ORIGINAL ARTICLE

Glutathione S-transferase M1 polymorphism and endometriosis susceptibility: A meta-analysis

Polymorphisme de la glutathione S-transférase M1 et susceptibilité à l'endométriose : une méta-analyse

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KEYWORDS
Endometriosis; GSTM1; Meta-analysis; Polymorphism

Summary

Background.—Many studies have investigated the association between glutathione S-transferase M1 (GSTM1) null genotype and the risk of endometriosis. However, the effect of the GSTM1 null genotype on endometriosis is still unclear because of apparent inconsistencies among those studies. A meta-analysis was performed to characterize the relationship more accurately. PubMed, Embase, and Web of Science were searched.

Objective.—To derive a more precise estimation of the relationship, a meta-analysis was performed.

Methods.—We estimated the summary odds ratio (OR) with a 95% confidence interval (95% CI) to assess the association. Up to 24 case-control studies with 2684 endometriosis cases and 3119 control cases were included into this meta-analysis.

Results.—Meta-analysis of the 24 studies showed that GSTM1 null genotype was associated with the risk of endometriosis (random effects OR = 1.66, 95% CI 1.23 to 2.24). In the subgroup analysis by ethnicity, increased risks were found for both Caucasians (OR = 1.26, 95% CI 1.04–1.51) and Asians (OR = 1.28, 95% CI 1.06–1.55). No evidence of publication bias was observed.

Conclusion.—In conclusion, this meta-analysis suggests that the GSTM1 null genotype increases the overall risk of endometriosis.

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Glutathione S-transferase M1 polymorphism and endometriosis susceptibility

Introduction

Endometriosis is defined as the presence of endometrial tissue outside the uterus, causing diverse diseases, including infertility, pelvic pain and dysmenorrhea. The prevalence of endometriosis has been found to range from 2 to 18% among women who seek tubal ligation and from 5 to 50% within infertile women [1]. Although retrograde menstruation is widely accepted as a major contributing factor in the pathogenesis of endometriosis, it has also been observed in up to 90% of menstruating women with patent fallopian tubes [2]. This implies that additional factors determine susceptibility to this condition, including endocrine and immunologic dysregulations and genetic and environmental factors [3].

The role of glutathione S-transferase (GST) polymorphisms as a risk factor for endometriosis has been extensively researched. GSTM1, one member of the GST family, was formerly termed GST1 or GST class ‘mu’ [4]. The increased interest in this particular gene is due to its contribution to the metabolism of dioxin as a phase II enzyme, indicating that the GSTM1 may increase susceptibility to endometriosis [5].

Although many studies have investigated the relationship between GSTM1 polymorphisms and endometriosis, the results are inconclusive, partially because of the possible small effect of the polymorphism on endometriosis risk and the relatively small sample size in each of published studies. Guo et al. [6] reported that there is no evidence that women with GSTM1 null genotype have increased risk of developing endometriosis as compared with women with other genotypes in their meta-analysis. However, their meta-analysis was only a small part of their original paper. When they performed the meta-analysis, the pooled sample size was relatively small and not enough information was available for more exhaustive subgroup analysis. Since then, additional ten studies with a large sample size about this polymorphism on endometriosis risk have been reported, which would greatly improve the power of the meta-analysis of this polymorphism. Subgroup analyses performed by ethnicity were also possible now. Therefore, we updated this meta-analysis to derive a more precise estimation of these associations.

Materials and methods

Search strategy

We conducted a comprehensive search of the PubMed, Embase, and Web of Science databases from its inception through April 2014. We combined search terms for GSTM1 polymorphism and endometriosis. The search terms included GST, GSTM1, glutathione S-transferase M1; gene, polymorphism, mutation, and variant; and endometriosis. The retrieved studies were manually screened in their entirety to assess their appropriateness according to the inclusion criteria. All references cited in the studies from the searches were also reviewed to identify additional published articles not indexed in the databases.

Study eligibility

The inclusion criteria were as follows:

- case control design with the genotyping of men with and without endometriosis;
- provides information on genotypic frequency;
- articles in English.

For studies with overlapping cases or controls, only the most recent and/or the largest study with extractable data was included into the meta-analysis. Studies investigating progression, severity, phenotype modification, response to treatment, or survival were excluded from this review. Genome scans that investigated linkages were also
excluded. In addition, family-based association studies were excluded because they used different study designs.

