Association between glutathione S-transferase M1/T1 gene polymorphisms and susceptibility to endometriosis: A systematic review and meta-analysis

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Abstract. Endometriosis is a polygenic/multifactorial disease caused by interactions between multiple genes and the environment. Findings from studies evaluating the association between the glutathione S-transferase (GST) M1/T1 null genotype and susceptibility to endometriosis are inconsistent. This meta-analysis updated and reevaluated the possible associations between GSTM1, GSTT1 and combined GSTM1/GSTT1 (null genotype versus wild-type) gene polymorphisms and susceptibility to endometriosis. The PubMed, Embase and Chinese BioMedical Literature databases and Google Scholar were searched for case-control genetic association studies on GSTM1/GSTT1 (null genotype versus wild-type) gene polymorphisms and endometriosis in comparison with non-endometriosis or healthy controls. Fixed-effect and random-effect meta-analytical techniques were conducted for the outcome measure and subgroup analyses. The meta-analysis demonstrated significant associations between the GSTM1 [odds ratio (OR)=1.56; 95% confidence interval (CI): 1.25-1.95; P<0.0001], GSTT1 (OR=1.31; 95% CI: 1.02-1.68; P=0.037) and GSTM1/GSTT1 (OR=1.68; 95% CI: 1.29-2.17; P<0.0001) null genotypes and increased risk for endometriosis. The results suggest that the GSTM1, GSTT1, and combined GSTM1/GSTT1 null genotypes increase susceptibility to endometriosis. Additional well-designed studies and precise analyses are warranted to confirm these findings.

Introduction

Endometriosis manifests as ectopic endometrial tissues outside the uterus. It is an intractable disease that causes infertility, dysmenorrhea and pelvic pain. Endometriosis occurs in 10% of women of childbearing age. Notably, the incidence of endometriosis has been rising in recent years (1). The pathogenesis of endometriosis remains to be elucidated.

Published reports indicate that endometriosis is a polygenic/multifactorial disease caused by interactions between multiple genes and the environment (2,3). In particular, a correlation has been identified between endometriosis and exposure to environmental toxins such as dioxin (4); dioxin and dioxin-like compounds have been implicated in the development of endometriosis (5,6). The phase II conjugation enzymes usually function to inactivate environmental toxins. Among these, glutathione S-transferase (GST) may be critical for the detoxification of dioxins. Human GSTs are classified into two distinct categories: Soluble or cytosolic and membrane-bound microsomal. The soluble or cytosolic GSTs are subdivided into seven families named α, µ, ω, π, σ, θ and ζ (7). Genes in several of these families are polymorphic, including: GSTA2 in the α family, GSTM1 and GSTM3 in the µ family, GSTP1 in the π family, GSTO, GSTT1, and GSTT2 in the θ family, and GSTZ1 in the ζ family. Heritable allelic differences in GSTM1, GSTM3, GSTT1 and GSTP1 may have marked relevance for individual susceptibility to disease. GSTM1 and GSTT1 are two candidate genes that may play an important role in the development of endometriosis. GSTM1 and GSTT1 are located on chromosomes 1p13.3 and 22q11.23, respectively. They are critical in the detoxification of the products of oxidative stress produced during the repair of the ovarian epithelium. GSTM1 and GSTT1 null alleles have reduced enzyme activity, a state that may contribute to inefficient detoxification of intermediates produced during stress. This may increase damage to various host genes and contribute to the pathogenesis of endometriosis (8,9).

A meta-analysis summarizing the literature up to the year 2005 suggested that the GSTT1 null genotype, but not the
GSTMI null genotype, was associated with an increased risk for endometriosis (7). In the years since 2005, additional reports investigating this topic have been published. The objective of the present study was to update the existing meta-analysis and reevaluate the possible associations between GSTMI, GSTT1 and combined GSTMI/GSTT1 (null genotype vs. wild-type) gene polymorphisms and susceptibility to endometriosis.

Materials and methods

Searches. For this systematic review and meta-analysis, PubMed (from January 1996 to January 2014), Embase (from January 1996 to January 2014), Chinese BioMedical Literature database (from January 1996 to January 2014) and Google Scholar (from January 1996 to January 2014) were searched. The following keywords were used: 'endometriosis', 'polymorphisms', 'glutathione S-transferases', 'GSTMI' and 'GSTT1' or their combinations.

Reference lists from articles identified by the electronic search were searched by hand. This process was performed iteratively until no additional articles could be identified.

Inclusion and exclusion criteria. Articles published in English or Chinese were included if they reported quantitative outcomes from case-control genetic association studies on GSTMI, GSTT1 or combined GSTMI/GSTT1 (null genotype vs. wild-type) gene polymorphisms and endometriosis versus non-endometriosis or healthy controls.

Studies were excluded if they were case reports, case-only studies, letters, reviews or meta-analyses; included subjects who were related; included cases of adenomyosis, which has unknown etiology (10); reported insufficient data; or were duplicate studies.

