Is There Any Association between Glutathione S-transferases M1 and Glutathione S-transferases T1 Gene Polymorphisms and Endometrial Cancer Risk? A Meta-analysis

Abstract
Epidemiological evidence on the association between genetic polymorphisms in glutathione S-transferases M1 (GSTM1) and T1 (GSTM1) genes and risk of endometrial cancer (EC) has been inconsistent. In this meta-analysis, we seek to investigate the relationship between GSTM1 and GSTT1 polymorphisms and the risk of EC. We searched Medline, PubMed, Web of Science, Embase, Chinese National Knowledge Infrastructure database, and Chinese Biomedical Literature database to identify eligible studies. The pooled odds ratios (ORs) with 95% confidence intervals (CIs) for the association were determined using a fixed- or random-effect model. Tests for heterogeneity of the results and sensitivity analyses were performed. A total of six case–control studies were included in the final meta-analysis of GSTM1 (1293 cases and 2211 controls) and GSTT1 (1286 cases and 2200 controls) genotypes. Overall, GSTM1 null genotype was not significantly associated with an increased risk of EC (OR = 1.00, 95% CI = 0.76–1.30, P = 0.982). Similarly, for GSTT1 deletion genotype, we observed no association under the investigated model in the overall analysis (OR = 0.91, 95% CI = 0.64–1.30, P = 0.619). Subgroup analysis also showed no significant association between the GSTM1 null genotype and EC risk in hospital-based design (OR = 1.26, 95% CI = 0.93–1.71, P = 0.131) and no relationship between GSTT1 null genotype with EC risk in population-based design (OR = 1.18, 95% CI = 0.79–1.76, P = 0.407). However, GSTM1 null genotype contributed to an increased EC risk in population-based design (OR = 0.76, 95% CI = 0.60–0.97, P = 0.027), while null GSTT1 in hospital-based studies (OR = 0.70, 95% CI = 0.52–0.93, P = 0.015). The present meta-analysis suggested that GST polymorphisms may not be involved in the etiology of EC. Large epidemiological studies with the combination of GSTM1 null, GSTT1 null, and design-specific with the development of EC are needed to prove our findings.

Keywords: Endometrial cancer, glutathione S-transferases M1, glutathione S-transferases T1, meta-analysis, susceptibility

Introduction
Endometrial cancer (EC) is the most common type of uterine cancer and gynecologic cancer worldwide. Conventionally, the classification of EC risks is major composed of prolonged unopposed estrogen stimulation, partial estrogen against drugs such as tamoxifen, late menopause, nulliparity, and obesity. However, epidemiologic studies showed that the combined effect of environmental factors and genetic factors plays a critical role in the development of EC. Recent evidence, mainly from molecular genetic analyses, suggests that invasive cancer has a substantial hereditary component.

The glutathione S-transferases (GSTs) enzymes are important phase II isoenzyme group which conjugate a broad range of electrophilic xenobiotic and carcinogenic compounds to glutathione. It has been assumed that GST functional variants that are related to a less effective detoxification of potential carcinogens may contribute to increased cancer susceptibility. Meanwhile, cytosolic GSTs (glutathione S-transferases M1 [GSTM1] and glutathione S-transferases T1 [GSTT1]) play a significant role in human carcinogenesis.

Endometrial carcinoma is a common malignant tumor in women, in which relatively little attention has been given to genetic susceptibility factors. In 1997, Esteller et al. first reported the GSTM1 and GSTT1 polymorphisms as potential risk factors for EC.
molecular markers and found that GSTM1 null genotype was associated with an increased risk of endometrial carcinoma.\[4\] However, Doherty et al. confirmed that GSTT1 but not GSTM1 null genotype was associated with the risk of EC.\[5\] Subsequently, several studies also reported the potential association between GSTM1 and GSTT1 polymorphisms and the EC risk.\[6-9\] However, the data show conflicting results that remain to be further clarified. Therefore, we conducted this meta-analysis to determine whether the deletion of GSTM1, GSTT1 has an impact on EC susceptibility.

Methods

Literature search and selection criteria

We searched PubMed, Embase, ISI Web of Science, China National Knowledge Infrastructure database, and Chinese Biomedical Literature database to identify relevant publications. The following search terms were used: ("glutathione S-transferase" OR "GST" OR "GSTM1" OR "GSTT1") and ("endometrial cancer" OR "endometrial carcinoma"). Relevant studies of GSTM1 or GSTT1 polymorphism and EC risk published in English or Chinese were all retrieved. In addition, the related articles of the reference lists were also screened for useful data. The literature search was updated on June 30, 2016.