### Statistical analysis

We calculated the overall odds ratio (OR) with the corresponding 95% confidence interval (CI) to assess the strength of the association between GSTM1 null genotype and risk of endometriosis. The significance of the pooled OR was determined using a Z test and a P value of less than 0.05 was considered significant. In our study, two models of meta-analysis for dichotomous outcomes were conducted: a random effects model and a fixed effects model [7]. The random effects model was conducted using the DerSimonian–Laird method, which assumes that studies were taken from populations with varying effect sizes and calculated the study weights both from intrastudy and interstudy variances [8]. The fixed effects model was conducted using the Mantel–Haenszel method, which assumes that studies were sampled from populations with the same effect size and adjusts the study weights based on intrastudy variance [9]. To assess the interstudy heterogeneity more precisely, both the $x^2$ based Q statistic test (Cochran’s Q statistic) to test for heterogeneity and the I2 statistic to quantify the proportion of the total variation due to heterogeneity were calculated [10]. The $I^2$ index, which expresses the percentage of the total variation across studies from heterogeneity, was calculated to assess the interstudy heterogeneity. The $I^2$ values 25%, 50%, and 75% were considered low, moderate, and high heterogeneity, respectively [9]. The random effects model was used to pool the results if the data exhibited moderate or high heterogeneity, whereas the fixed effects model was used to pool the results when the $I^2$ was less than 50%. A Galbraith plot was used to spot outliers as major sources of interstudy heterogeneity [11]. To validate the credibility of the outcomes in this meta-analysis, sensitivity analysis was performed through sequential omission of individual studies or by omitting low quality studies [12]. For additional analyses, the cases and controls were subgrouped based on their ethnicity. Racial/ethnic descent was categorized into Caucasians, Asians and mixed according to ethnicity classifications for genetic studies [13]. Publication bias was determined using a Begg’s funnel plot, wherein the standard error of log OR of each study was plotted against its log OR, and an asymmetric plot suggests possible publication bias. In addition, funnel plot asymmetry was assessed using an Egger’s Linear Regression Test [14]. All analyses were performed using STATA version 12.0 (StataCorp LP, College Station, Texas). A $P$ value $< 0.05$ was considered statistically significant, except where otherwise specified.
Table 1  Characteristics of studies on GSTM1 null genotype and endometriosis risk.
Caractéristiques des études sur le gène nul de la GSTM1 et le risque d’endométriose.