Selection of studies. Two reviewers (XYX and ZSJ) independently examined titles and abstracts to select eligible studies. Records were removed that were ongoing or unpublished studies, or were published as abstracts or conference proceedings. Where data sets were overlapping or duplicated, only the most recent information was included. The full text of potentially relevant studies was retrieved. Two reviewers (XYX and HJG) independently examined the full text records to determine which studies met the inclusion criteria. Disagreement about the selection of studies was resolved by discussion and consensus.

Data extraction and management. Two reviewers (XYX and ZSJ) independently extracted data from eligible studies including the first author's last name, publication year, study location, ethnicity, matching variability, diagnostic criteria, stages of disease, source of controls, numbers of cases and controls, and numbers and/or percentages of null genotypes. Disagreement about data extraction was resolved by discussion and consensus.

Assessment of quality of evidence in included studies. Two reviewers (YYL and HJG) independently assessed quality of evidence in the included studies using the 9-star Newcastle-Ottawa Scale, which considers selection, comparability and outcome evaluation criteria.

Results

Screening and selection. The searches identified 120 articles. Titles and abstracts were screened, and 36 studies were identified as potentially eligible for inclusion. The full text articles for these studies were retrieved. Following analysis of the full text articles, four studies were excluded and 32 studies were found to be eligible for inclusion according to the criteria used for considering studies in this review (Fig. 1).

Included studies. The characteristics of the included studies are shown in Table 1. There were 32 case-control genetic association studies involving 3,990 cases of endometriosis and 4,625 controls. One publication addressed two groups of subjects with different ethnicities and was considered as two case-control genetic association studies (12); thus, the total number of studies was considered to be 33. Studies included data relevant to the GSTMI genotype, GSTT1 genotype or the combined GSTMI/GSTT1 genotype. Of the 32 eligible studies, 20 were conducted in Asia (12-31), eight in Europe (32-39), two in North America (40,41), and two in South America (42,43). The evidence reported in 23 studies was identified as high-quality, and that in 10 studies was identified as low-quality.

Assessment of heterogeneity. Heterogeneity was assessed using the χ² test and I² test. The I² statistic was interpreted as follows: I²=0-40%, heterogeneity may not be important; I²= 30-60%, heterogeneity may be moderate; I²=50-90%, heterogeneity may be substantial; and I²=75-100%, considerable heterogeneity (11). If heterogeneity was present, meta-regression was used to find the source.

Assessment of reporting biases. A funnel plot of effect estimates against their standard errors (SEs) was created to assess possible reporting bias between studies. Funnel plot asymmetry was assessed using Egger's linear regression test and Begg's rank correlation test; P<0.05 suggested publication bias.

GSTMI/GSTT1 and risk for endometriosis. Two reviewers (XYX and HJG) independently combined data from trials using a fixed-effect model (DerSimonian and Laird method) when there was no significant heterogeneity in populations (I²<50%) and a random-effect model (Mantel-Haenszel method) when there was considerable heterogeneity. Variables were synthesized using odds ratios (ORs). A P-value of 0.05 was used as the cut-off value to determine statistical significance, and data are presented as the estimated OR with 95% confidence intervals (CIs). All statistical analyses were performed using STATA software, version 12.0 (StataCorp, College Station, TX, USA). Inconsistencies in data analysis were resolved through consensus and discussion with a third reviewer (ZSJ).

Sensitivity and subgroup analyses. Sensitivity analyses were performed to explore the impact of excluding outlying results. Subgroup analyses were performed by stratifying patients according to ethnicity (Caucasian, Asian or mixed), characteristics of controls (hospital patients or healthy individuals), and quality of evidence (high-quality or low-quality).
Excluded studies. Of the 36 studies that were relevant to the GSTM1/GSTT1 genotype and endometriosis, four were excluded. Of these, three were duplicates (13,14,32), and one included subjects who were related (44).

GSTM1/GSTT1 and risk for endometriosis

GSTM1 genotype. Data reporting on the GSTM1 gene polymorphism are described in 33 case-control studies (3,990 cases of endometriosis and 4,625 controls). The meta-analysis demonstrated that there was a significant association between the GSTM1 null genotype and an increased risk for endometriosis (OR=1.56; 95% CI: 1.25-1.95; P<0.0001; Fig. 2A).

Subgroup analyses stratified by ethnicity (Caucasian: OR=1.599; 95% CI: 1.205-2.122; P=0.001; Asian: OR=1.772; 95% CI: 1.242-2.528; P=0.002), source of controls (hospital patients: OR=1.561; 95% CI: 1.151-2.117; P=0.004; healthy individuals: OR=1.569; 95% CI: 1.131-2.176; P=0.007), and quality of evidence (high-quality: OR=1.563; 95% CI: 1.253-1.949; P<0.0001) confirmed this finding.