To get all eligible articles, the following inclusion criteria must be met: (a) cohort or case–control studies; (b) evaluating the relationship between GSTs (GSTM1 and GSTT1) and EC risk; (c) providing sufficient information to calculate pooling odds ratios (ORs) with 95% confidence intervals (CIs); (d) the published language must be in English or Chinese. The following information was extracted from each study: (a) name of the first author; (b) year of publication; (c) country of origin. Two authors independently assessed the articles for compliance with the inclusion criteria and resolved discrepancies by discussion until a consistent decision was reached. We used the modified Newcastle–Ottawa quality scale (NOS)\[10\] to assess the quality of each study.

Statistical analysis

The association between GSTM1 and GSTT1 polymorphisms and risk of EC was estimated by calculating pooled ORs with 95% CI under an additive model. The significance of pooled ORs was checked using the Z-test. Heterogeneity between studies was measured by $Q$ statistic ($P < 0.05$ represented significant results) and $I^2$ statistic ($I^2 < 25\%$, no heterogeneity; $25\% < I^2 < 50\%$, moderate heterogeneity; $50\% < I^2 < 75\%$, large heterogeneity, and $75\% < I^2 < 100\%$, extreme heterogeneity).\[11\] A random-(DeSimonian–Laird method)\[12\] or fixed-(Mantel–Haenszel method)\[13\] effect model was used to calculate the pooled OR in the presence ($P < 0.05$) or absence ($P > 0.05$) of heterogeneity, respectively. The following categories were conducted to find the source of heterogeneity: source of controls, ethnicity, study numbers, and quality score of the papers (NOS). The leave-one-out sensitivity analysis was performed to assess the stability of results. Publication bias was assessed by funnel’s plot\[14\] and Egger’s linear regression\[15\] ($P < 0.05$ was considered statistically significant). Statistical analyses were performed using STATA version 12.0 (StataCorp, College Station, TX, USA).

Results

Characteristics of the studies

A total of six published articles were identified to be eligible to be included for the final meta-analysis. A flowchart summarizing the selection details is depicted in Figure 1.

Of the six studies, three studies were population-based design, and the other three were based on the hospital. All studies investigated both GSTM1 and GSTT1 polymorphism and were based on Caucasians, except for one on Asians. For genotyping methods, three articles adopt polymerase chain reaction (PCR), while the other three were real-time PCR, PCR-restriction fragment length polymorphism, and multi-PCR, respectively. More detailed characteristics of the included studies are presented in Table 1.

![Figure 1: Flowchart of study selection](image-url)
Quantitative synthesis

Glutathione S-transferases M1 polymorphism

The association of GSTM1 polymorphism with risk of EC was estimated in 1293 cases and 2211 controls. The overall results showed no significant association of null GSTM1 carries with the EC risk (OR = 1.00; 95% CI = 0.76–1.30) [Figure 2a]. There was heterogeneity in studies (F = 64.8%, P = 0.014), and a random-effects model was used [Table 2].

Glutathione S-transferases T1 polymorphism

As for GSTT1 polymorphism, a total of 1286 cases and 2200 controls from the six studies were included. The overall results also showed no significant association between GSTT1 null genotype and EC risk (OR = 0.91, 95% CI = 0.64–1.30) [Figure 2b]. A random-effects model was introduced as there was significant heterogeneity in studies (F = 70.4%, P = 0.005) [Table 2].

Subgroup analysis

We performed meta-regression with the introduction of ethnicity (Caucasians vs. Asians), study design (population-based vs. hospital-based), number of cases (≥200 vs. <200), and the score quality of the papers (≥6 vs. ≤6) to identify the potential source of heterogeneity. Results showed that the study design was the only source that contributed to the heterogeneity. For GSTM1 null genotype, subgroup analysis results showed no significant association with EC in hospital-based design (OR = 1.26, 95% CI = 0.93–1.71, P = 0.131) [Figure 3a], whereas results showed no association of GSTT1 deletion with EC in population-based design (OR = 1.18, 95% CI = 0.79–1.76, P = 0.407) [Figure 3b]. Moreover, the heterogeneity dropped in both groups. However, there were significant associations of EC with the null of genotype GSTM1 in population-based studies