<table>
<thead>
<tr>
<th>Study</th>
<th>ID</th>
<th>Country</th>
<th>Population</th>
<th>Case age mean (range)</th>
<th>Control age (range)</th>
<th>Case null</th>
<th>Case present</th>
<th>Control null</th>
<th>Control present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baranov et al., 1996</td>
<td>1</td>
<td>Russia</td>
<td>Caucasian</td>
<td>ND</td>
<td>18–40</td>
<td>34</td>
<td>8</td>
<td>26</td>
<td>41</td>
</tr>
<tr>
<td>Baranov et al., 1999</td>
<td>2</td>
<td>Russia</td>
<td>Caucasian</td>
<td>ND</td>
<td>ND</td>
<td>88</td>
<td>62</td>
<td>42</td>
<td>57</td>
</tr>
<tr>
<td>Baranova et al., 1999</td>
<td>3</td>
<td>France</td>
<td>Caucasian</td>
<td>ND</td>
<td>ND</td>
<td>50</td>
<td>15</td>
<td>33</td>
<td>39</td>
</tr>
<tr>
<td>Baxter et al., 2001</td>
<td>4</td>
<td>South-east England</td>
<td>Caucasian</td>
<td>ND</td>
<td>ND</td>
<td>40</td>
<td>44</td>
<td>107</td>
<td>112</td>
</tr>
<tr>
<td>Hadfield et al., 2001</td>
<td>5</td>
<td>England</td>
<td>Caucasian</td>
<td>40–50</td>
<td>40–50</td>
<td>59</td>
<td>73</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>Ivashchenko et al., 2003</td>
<td>6</td>
<td>Russia</td>
<td>Caucasian</td>
<td>ND</td>
<td>ND</td>
<td>42</td>
<td>32</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>Arvanitis et al., 2003</td>
<td>7</td>
<td>Greece</td>
<td>Caucasian</td>
<td>27.2 (21–37)</td>
<td>34.5 (26–53)</td>
<td>161</td>
<td>114</td>
<td>181</td>
<td>165</td>
</tr>
<tr>
<td>Lin et al., 2003</td>
<td>8</td>
<td>China (Han nationality)</td>
<td>Asian</td>
<td>ND</td>
<td>ND</td>
<td>49</td>
<td>19</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Peng et al., 2003</td>
<td>9</td>
<td>China (Han nationality)</td>
<td>Asian</td>
<td>ND (21–45)</td>
<td>ND (23–41)</td>
<td>50</td>
<td>26</td>
<td>37</td>
<td>43</td>
</tr>
<tr>
<td>Morizane et al., 2004</td>
<td>10</td>
<td>Japan</td>
<td>Asian</td>
<td>ND</td>
<td>ND</td>
<td>50</td>
<td>26</td>
<td>37</td>
<td>43</td>
</tr>
<tr>
<td>Hsieh et al., 2004</td>
<td>11</td>
<td>Taiwan</td>
<td>Asian</td>
<td>ND</td>
<td>ND</td>
<td>95</td>
<td>55</td>
<td>8</td>
<td>151</td>
</tr>
<tr>
<td>Ding et al., 2004</td>
<td>12</td>
<td>China (Uygur nationality)</td>
<td>Asian</td>
<td>ND (25–45)</td>
<td>ND (21–45)</td>
<td>21</td>
<td>20</td>
<td>57</td>
<td>50</td>
</tr>
<tr>
<td>Ding et al., 2004</td>
<td>13</td>
<td>China (Han nationality)</td>
<td>Asian</td>
<td>ND (24–46)</td>
<td>ND (33–46)</td>
<td>46</td>
<td>34</td>
<td>55</td>
<td>50</td>
</tr>
<tr>
<td>Hur et al., 2005</td>
<td>14</td>
<td>South Korea</td>
<td>Asian</td>
<td>ND</td>
<td>ND</td>
<td>112</td>
<td>82</td>
<td>145</td>
<td>114</td>
</tr>
<tr>
<td>Frare et al., 2013</td>
<td>15</td>
<td>Brazil</td>
<td>Mixed</td>
<td>33.2 (ND)</td>
<td>37.4 (ND)</td>
<td>25</td>
<td>25</td>
<td>34</td>
<td>12</td>
</tr>
<tr>
<td>Hosseinzadeh et al., 2011</td>
<td>16</td>
<td>Iran</td>
<td>Caucasian</td>
<td>33 (18–35)</td>
<td>32 (ND)</td>
<td>87</td>
<td>33</td>
<td>80</td>
<td>120</td>
</tr>
<tr>
<td>Huang et al., 2010</td>
<td>17</td>
<td>Taiwan</td>
<td>Asian</td>
<td>34.3 (ND)</td>
<td>36.2 (ND)</td>
<td>12</td>
<td>16</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Kim et al., 2007</td>
<td>18</td>
<td>Korea</td>
<td>Asian</td>
<td>29.6 (19–40)</td>
<td>36.9 (16–55)</td>
<td>183</td>
<td>133</td>
<td>146</td>
<td>110</td>
</tr>
<tr>
<td>Matsuzaka et al., 2012</td>
<td>19</td>
<td>Australia</td>
<td>Mixed</td>
<td>38 (ND)</td>
<td>43 (ND)</td>
<td>43</td>
<td>54</td>
<td>67</td>
<td>76</td>
</tr>
<tr>
<td>Roya et al., 2009</td>
<td>20</td>
<td>Indian</td>
<td>Mixed</td>
<td>28.5 (ND)</td>
<td>28.4 (ND)</td>
<td>26</td>
<td>71</td>
<td>71</td>
<td>87</td>
</tr>
<tr>
<td>Sachan et al., 2013</td>
<td>21</td>
<td>Indian</td>
<td>Mixed</td>
<td>ND</td>
<td>ND</td>
<td>27</td>
<td>39</td>
<td>16</td>
<td>84</td>
</tr>
<tr>
<td>Seifati et al., 2011</td>
<td>22</td>
<td>Iran</td>
<td>Caucasian</td>
<td>28.95 (24–43)</td>
<td>29.82 (17–52)</td>
<td>51</td>
<td>50</td>
<td>74</td>
<td>68</td>
</tr>
<tr>
<td>Vichi et al., 2012</td>
<td>23</td>
<td>Italy</td>
<td>Caucasian</td>
<td>ND (18–45)</td>
<td>ND</td>
<td>104</td>
<td>77</td>
<td>85</td>
<td>77</td>
</tr>
<tr>
<td>Wu et al., 2011</td>
<td>24</td>
<td>Taiwan</td>
<td>Asian</td>
<td>30.9 (ND)</td>
<td>29.1 (ND)</td>
<td>57</td>
<td>64</td>
<td>52</td>
<td>119</td>
</tr>
</tbody>
</table>
Figure 2  Forest plots showed associations between GSMT1 null genotype and risk of endometriosis. A. Analysis of total studies. B. Analysis of total studies after adjustment for heterogeneity.