Subgroup analysis stratified for mixed ethnicity (two case control studies involving 111 cases of endometriosis and 78 controls) demonstrated a significant association between the GSTM1 null genotype and a decreased risk for endometriosis (OR=0.404; 95% CI: 0.219-0.745; P=0.004; Table II). Compared with individual Caucasian and Asian populations, the difference was statistically significant (P<0.001; data shown in Table III).

GSTT1 genotype. Data reporting on the GSTT1 gene polymorphism are described in 18 case-control studies (2,371 cases of endometriosis and 2,490 controls). The meta-analysis demonstrated a significant association between the GSTT1 null genotype and an increased risk for endometriosis (OR=1.31; 95% CI: 1.02-1.68; P=0.037; Fig. 2B).

Subgroup analysis stratified by ethnicity demonstrated a significant association between the GSTT1 null genotype and an increased risk for endometriosis among Asians (OR=1.573; 95% CI: 1.186-2.085; P=0.002), but not among Caucasians (OR=1.124; 95% CI: 0.745-1.697; P=0.577).

Subgroup analyses stratified by the source of controls found no significant association between the GSTT1 null genotype and an increased risk for endometriosis among hospital-based studies (OR=1.284; 95% CI: 0.963-1.712; P=0.089) or among healthy individuals (OR=1.315; 95% CI: 0.767-2.254; P=0.320).

Subgroup analyses stratified by quality of evidence demonstrated a significant association between the GSTT1 null genotype and an increased risk for endometriosis among studies considered high-quality evidence (OR=1.376; 95% CI: 1.020-1.858; P=0.037), but not among studies considered low-quality evidence (OR=1.121, 95% CI: 0.646-1.944; P=0.684; Table II).

Combined GSTM1/GSTT1 genotype. Data reporting on the combined GSTM1/GSTT1 gene polymorphism are described in eight case-control studies (1,083 cases of endometriosis and 1,222 controls). The meta-analysis demonstrated a significant
Table I. Characteristics of included studies on the *GSTM1*, *GSTT1* and combined *GSTM1/GSTT1* gene polymorphisms.

| First author, year | Ethnicity | Countries | Source of controls          | Quality | Cases/controls | Cases/controls | Cases/controls | Cases/controls | Refs. |
|--------------------|-----------|-----------|-----------------------------|---------|----------------|----------------|----------------|----------------|-------|
| Baranov, 1996      | Caucasian | Russia    | Healthy individuals         | Low     | 42/34          | 67/26          |                |                | (33)  |
| Baranova, 1999     | Caucasian | Russia, France | Hospital patients         | High    | 65/50          | 72/33          |                |                | (32)  |
| Baranov, 1999      | Caucasian | Russia    | Healthy individuals         | Low     | 150/88         | 99/42          |                |                | (34)  |
| Hadfield, 2001     | Caucasian | UK        | Hospital patients          | High    | 132/59         | 52/27          | 116/29         | 50/14          | (35)  |
| Baxter, 2001       | Caucasian | England   | Healthy individuals         | High    | 84/40          | 219/107        |                |                | (36)  |
| Bischoff, 2002     | Caucasian | USA       | Hospital patients          | Low     | 62/13          | 36/20          |                |                | (40)  |
| Ivaschenko, 2003   | Caucasian | Russia    | Hospital patients          | High    | 74/42          | 40/17          | 74/27          | 40/6           | (37)  |
| Arvanitis, 2003    | Caucasian | Greece    | Healthy individuals         | High    | 275/161        | 346/181        | 275/24         | 346/31         | (38)  |
| Peng, 2003         | Asian     | China     | Hospital patients          | High    | 76/50          | 80/37          |                |                | (15)  |
| Lin, 2003          | Asian     | China     | Hospital patients          | High    | 68/49          | 28/12          | 68/53          | 28/9           | (16)  |
| Morizane, 2004     | Asian     | Japan     | Healthy individuals         | Low     | 108/57         | 173/89         | 108/52         | 173/71         | (17)  |
| Hsieh, 2004        | Asian     | China     | Hospital patients          | High    | 150/95         | 159/8          |                |                | (18)  |
| De Carvalho, 2004  | Mixed     | Brazil    | Hospital patients          | Low     | 61/21          | 32/17          |                |                | (42)  |
| Ding, 2004         | Asian     | China     | Healthy individuals         | High    | 80/46          | 105/55         | 80/59          | 105/47         | (12)  |
| Ding, 2004         | Asian     | China     | Healthy individuals         | High    | 41/21          | 107/57         | 41/15          | 107/32         | (41)  |
| Babu, 2005         | Caucasian | India     | Hospital patients          | High    | 310/121        | 215/64         | 310/42         | 215/34         | (19)  |
| Hur, 2005          | Asian     | Korea     | Hospital patients          | Low     | 194/112        | 259/145        | 194/104        | 259/125        | (20)  |
| Aban, 2007         | Caucasian | Turkey    | Hospital patients          | High    | 150/88         | 150/65         | 150/59         | 150/44         | (21)  |
| Chang, 2007        | Asian     | China     | Hospital patients          | High    | 74/48          | 65/30          | 74/46          | 65/32          | (30)  |
| Kim, 2007          | Asian     | Korea     | Hospital patients          | High    | 316/183        | 256/146        | 316/178        | 256/124        | (22)  |
| Rozati, 2009       | Caucasian | India     | Hospital patients          | High    | 97/26          | 102/15         |                |                | (13)  |
| Yang, 2009         | Asian     | China     | Hospital patients          | Low     | 216/134        | 216/100        |                |                | (14)  |
| Cao, 2009          | Asian     | China     | Hospital patients          | High    | 51/33          | 102/61         | 51/22          | 102/39         | (23)  |
| Wu, 2009           | Asian     | China     | Hospital patients          | High    | 96/63          | 85/40          |                |                | (24)  |
| Huang, 2010        | Asian     | China     | Hospital patients          | High    | 28/12          | 29/10          |                |                | (25)  |
| Trabert, 2011      | Caucasian | USA       | Healthy individuals         | High    | 254/137        | 567/268        |                |                | (41)  |
| Hosseinzadeh, 2011 | Caucasian | Iran      | Healthy individuals         | High    | 120/87         | 200/80         |                |                | (26)  |
| Wu, 2012           | Asian     | China     | Healthy individuals         | low     | 121/57         | 171/52         | 121/40         | 171/33         | (27)  |
| Seifati, 2012      | Caucasian | Iran      | Hospital patients          | High    | 101/51         | 142/74         |                |                | (28)  |
| Vichi, 2012        | Caucasian | Italy     | Hospital patients          | High    | 181/104        | 162/85         | 181/20         | 162/32         | (39)  |
| Matsuzaka, 2012    | Asian     | Japan     | Hospital patients          | High    | 97/43          | 143/67         | 97/38          | 143/56         | (29)  |
| Frare, 2013        | Mixed     | Brazil    | Healthy individuals         | Low     | 50/25          | 46/34          | 50/16          | 46/27          | (43)  |
| Sachan, 2013       | Caucasian | Iran      | Healthy people              | Low     | 66/27          | 100/16         |                |                | (31)  |

