.** The Hardy-Weinberg equilibrium (HWE) for each of the included study was calculated using the Online Encyclopedia for Genetic Epidemiology software (Rodriguez et al., 2009). Odd ratio (OR) with 95% confidence interval (95% CI) was calculated for five genetic comparison models including the allelic (c2 vs. c1), heterozygous (c1/c2 vs. c1/c1), homozygous (c2/c2 vs. c1/c1), dominant (c1/c2+c2/c2 vs. c1/c1) and recessive (c1/c1+c1/c2 vs. c2/c2) using the Comprehensive Meta Analysis Ver. 2.2.064 (Biostat, Inc., USA). The heterogeneity of each genetic comparison study was determined by the $I^2$ (a value in %) and Q (a p-value) statistical tests. Fixed effect model (Mantel-Haenszel method) was used to calculate the pooled OR (Mantel & Haenszel, 1959) for $F$ value less than 50% and $p \geq 0.10$ while the random effect model (DerSimonian-Laird method) was used to calculate the pooled OR for $F$ value more than 50% and $p < 0.10$ (DerSimonian & Laird, 1986). Publication bias was determined using the funnel plot supported with Egger’s test (Egger et al., 1997) and Begg’s test (Begg & Mazumdar, 1994), and a p-value>0.05 was considered not statistically significant for both tests. All p-values in this meta-analysis were two-sided.

RESULTS AND DISCUSSION

Eight studies with a total of 7257 subjects (3650 breast cancer cases and 3607 controls) were included in this meta-analysis after literature search based on the inclusion criteria and detailed assessment (Anderson et al., 2012; Choi et al., 2003; Chong et al., 2016; Khedhaier et al., 2008; McCarty et al., 2012; Sangrajrang et al., 2010; Wu et al., 2006; Zgheib et al., 2013) (Figure 1). Among all studies, four were conducted in Asian populations, two were in non-Asian populations and two were in mixed populations. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was the most frequent approach used for genotyping among all studies followed by hydrolysis-probe real-time
PCR and MassARRAY. All study populations were within the HWE except for study by McCarty et al. (2012). The characteristic of the included studies was summarized in Table 1.

![Diagram of literature searching process](https://example.com/diagram.png)

**Figure 1.** Schematic diagram of literature searching process for this meta-analysis.

In this meta-analysis, all pooled ORs were calculated using the fixed model (Mantel-Haenszel method) based on the absent of heterogeneity among all included studies (Table 2). The variant c2 allele was not associated with the risk of breast cancer in the allelic comparison model (OR=0.968, 95% CI=0.855-1.097; p=0.612) (Figure 2). Besides that, forest plot for all genotypic comparison models including the heterozygous (OR=1.003, 95% CI=0.869-1.159; p=0.963) (Figure 3), homozygous (OR=0.792, 95% CI=0.519-1.207; p=0.278) (Figure 4), dominant (OR=0.987, 95% CI=0.858-1.134; p=0.851) (Figure 5) and recessive (OR=1.265, 95% CI=0.831-1.924; p=0.273) (Figure 6) revealed no significant evidence to associate the CYP2E1 rs2031920 and rs3813867 polymorphisms with breast cancer risk. Although previous studies showed that the presence of at least one c2 allele increased the risk of breast cancer in the Malaysian population (Chong et al., 2016) and subjects with at least one c1 allele elevated the risk of breast cancer in the Taiwanese population (Wu et al., 2006), the findings of this meta-analysis were statistically reliable and robust as there was no individual study significantly influenced the pooled OR when individual studies were subsequently removed in the sensitivity test.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Population</th>
<th>Sample size (case/control)</th>
<th>Case</th>
<th>Control</th>
<th>HW E (χ²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al. (2012)</td>
<td>Mixed</td>
<td>883/927</td>
<td>c1/c1 817</td>
<td>c1/c2 63</td>
<td>c2/c2 3</td>
</tr>
<tr>
<td>Chong et al. (2016)</td>
<td>Malaysian</td>
<td>71/260</td>
<td>c1/c1 40</td>
<td>c1/c2 29</td>
<td>c2/c2 2</td>
</tr>
<tr>
<td>Khedhaier et al. (2008)</td>
<td>Tunisian</td>
<td>304/244</td>
<td>c1/c1 296</td>
<td>c1/c2 8</td>
<td>c2/c2 0</td>
</tr>
<tr>
<td>McCarty et al. (2012)</td>
<td>Mixed</td>
<td>1028/1048</td>
<td>c1/c1 957</td>
<td>c1/c2 64</td>
<td>c2/c2 7</td>
</tr>
<tr>
<td>Sangrajrang et al. (2010)</td>
<td>Thai</td>
<td>546/473</td>
<td>c1/c1 400</td>
<td>c1/c2 135</td>
<td>c2/c2 11</td>
</tr>
<tr>
<td>Wu et al. (2006)</td>
<td>Taiwanese</td>
<td>262/225</td>
<td>c1/c1 162</td>
<td>c1/c2 94</td>
<td>c2/c2 6</td>
</tr>
<tr>
<td>Zgheib et al. (2013)</td>
<td>Lebanese</td>
<td>227/98</td>
<td>c1/c1 219</td>
<td>c1/c2 8</td>
<td>c2/c2 0</td>
</tr>
</tbody>
</table>

*In Hardy-Weinberg equilibrium (HWE) if χ² < 3.84.*
Table 2. Association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with the risk of breast cancer in different comparison models.