Graphique en forêt montrant l’association entre le génotype nul de la GSTM1 et le risque d’endométriose.

Results

Characteristics of included studies

A total of 23 publications met the inclusion criteria [5,15–36] (Fig. 1). In Ding’s study [22], the ORs were presented separately according to the different subgroup. Therefore, each group was considered separately for subgroup analysis. Hence, a total of 24-case control studies including 2684 endometriosis cases and 3119 control cases were included into the meta-analysis. Table 1 shows a brief description of these 24 case control studies, 10 (41.7%) involved Caucasian populations, 10 (41.7%) involved Asian populations and four (16.6%) involved mixed populations.
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Table 2  Summary of odds ratios (OR) with confidence interval (CI) in the meta-analysis.
Résumé des OR avec leurs intervalles de confiance.

<table>
<thead>
<tr>
<th>Null versus present</th>
<th>Studies</th>
<th>Odds ratio</th>
<th>Model</th>
<th>Heterogeneity</th>
<th>Egger’s test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>$P_{OR}$</td>
<td>$I^2$ (%)</td>
<td>$P_{I}$</td>
<td>$t$ value</td>
</tr>
<tr>
<td>Total studies</td>
<td>24</td>
<td>1.66 (1.23--2.24)</td>
<td>0.001</td>
<td>Random</td>
<td>85.7</td>
</tr>
<tr>
<td>Total studies (adjustment for heterogeneity)</td>
<td>17</td>
<td>1.12 (1.05--1.18)</td>
<td>0.000</td>
<td>Fixed</td>
<td>38.4</td>
</tr>
<tr>
<td>Caucasians</td>
<td>10</td>
<td>1.71 (1.17--2.51)</td>
<td>0.006</td>
<td>Random</td>
<td>80.2</td>
</tr>
<tr>
<td>Caucasians (adjustment for heterogeneity)</td>
<td>6</td>
<td>1.26 (1.04--1.51)</td>
<td>0.017</td>
<td>Fixed</td>
<td>12.2</td>
</tr>
<tr>
<td>Asians</td>
<td>10</td>
<td>2.08 (1.22--3.53)</td>
<td>0.007</td>
<td>Random</td>
<td>88.2</td>
</tr>
<tr>
<td>Asians (adjustment for heterogeneity)</td>
<td>8</td>
<td>1.28 (1.06--1.55)</td>
<td>0.010</td>
<td>Fixed</td>
<td>47.8</td>
</tr>
<tr>
<td>Mixed</td>
<td>4</td>
<td>0.85 (0.34--2.14)</td>
<td>0.732</td>
<td>Random</td>
<td>87.8</td>
</tr>
<tr>
<td>Mixed (adjustment for heterogeneity)</td>
<td>3</td>
<td>0.55 (0.32--0.97)</td>
<td>0.040</td>
<td>Random</td>
<td>59.4</td>
</tr>
</tbody>
</table>

$P_{I}$, the $P$ value of heterogeneity test.

(Table 1). The number of cases varied from 41 to 316, and the number of controls varied from 28 to 346 (Table 1). Controls were mainly healthy populations and matched for age.

Main results

The data exhibited obvious inter-study heterogeneity among the 24 studies ($I^2 = 85.7\%$); thus, the random effects model was used to pool data. Meta-analysis showed that GSTM1 null genotype was associated with risk of endometriosis ($OR = 1.66, 95\% CI 1.23 to 2.24$) (Fig. 2a). Sensitivity analyses through sequential omission of individual studies suggested that the significance of overall combined ORs was unstable. Subgroup analyses by ethnicity suggested GSTM1 null genotype was associated risk of endometriosis both in Caucasians and Asians (Table 2).

For the meta-analysis of all the studies, seven studies were identified by the Galbraith plot as possible sources of heterogeneity. No obvious inter-study heterogeneity was observed among the remaining 17 studies ($I^2 = 38.4\%$); thus, the fixed effects model was used to pool the ORs. The meta-analysis showed that the GSTM1 null genotype was still associated with an increased risk of endometriosis ($OR = 1.12, 95\% CI 1.05--1.18$) (Fig. 2b). Sensitivity analyses by omitting those studies also did not materially alter the overall combined ORs.