Null genotype vs. wild type gene polymorphisms and susceptibility to endometriosis. *GSTM1*, glutathione S-transferase µ1; *GSTT1*, glutathione S-transferase θ1.
association between the combined \textit{GSTM1}/\textit{GSTT1} null genotype and an increased risk for endometriosis (OR=1.68, 95% CI: 1.29-2.17; P<0.0001; Fig. 2C).

This association was unchanged by subgroup analyses stratified by source of controls (hospital-based studies: OR=1.797; 95% CI: 1.082-2.989; P=0.024; healthy individuals: OR=1.569 [1.131-2.176]; P=0.007) or quality of evidence (high-quality evidence: OR=1.753; 95% CI: 1.265-2.430; P=0.001; low-quality evidence: OR=1.542; 95% CI: 1.009-2.356; P=0.045; Table II).

Subgroup analysis stratified by ethnicity demonstrated a significant association between the combined \textit{GSTM1}/\textit{GSTT1} null genotype and an increased risk for endometriosis among Asian populations (OR=1.898; 95% CI: 1.404-2.565; P<0.001), but not among Caucasian populations (OR=1.185; 95% CI: 0.717-1.961; P=0.508).

Publication bias. Visual inspection of a Funnel plot, Egger's test and Begg's rank correlation test revealed no significant publication bias for the \textit{GSTM1}, \textit{GSTT1} and combined \textit{GSTM1}/\textit{GSTT1} studies (Fig. 3; Table IV).

Heterogeneity analysis. There was evidence of significant heterogeneity (I^2>50%) between studies of \textit{GSTM1} and \textit{GSTT1}, and those used in subgroup analyses, although not among studies of \textit{GSTM1}/\textit{GSTT1} combined (Table IV). Therefore, the random-effect model was used in all analyses with the exception of the analysis of combined \textit{GSTM1}/\textit{GSTT1} gene polymorphisms. For the \textit{GSTM1} and \textit{GSTT1} gene polymorphisms, a meta-regression was conducted in which publication year, ethnicity, source of controls, sample size, and quality of evidence were covariates. All the covariates were entered into the meta-regression model simultaneously,
Figure 2. Association between GSTM1, GSTT1 and the combined GSTM1/GSTT1 null genotypes and susceptibility to endometriosis. (A) A total of 33 studies described the association between the GSTM1 null genotype and susceptibility to endometriosis [odds ratio (OR)=1.56; 95% confidence interval (CI): 1.25-1.95; P<0.0001]; and (B) 18 studies described the association between the GSTT1 null genotype and susceptibility to endometriosis (OR=1.31; 95% CI: 1.02-1.68; P=0.037). GSTM1, glutathione S-transferase µ1; GSTT1, glutathione S-transferase θ1.
and the covariates that had the highest P-values were omitted one at a time in order to identify any sources of heterogeneity among them. However, the meta-regression analysis did not identify any of these covariates as a significant source of heterogeneity (Figs. 4 and 5).