<table>
<thead>
<tr>
<th>Genetic comparison</th>
<th>Model</th>
<th>OR (95% CI); p</th>
<th>Heterogeneity ($I^2$); p</th>
<th>Egger’s test (t); p</th>
<th>Begg’s test (z); p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allelic (c2 vs. c1)</td>
<td>Fixed</td>
<td>0.968 (0.855-1.097); 0.612</td>
<td>24.693; 0.232</td>
<td>0.297; 0.776</td>
<td>0.742; 0.458</td>
</tr>
<tr>
<td>Heterozygous (c1/c2 vs. c1/c1)</td>
<td>Fixed</td>
<td>1.003 (0.869-1.159); 0.963</td>
<td>9.140; 0.359</td>
<td>0.064; 0.951</td>
<td>0.742; 0.458</td>
</tr>
<tr>
<td>Homozygous (c2/c2 vs. c1/c1)</td>
<td>Fixed</td>
<td>0.792 (0.519-1.207); 0.278</td>
<td>39.445; 0.143</td>
<td>1.573; 0.191</td>
<td>1.315; 0.188</td>
</tr>
<tr>
<td>Dominant (c1/c2+c2/c2 vs. c1/c1)</td>
<td>Fixed</td>
<td>0.987 (0.858-1.134); 0.851</td>
<td>15.360; 0.309</td>
<td>0.199; 0.849</td>
<td>0.495; 0.621</td>
</tr>
<tr>
<td>Recessive (c1/c1+c1/c2 vs. c2/c2)</td>
<td>Fixed</td>
<td>1.265 (0.831-1.924); 0.273</td>
<td>38.570; 0.149</td>
<td>1.388; 0.238</td>
<td>0.939; 0.348</td>
</tr>
</tbody>
</table>

Figure 2. Forest plot showing the association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk under allelic comparison model (c2 vs. c1).
Figure 3. Forest plot showing the association of CYP2E1 rs2031920 and rs3813867 polymorphisms with breast cancer risk under heterozygous comparison model (c1/c2 vs. c1/c1).

Figure 4. Forest plot showing the association of CYP2E1 rs2031920 and rs3813867 polymorphisms with breast cancer risk under homozygous comparison model (c2/c2 vs. c1/c1). Two studies were excluded as no data on c2/c2 genotype was reported (Khedhaier et al., 2008; Zgheib et al., 2013).
Figure 5. Forest plot showing the association of CYP2E1 rs2031920 and rs3813867 polymorphisms with breast cancer risk under dominant comparison model (c1/c2+c2/c2 vs. c1/c1).

Figure 6. Forest plot showing the association of CYP2E1 rs2031920 and rs3813867 polymorphisms with breast cancer risk under recessive comparison model (c1/c1+c1/c2 vs. c2/c2). Two studies were excluded as no data on c2/c2 genotype was reported (Khedhaier et al., 2008; Zgheib et al., 2013).

Funnel plot was performed to test for existing of publication bias in this meta-analysis. The shape of funnel plot under allelic comparison model showed a low possibility of publication bias (Figure 7), which further supported by statistical evidences from Egger’s test ($t=0.297; p=0.776$) and Begg’s test ($z=0.742; p=0.458$). The results of Egger’s test and Begg’s test for comparison of heterozygous, homozygous, dominant and recessive models also revealed no evidence of publication bias (Table 2). Therefore, we concluded that no publication bias was detected in this meta-analysis.

There are several limitations that should be addressed in this meta-analysis. First, only eight studies with a total of 3650 breast cancer cases and 3607 controls were included in this meta-analysis, which is relatively small. Second, studies that included in this meta-analysis were only obtained from the PubMed database and limited to articles that published in English. Therefore, there is possible for studies that published in other databases or other languages that meet the inclusion criteria but did not include in this meta-analysis. Third, this meta-analysis did not perform subgroup analyses for ethnicity, menopausal
status, lifestyle and environmental factors that have been reported to associate with breast cancer risk (Farvid et al., 2016; Kim et al., 2016; White et al., 2016).

Figure 7. Funnel plot showing the association of CYP2E1 rs2031920 and rs3813867 polymorphisms with breast cancer risk under allelic comparison model (c2 vs. c1) with t=0.297 and p=0.776 in Egger’s test, and z=0.742 and p=0.458 in Begg’s test.

CONCLUSION

In conclusion, this meta-analysis revealed that the CYP2E1 rs2031920 and rs3813867 polymorphisms were not associated with breast cancer risk. A comprehensive meta-analysis by including the subgroup analyses such as ethnicity, menopausal status, lifestyle and environmental factors is needed to understand the interplay between these factors and CYP2E1 polymorphisms with the risk of breast cancer.

REFERENCES