### Table 1: Characteristics of the publications identified for the meta-analysis

<table>
<thead>
<tr>
<th>Study, country</th>
<th>Ethnicity</th>
<th>Study design</th>
<th>Sample size (case/control)</th>
<th>Genotyping method</th>
<th>Genotype distribution (case/control)</th>
<th>NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esteller et al., 1997, Spain</td>
<td>Caucasian</td>
<td>Hospital</td>
<td>80/60</td>
<td>PCR</td>
<td>GSTM1 null: 51/28, GSTM1 present: 29/32, GSTT1 null: 19/12, GSTT1 present: 61/48</td>
<td>5</td>
</tr>
<tr>
<td>Ashton et al., 2010, Australia</td>
<td>Caucasian</td>
<td>Population</td>
<td>191/286</td>
<td>PCR</td>
<td>GSTM1 null: 84/161, GSTM1 present: 107/125, GSTT1 null: 107/125, GSTT1 present: 33/47</td>
<td>8</td>
</tr>
<tr>
<td>Du et al., 2010, China</td>
<td>Asian</td>
<td>Hospital</td>
<td>171/201</td>
<td>Multi-PCR</td>
<td>GSTM1 null: 109/114, GSTM1 present: 62/87, GSTT1 null: 70/109, GSTT1 present: 101/92</td>
<td>7</td>
</tr>
<tr>
<td>Karageorgi et al., 2011, USA</td>
<td>Caucasian</td>
<td>Hospital</td>
<td>441/1237</td>
<td>Real-time PCR</td>
<td>GSTM1 null: 232/621, GSTM1 present: 195/556, GSTT1 null: 64/239, GSTT1 present: 356/927</td>
<td>5</td>
</tr>
</tbody>
</table>

PCR=Polymerase chain reaction, RFLP=Restriction fragment length polymorphism, NOS=Newcastle-Ottawa quality scale, GSTM1=Glutathione S-transferases M1, GSTT1=Glutathione S-transferases T1

[Figure 2: (a) Forest plot for the overall association between glutathione S-transferases M1 null genotype and endometrial cancer risk. (b) Forest plot for the overall association between glutathione S-transferases T1 null genotype and endometrial cancer risk]
The facts that the GSTs have broad and overlapping substrate specificities and that allelic variants associated with less effective detoxification of potential carcinogens may confer an increased susceptibility to cancer.\[16,17\] Previous research showed conflicting results that cannot reach an agreement.\[4-9\] We performed this meta-analysis to clarify new developments and produce more powerful estimation on the association between the GSTs gene polymorphisms and EC susceptibility. With more comprehensive data, the analysis showed no significant association between the GSTs and EC susceptibility.

Ozerkan \textit{et al.} reported that GSTM1 and GSTT1 null genotype was not related with EC and no association could be demonstrated between GSTM1 and GSTT1 polymorphisms and clinical stages of EC as well.\[9\] Karageorgi \textit{et al.} found that GSTM1 copy did not influence GSTT1 null genotype was not related with EC and no association could be demonstrated between GSTM1 and GSTT1 polymorphisms and clinical stages of EC as well.\[9\] Karageorgi \textit{et al.} found that GSTM1 copy did not influence GSTT1 null genotype and endometrial cancer risk. It is believed that the null genotypes decrease the enzymatic activity and have been proposed to increase cancer risk due to the inability to detoxify metabolites of estrogen.\[6\] Our studies revealed no association between GSTM1/GSTT1 and EC risk. Interestingly, Ashton \textit{et al.} found that the deletion of GSTM1 was associated with a decrease in EC risk. It is believed that the null genotypes decrease the enzymatic activity and have been proposed to increase cancer risk due to the inability to detoxify metabolites of estrogen.\[6\] Our studies revealed no association between GSTM1/GSTT1 and EC risk.

\section*{Discussion}

Table 2: Meta-analysis of the association between glutathione S-transferases M1, glutathione S-transferases T1 polymorphisms, and the risk of endometrial cancer

<table>
<thead>
<tr>
<th>SNP</th>
<th>Null versus present</th>
<th>Study sample (case/control)</th>
<th>OR</th>
<th>95% CI</th>
<th>Heterogeneity</th>
<th>$P_z$</th>
<th>$P_E$</th>
<th>$P_B$</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1</td>
<td>Total</td>
<td>1293/2211</td>
<td>1.00</td>
<td>0.76-1.30</td>
<td>64.80</td>
<td>0.014</td>
<td>0.982</td>
<td>0.677</td>
</tr>
<tr>
<td>Subgroup-analysis</td>
<td>Hospital-based</td>
<td>678/1438</td>
<td>1.26</td>
<td>0.93-1.71</td>
<td>43.10</td>
<td>0.173</td>
<td>0.131</td>
<td>0.093</td>
</tr>
<tr>
<td></td>
<td>Population-based</td>
<td>615/773</td>
<td>0.76</td>
<td>0.60-0.97</td>
<td>13.00</td>
<td>0.317</td>
<td>0.027*</td>
<td>0.759</td>
</tr>
<tr>
<td>GSTT1</td>
<td>Total</td>
<td>1286/2200</td>
<td>0.91</td>
<td>0.64-1.30</td>
<td>70.40</td>
<td>0.005*</td>
<td>0.619</td>
<td>0.641</td>
</tr>
<tr>
<td>Subgroup-analysis</td>
<td>Hospital-based</td>
<td>671/1427</td>
<td>0.70</td>
<td>0.52-0.93</td>
<td>24.20</td>
<td>0.268</td>
<td>0.015*</td>
<td>0.317</td>
</tr>
<tr>
<td></td>
<td>Population-based</td>
<td>615/773</td>
<td>1.18</td>
<td>0.79-1.76</td>
<td>44.10</td>
<td>0.167</td>
<td>0.407</td>
<td>0.163</td>
</tr>
</tbody>
</table>