**Sensitivity analysis.** To explore the effects of individual studies on the pooled OR estimates, a sensitivity analysis was performed, with the omission of one study at a time. The OR estimates for the *GSTM1* polymorphism were not notably altered (Fig. 6A). The OR estimates for the *GSTT1* and

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**Figure 2. Continued.** (C) Eight studies described the association between the combined *GSTM1/GSTT1* null genotypes and susceptibility to endometriosis [odds ratio (OR)=1.68; 95% confidence interval (CI): 1.29-2.17; P<0.0001]. GSTM1, glutathione S-transferase μ1; GSTT1, glutathione S-transferase θ1.

**Figure 3. Assessment of publication bias for studies on (A) *GSTM1*, (B) *GSTT1* and (C) combined *GSTM1/GSTT1* genotypes. GSTM1, glutathione S-transferase μ1; GSTT1, glutathione S-transferase θ1.
### Table III. Comparisons of subgroup analyses for GSTM1, GSTT1 and combined GSTM1/GSTT1 studies.

#### A. Analysis of the GSTM1 gene

| Subgroup                | Subjects | GSTM1 (n) |  |  |  |  |
|-------------------------|----------|-----------|---|---|---|---|
|                         |          | Null      | Normal | $\chi^2$ | P-value |
| **Ethnicity**           |          |           |         |           |         |
| Caucasian               | Cases    | 1,128     | 1,035   | 3.245     | 0.72a   |
|                         | Controls | 1,120     | 1,449   |           |         |
| Asian                   | Cases    | 1,003     | 713     |           |         |
|                         | Controls | 909       | 1,069   |           |         |
| **Mixed**               | Cases    | 46        | 65      | 18.737    | <0.001b |
|                         | Controls | 51        | 27      | 23.467    | <0.001c |
| **Source of controls**  |          |           |         |           |         |
| Hospital patients       | Cases    | 1,397     | 1,202   | 0.130     | 0.718   |
|                         | Controls | 1,073     | 1,352   |           |         |
| Healthy individuals     | Cases    | 780       | 611     |           |         |
|                         | Controls | 1,007     | 1,193   |           |         |
| **Quality**             |          |           |         |           |         |
| High quality            | Cases    | 1,609     | 1,311   | 0.825     | 0.364   |
|                         | Controls | 1,539     | 1,887   |           |         |
| Low quality             | Cases    | 568       | 502     |           |         |
|                         | Controls | 541       | 658     |           |         |

#### B. Analysis of the GSTT1 gene

| Subgroup                | Subjects | GSTT1 (n) |  |  |  |  |
|-------------------------|----------|-----------|---|---|---|---|
|                         |          | Null      | Normal | $\chi^2$ | P-value |
| **Ethnicity**           |          |           |         |           |         |
| Caucasian               | Cases    | 214       | 957     | 6.766     | 0.009   |
|                         | Controls | 168       | 867     |           |         |
| Asian                   | Cases    | 607       | 543     |           |         |
|                         | Controls | 568       | 841     |           |         |
| **Source of controls**  |          |           |         |           |         |
| Hospital patients       | Cases    | 631       | 1,065   | 0.638     | 0.425   |
|                         | Controls | 522       | 1,020   |           |         |
| Healthy individuals     | Cases    | 206       | 469     |           |         |
|                         | Controls | 241       | 707     |           |         |
| **Quality**             |          |           |         |           |         |
| High quality            | Cases    | 625       | 1,273   | 0.062     | 0.803   |
|                         | Controls | 507       | 1,334   |           |         |
| Low quality             | Cases    | 212       | 261     |           |         |
|                         | Controls | 256       | 393     |           |         |

#### C. Analysis of GSTM1+GSTT1 genes

| Subgroup                | Subjects | GSTM1+GSTT1 (n) |  |  |  |  |
|-------------------------|----------|-----------------|---|---|---|---|
|                         |          | GSTM1+GSTT1     | $\chi^2$ | P-value |
| **Ethnicity**           |          | (n)             |           |         |
| Caucasian               | Cases    | 41              | 618       | 7.642    | 0.006   |
|                         | Controls | 29              | 572       |           |         |
Table III. Continued.