For meta-analysis of Caucasian studies, four studies were spotted by Galbraith plot as the possibly major sources of heterogeneity. There was no between-study heterogeneity among those remained six studies ($I^2 = 12.2\%$), thus the fixed-effects model was used to pool the ORs. Meta-analysis showed GSTM1 null genotype was associated increased risk of endometriosis ($OR = 1.26, 95\% CI 1.04--1.51$) (Fig. 3). Sensitivity analyses by omitting those studies also did not materially alter the overall combined ORs.

For meta-analysis of Asian studies, two studies were spotted by Galbraith plot as the possibly major sources of heterogeneity. After the exclusion of these two studies, heterogeneity was significantly decreased ($I^2 = 47.8\%$). The meta-analysis showed that the GSTM1 null genotype was still associated with a risk of endometriosis ($OR = 1.28, 95\% CI 1.06--1.55$) (Table 2). Sensitivity analyses through the omission of these two studies also did not materially alter the overall combined ORs.

Publication bias

Egger’s test was performed to access the publication bias of literatures. The results still did not suggest any evidence of publication bias (Table 2).

Discussion

Endometriosis represents a classical example of complex diseases with substantial genetic contribution [6]. Increased susceptibility to endogenous estrogens or exogenous dioxin-like compounds may confer susceptibility to endometriosis [37].

However, the exact mechanism is still being explored and debated. As a powerful statistical method, meta-analysis can help to pool the results of individual studies and can obtain a more precise estimate of relationships among research themes [38]. The primary purpose of this meta-analysis was to summarize the effect size results from a number of independent case-control studies to arrive at summary conclusions.

In this meta-analysis, we included ten additional case-control studies, which allowed for a greater number of subjects (2684 cases of endometriosis and 3119 controls) and, hence, a more detailed and accurate risk estimation than in prior meta-analyses, for which the literature ended in 2004 (14 studies including 1539 endometriosis cases and 1805 controls) [39].
Heterogeneity is a potential problem when interpreting the results of all meta-analyses, and determining the sources of heterogeneity is one of the most important goals of meta-analysis [40]. In this present meta-analysis, we assessed the between-study heterogeneity by using three different methods including the chi-square based Q statistic test (Cochran’s Q statistic) to test for heterogeneity, the I² statistic to quantify the between-study heterogeneity, and Galbraith plots to spot outliers as the possible major sources of heterogeneity. Generally, we found clear heterogeneity in the meta-analysis of 24 studies ($I^2 = 85.7\%$). To find the major sources of heterogeneity, we first performed subgroup

**Figure 3** Galbraith plots of association between GSTM1 null genotype and endometriosis risk. A. Galbraith plot of meta-analysis of total studies. B. Galbraith plot of meta-analysis of Caucasian studies. C. Galbraith plot of meta-analysis of Asian studies (each number of ID represented a respective and separate study included into meta-analysis).

*Graphiques de Galbraith de la méta-analyse de l’ensemble des études (A) et des études de populations caucasiennes (B) et asiatiques (C). Chaque nombre ID représente une étude spécifique incluse dans la méta-analyse.*
meta-analyses by ethnicity. Subgroup analyses showed that the heterogeneity was still significant in all subgroup analyses. Thus, a Galbraith plot was used to identify those studies contributing the greatest amount to that heterogeneity to investigate potential causes. Seven studies were identified by the Galbraith plot as possible sources of heterogeneity (Fig. 2) [15, 16, 24, 28, 30, 35, 36]. No obvious inter-study heterogeneity was observed among the remaining 17 studies ($I^2 = 38.4\%$). A possible explanation of heterogeneity might be differences in study design or in the population included. These seven studies may have included controls that had different risks for developing endometriosis. After adjusting for heterogeneity, the meta-analysis showed that the GSTM1 null genotype was still associated with an increased risk of endometriosis (fixed effects OR = 1.12, 95% CI 1.05–1.18), which is consistent with previous results [39].

However, this meta-analysis has some limitations. First, the inclusion criteria for the controls differed among the different studies. The controls in some studies were selected from asymptomatic healthy individuals, whereas other studies selected non-endometriosis individuals. Second, in the subgroup analysis, the number of individuals in mixed population was relatively small; therefore, they are insufficiently robust for determining the actual association. Third, only published studies were included in this meta-analysis. Therefore, publication bias may have occurred, even though statistical analysis did not detect it.

In conclusion, this meta-analysis suggests that the GSTM1 null genotype increases the overall risk of endometriosis. Moreover, gene–gene and gene–environment interactions should also be considered in the analysis. Further studies that consider these factors may provide a better, more comprehensive understanding of the association between the GSTM1 polymorphism and endometriosis.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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