*Statistical significance. $P_z$=P for Z-test, $P_E$=P for Egger’s test, $P_B$=P for Begg’s test. SNP=Single nucleotide polymorphism, OR=Odds ratio, 95% CI=95% confidence interval, GSTM1=Glutathione S-transferases M1, GSTT1=Glutathione S-transferases T1

Figure 3: (a) Relationship between glutathione S-transferases M1 null genotype and endometrial cancer risk by study design. (b) Relationship between glutathione S-transferases T1 null genotype and endometrial cancer risk by study design

(OR = 0.76, 95% CI = 0.60-0.97, $P = 0.027$) and with the null genotype of GSTT1 polymorphism in hospital-based studies (OR = 0.70, 95% CI = 0.52-0.93, $P = 0.015$). More details are presented in Table 2.

\section*{Sensitivity analysis and potential publication bias}

We performed a sensitivity analysis by excluding sequentially one study at a time to inspect the stability and reliability of our meta-analysis results. The recalculated ORs and 95% CIs did not change significantly, suggesting the results were robust and convincing. Publication bias was measured by both funnel plot and Egger’s test. Results are shown in Figure 4a and b. The outlines did not reveal any evidence for publication bias (all $P > 0.05$). Details are shown in Table 2.
Yin and Chen: The relationship between GSTM1, GSTT1, and endometrial cancer risk

in hospital-based design, and the heterogeneity dropped in both subgroups. The results were similar to some previous studies and simultaneously inconsistent to other studies. Therefore, it is more likely that the inconsistent results among those studies were attributed to study design.

The present meta-analysis included 1307 cases and 2271 controls, which increased the statistical power. Pooled results revealed that GSTs had no association with the risk of EC, which was consistent with the reports from Ozerkan et al. To determine the potential causes for the instability of the results, we performed subgroup analysis by source of the control. Results showed similar nonsignificant differences in hospital-based design of GSTM1 deletion and population-based design of GSTT1 deletion. However, results also showed that GSTM1 null polymorphism was significantly associated with EC risk in hospital-based design. The exact mechanisms of all GSTs are not clear; however, it appears that GSTs deactivate metabolites of estrogen formed during phase II estrogen metabolism. The null genotypes decrease the enzymatic activity and have been proposed to increase cancer risk due to the inability to detoxify metabolites of estrogen.

Moreover, the GSTs consist of seven classes and 60% of them are homology; therefore, it is possible that other GSTs catalyze the conjugation of glutathione of a wide variety of metabolites. In other words, the nonsignificant relationship between GSTM1 and GSTT1 deletion genotype and EC implies that a role of functional genes might also be involved in activating compounds acted as endometrial carcinogens. In addition, the effects might also be blinded by chance or other unidentified factors involved in the endometrial carcinogenesis. Hence, our results should be interpreted with caution.

However, our study also has limitations. First, the subjects were mainly Caucasians, which may impose restrictions on applied to other ethnicities. Second, due to the limited sample size, it might not provide sufficient power to detect the weak association between the null GSTM1 and GSTT1 polymorphism and EC risk. Third, complex environmental factors also played critical roles in developing carcinoma, but we could not evaluate the interactive effect between genes and environment in our meta-analysis. Fourth, the present meta-analysis found no association between the GSTs genetic polymorphisms that may contribute to EC risk, but subgroup analysis did, and there were heterogeneity in the overall results and subgroup analysis, which might impair the stability of the study. In addition, the presence of other unidentified causal factors involved in the EC development, a more precise evaluation needs to be adjusted by other covariants if available. All those potential factors might affect the results of our meta-analysis.

**Conclusions**

On the basis of the statistical evidence, results from this meta-analysis showed that both GSTM1 genotype and GSTT1 genotype had no significant association with the development of EC. However, stratifying analysis by study design showed GSTM1 null genotype has a moderately strong association with EC risk in population-based design, while GSTT1 null genotype shows moderately increased risk for EC with no heterogeneity (both $\hat{P} < 25\%$).
Furthermore, epidemiological studies considering large sample size, well-matched controls, standardized unbiased genotyping methods would be required to precisely estimate the effect size of the association between EC risk and GSTM1 and GSTT1 polymorphisms.

Financial support and sponsorship
This work is supported by Natural Science Foundation of China named “The research about the role of EFEMP1 in ovarian cancer invasion, metastasis, and angiogenesis” (No: 81202056).

Conflicts of interest
There are no conflicts of interest.

Received: 29 Oct 15 Accepted: 28 Feb 17 Published: 23 Jun 17

References