| Subgroup          | Subjects | Null | Normal | χ²          | P-value |
|-------------------|----------|------|--------|-------------|---------|
| Asian             | Cases    | 124  | 300    |             |         |
|                   | Controls | 109  | 152    |             |         |
| Source of controls|          |      |        | 0.091       | 0.763   |
| Hospital patients | Cases    | 57   | 401    |             |         |
|                   | Controls | 26   | 294    |             |         |
| Healthy individuals| Cases    | 108  | 517    |             |         |
|                   | Controls | 112  | 790    |             |         |
| Quality           |          |      |        | 0.022       | 0.882   |
| High quality      | Cases    | 112  | 542    |             |         |
|                   | Controls | 80   | 598    |             |         |
| Low quality       | Cases    | 53   | 176    |             |         |
|                   | Controls | 58   | 286    |             |         |

*Caucasians vs. Asians,* *Caucasians vs. mixed,* *Asians vs. mixed.*

Table IV. Heterogeneity and publication bias of GSTM1, GSTT1 and combined GSTM1/GSTT1 studies.

| Group                              | Heterogeneity | Publication bias (P-value) |
|------------------------------------|---------------|---------------------------|
|                                    | F² value (%)  | P-value                   | Egger's test | Begg's funnel plot |
| Total studies                      |               |                          |             |                   |
| GSTM1 genotype                     | 81.8          | <0.001                    | 0.313        | 0.412             |
| GSTT1 genotype                     | 69.9          | <0.001                    | 0.557        | 0.705             |
| GSTM1+GSTT1 genotype               | 44.7          | 0.081                     | 0.170        | 1.000             |
| Caucasian                          |               |                          |             |                   |
| GSTM1 genotype                     | 79.2          | <0.001                    | 0.454        | 0.322             |
| GSTT1 genotype                     | 64.7          | 0.009                     | 0.339        | 0.764             |
| GSTM1+GSTT1 genotype               | 58.5          | 0.090                     | 0.021        | 0.296             |
| Asian                              |               |                          |             |                   |
| GSTM1 genotype                     | 83.8          | <0.001                    | 0.098        | 0.083             |
| GSTT1 genotype                     | 62.4          | 0.004                     | 0.160        | 0.210             |
| GSTM1+GSTT1 genotype               | 13.6          | 0.081                     | 0.340        | 0.806             |
| Mixed                              |               |                          |             |                   |
| GSTM1 genotype                     | 0.0           | 0.664                     | <0.001       | 0.317             |
| Controls from hospital patients    |               |                          |             |                   |
| GSTM1 genotype                     | 83.6          | <0.001                    | 0.390        | 0.506             |
| GSTT1 genotype                     | 65.9          | 0.001                     | 0.335        | 0.451             |
| GSTM1+GSTT1 genotype               | 62.2          | 0.071                     | 0.585        | 1.000             |
| Controls from healthy individuals  |               |                          |             |                   |
| GSTM1 genotype                     | 79.4          | <0.001                    | 0.598        | 0.784             |
| GSTT1 genotype                     | 78.4          | <0.001                    | 0.431        | 0.707             |
| GSTM1+GSTT1 genotype               | 45.9          | 0.116                     | 0.531        | 1.000             |
| High quality                       |               |                          |             |                   |
| GSTM1 genotype                     | 80.9          | <0.001                    | 0.042        | 0.068             |
| GSTT1 genotype                     | 69.9          | <0.001                    | 0.530        | 0.189             |
| GSTM1+GSTT1 genotype               | 49.0          | 0.081                     | 0.641        | 1.000             |
| Low quality                        |               |                          |             |                   |
| GSTM1 genotype                     | 85.1          | <0.001                    | 0.788        | 0.516             |
| GSTT1 genotype                     | 77.2          | 0.004                     | 0.347        | 1.000             |
| GSTM1+GSTT1 genotype               | 62.9          | 0.101                     |             |                   |

GSTM1, glutathione S-transferase µ1; GSTT1, glutathione S-transferase θ1.
Figure 4. Meta-regression for GSTM1 studies, with publication year, ethnicity, source of controls, sample size, and quality of evidence as covariates. All covariates were entered into the meta-regression model simultaneously, and the covariates with the highest P-values were omitted one at a time to identify sources of heterogeneity. The meta-regression did not identify any of these covariates as a significant source of heterogeneity. Variables were omitted in the following order: Size (A→B), source (B→C), publication year (C→D), ethnicity (D→E). GSTM1, glutathione S-transferase µ1.
Figure 5. Meta-regression for GSTT1 studies, with publication year, ethnicity, source of controls, sample size, and quality of evidence as covariates. All covariates were entered into the meta-regression model simultaneously, and covariates with the highest P-values were omitted one at a time to identify sources of heterogeneity. Meta-regression identified publication year as a significant source of heterogeneity (P=0.048), but after omitting this covariate heterogeneity remained substantial ($I^2=67.21\%$). Variables were omitted in the order: Source (A→B), quality (B→C), ethnicity (C→D), size (D→E). GSTT1, glutathione S-transferase θ1.

| A | meta-reg logr quality ethnicity source publish size, wese(s_eislogS) hest(reml) |
|---|---|---|---|---|---|---|
|  | Meta-regression | Number of obs = | 10 |
|  | REML estimate of between-study variance | total = | 2.886 |
|  | % residual variation due to heterogeneity | $I^2$-squared_res = | 67.21 |
|  | Proportion of between-study variance explained | Adj R-squared = | 6.39 |
|  | Joint test for all covariates | Model F(5,12) = | 1.16 |
|  | With Knapp-Heirtz modification | Pmo > F = | 0.392 |
|  | logor | Coef. | Std. Err. | t | P>|t| | [%95 Conf Interval] |
| quality | -1.159369 | .4520592 | -2.55 | 0.012 | -2.147592 | .029687 |
| ethnicity | .271699 | .4057281 | 0.67 | 0.515 | -1.083262 | 1.515742 |
| source | -0.037378 | .3782783 | 0.21 | 0.833 | -0.504479 | .764733 |
| publish | -1.370493 | .0633561 | -5.00 | 0.000 | -1.905493 | .503450 |
| size | -0.064926 | .098686 | -6.65 | 0.000 | -1.279083 | -0.059799 |
| _cons | 1.225154 | .7635397 | 1.62 | 0.101 | -0.424506 | 2.87487 |

| B | meta-reg logr quality ethnicity publish size, wese(s_eislogS) hest(reml) |
|---|---|---|---|---|---|---|
|  | Meta-regression | Number of obs = | 10 |
|  | REML estimate of between-study variance | total = | 2.512 |
|  | % residual variation due to heterogeneity | $I^2$-squared_res = | 69.50 |
|  | Proportion of between-study variance explained | Adj R-squared = | 2.39 |
|  | Joint test for all covariates | Model F(4,13) = | 1.53 |
|  | With Knapp-Heirtz modification | Pmo > F = | 0.294 |
|  | logor | Coef. | Std. Err. | t | P>|t| | [%95 Conf Interval] |
| quality | -1.157198 | .4061806 | -2.96 | 0.057 | -1.967305 | .695535 |
| ethnicity | .250402 | .3759533 | 0.67 | 0.515 | -1.306044 | 1.006217 |
| publish | -1.549974 | .0720523 | -2.07 | 0.093 | -1.646879 | 0.000498 |
| size | -0.051666 | .0953939 | -0.69 | 0.501 | -0.268154 | 0.157511 |
| _cons | 1.172368 | .6945833 | 1.69 | 0.113 | -0.327205 | 2.673733 |

| C | meta-reg logr ethnicity publish size, wese(s_eislogS) hest(reml) |
|---|---|---|---|---|---|---|
|  | Meta-regression | Number of obs = | 10 |
|  | REML estimate of between-study variance | total = | 2.216 |
|  | % residual variation due to heterogeneity | $I^2$-squared_res = | 67.69 |
|  | Proportion of between-study variance explained | Adj R-squared = | 10.26 |
|  | Joint test for all covariates | Model F(3,14) = | 2.05 |
|  | With Knapp-Heirtz modification | Pmo > F = | 0.157 |
|  | logor | Coef. | Std. Err. | t | P>|t| | [%95 Conf Interval] |
| ethnicity | .1674777 | .3160203 | 0.53 | 0.605 | -0.510712 | .845667 |
| publish | -1.625938 | .0767044 | -2.12 | 0.052 | -3.276219 | 0.007704 |
| size | -0.722779 | .0891892 | -8.1 | 0.000 | -1.643175 | 0.108017 |
| _cons | 1.073036 | .6596585 | 1.66 | 0.130 | -0.317559 | 2.86222 |

| D | meta-reg logr publish size, wese(s_eislogS) hest(reml) |
|---|---|---|---|---|---|---|
|  | Meta-regression | Number of obs = | 10 |
|  | REML estimate of between-study variance | total = | 2.943 |
|  | % residual variation due to heterogeneity | $I^2$-squared_res = | 66.80 |
|  | Proportion of between-study variance explained | Adj R-squared = | 24.68 |
|  | Joint test for all covariates | Model F(2,15) = | 3.05 |
|  | With Knapp-Heirtz modification | Pmo > F = | 0.077 |
|  | logor | Coef. | Std. Err. | t | P>|t| | [%95 Conf Interval] |
| publish | -1.140563 | .0653113 | -2.16 | 0.046 | -2.512709 | -0.028556 |
| size | -0.925623 | .0773951 | -11.19 | 0.000 | -2.596831 | 0.073486 |
| _cons | 1.337196 | .4586598 | 2.92 | 0.001 | 0.359908 | 2.314833 |

| E | meta-reg logr publish, wese(s_eislogS) hest(reml) |
|---|---|---|---|---|---|---|
|  | Meta-regression | Number of obs = | 10 |
|  | REML estimate of between-study variance | total = | 2.102 |
|  | % residual variation due to heterogeneity | $I^2$-squared_res = | 67.21 |
|  | Proportion of between-study variance explained | Adj R-squared = | 21.67 |
|  | With Knapp-Heirtz modification |
|  | logor | Coef. | Std. Err. | t | P>|t| | [%95 Conf Interval] |
| publish | -1.141961 | .0661479 | -2.16 | 0.046 | -2.512709 | -0.028556 |
| _cons | 1.080376 | .3760155 | 2.70 | 0.012 | 0.323584 | 1.814953 |
Figure 6. Sensitivity analyses investigating the association between the (A) GSTM1, (B) GSTT1 and (C) combined GSTM1/GSTT1 null genotypes and susceptibility to endometriosis; one study was omitted at a time. GSTM1, glutathione S-transferase µ1; GSTT1, glutathione S-transferase θ1.
combined GSTM1/GSTT1 polymorphisms were altered when studies were excluded (Fig. 6B and C).

Discussion

In the present study, a meta-analysis of data from 33 studies was conducted to examine the associations between the GSTM1, GSTT1 and combined GSTM1/GSTT1 null genotypes and susceptibility to endometriosis. The risk for endometriosis was significantly increased in the presence of the GSTM1, GSTT1 and combined GSTM1/GSTT1 null genotypes compared with the wild-type. Subgroup analyses stratified by ethnicity, source of controls and quality of evidence confirmed this finding among several subgroups, but particularly among studies considered high-quality evidence. Notably, among patients of mixed ethnicity, the GSTM1 null genotype was significantly associated with a decreased risk for endometriosis compared with the wild-type.

A similar meta-analysis of 23 studies performed in 2005 demonstrated an increased risk for endometriosis in women with the GSTT1 null genotype (8). However, the authors requested that their findings be interpreted with caution as asymmetry in the funnel plot was evident, which was likely due to publication bias (8). This previous study did not include subgroup analyses or an evaluation of the combined GSTM1/GSTT1 null genotype-endometriosis association.

Previous meta-analyses have found that the GSTM1/GSTT1 gene polymorphism is associated with cervical cancer (45), breast cancer (46), bladder cancer (47), gastric cancer (48,49) and acute leukemia (50). In accordance with the observations of the present study, several studies have shown that the GSTM1 (OR=32.6, 95% CI: 15.07-70.32, P<0.0001) (17) and GSTT1 (OR>3; P<0.0001) (18) null genotypes are associated with an increased risk for endometriosis. However, other reports suggest the GSTM1 (OR=0.21, 95% CI: 0.09-0.52, P<0.0001; OR=0.38, 95% CI: 0.15-0.83, P<0.0001) (40,43), GSTT1 (OR=5; P<0.0001) (16) and combined GSTM1/GSTT1 null genotypes associated with a decreased risk for endometriosis. These divergent results may be explained by differences in GSTM1/GSTT1 null genotype frequencies and study locations. The frequency of the GSTM1/GSTT1 null genotype may vary from 10 to 65% depending on the region and population studied (51). Different study locations may introduce confounding variables associated with variations in lifestyles and exposures to toxic substances of the study populations.

The results of the present study must be interpreted with caution due to the presence of substantial heterogeneity. Among analyses of the studies of GSTM1 and GSTT1, the cause of heterogeneity remains unclear, despite meta-regression analyses being conducted. Among the analyses of combined GSTM1/GSTT1 studies, subgroup and sensitivity analyses suggested that studies that included patients with advanced stage endometriosis caused most of the variability. Publication bias was unlikely to have influenced the findings.

In addition to the heterogeneity, there were several limitations to this study. Firstly, the composition of the endometriosis patient and control populations varied between studies. For instance, some studies included only patients with advanced endometriosis (17-20,22,27,35), while control populations consisted of a mixture of infertile (29), postmenopausal (43) and premenopausal (18,35) women, and newborn babies that had not been exposed to the environment (17). Furthermore, patients and controls were not always accurately matched by age or environmental exposures. Secondly, gene-gene or gene-environment interactions may jointly increase the risk for endometriosis; therefore, different lifestyle and environmental factors may contribute to differential genotypic frequencies in cases and controls. Attempts were made to mitigate inaccuracies associated with this limitation through a subgroup analysis stratified according to ethnicity. Thirdly, this study was based on published articles. As a positive result is more likely to be published, publication bias is an inherent limitation of all meta-analyses irrespective of the outcomes of the Egger's linear regression test and Begg's rank correlation test.

In conclusion, the present meta-analysis shows the GSTM1, GSTT1 and combined GSTM1/GSTT1 null genotypes are likely associated with increased susceptibility to endometriosis. These data are in contrast to those reported previously. Therefore, further studies reporting higher quality evidence are necessary to verify these conclusions.

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