Glutathione S-transferase M1*null genotype but not myeloperoxidase promoter G–463A polymorphism is associated with higher susceptibility to endometriosis

Yao-Yuan Hsieh¹,⁴, Chi-Chen Chang¹, Fuu-Jen Tsai², Cheng-Chieh Lin³, Jiun-Ming Chen² and Chang-Hai Tsai²,⁵

¹Department of Obstetrics and Gynecology, ²Department of Pediatrics and Medical Genetics, ³Department of Family Medicine, China Medical University Hospital, Taichung, and ⁴Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan
⁵To whom correspondence should be addressed at: Department of Pediatrics and Medical Genetics, China Medical University Hospital, No.2 Yuh-Der Road, Taichung, Taiwan. E-mail: d0704@www.cmuh.org.tw

Glutathione S-transferase M1 (GSTM1), one member of the GST family, is responsible for metabolism of xenobiotics and carcinogens. Myeloperoxidase (MPO) plays an important role in the oxidation and activation of carcinogens and nitric oxide. Allelic variants of GSTM1 and MPO gene polymorphisms might impair detoxification function and increase the susceptibility to endometriosis. We aimed to investigate if these polymorphisms are useful markers for predicting endometriosis susceptibility. Women were divided into two groups: (i) endometriosis (n = 150); (ii) non-endometriosis (n = 159). Polymorphisms for GSTM1 and MPO were amplified by polymerase chain reaction and detected by electrophoresis after restriction digestion. The relative frequencies of the GSTM1*wild (+/+ , +/0)/null (0/0) genotypes and MPO–463*G/A gene polymorphisms between both groups were compared. The distribution of GSTM1 polymorphisms was significantly different between the two groups. Proportions of GSTM1*wild/null alleles in both groups were: (i) 36.7/63.3%; (ii) 95/5% (P = 0.001). In contrast, MPO–463 genotypes were not significantly different between the two groups. Proportions of MPO*A homozygote/heterozygote/G homozygote in both groups were: (i) 2.7/17.4/79.9% and (ii) 1.9/17/81.1% (P > 0.05). We conclude that the GSTM1*null genotype is associated with a higher risk of endometriosis development. MPO–463*/A gene polymorphism is not related to the susceptibility of endometriosis.

Key words: endometriosis/gene polymorphism/glutathione S-transferase (GSTM1)/myeloperoxidase (MPO)

Introduction

Endometriosis, a common polygenic/multifactorial disease, might be caused by an interaction between multiple genes as well as the environment (Bischoff and Simpson, 2000). Endometriosis displays features similar to malignancy, including local invasion and aggressive spread to distant organs. Tumor suppressor genes play a role in the regulation of cell growth and prevention of carcinogenesis. The altered tumor suppressor genes might be related to the development of endometriosis. Genetic alterations have been identified in endometriotic lesions, which might contribute to their initiation and progression (Jiang et al., 1998). It is logical to suspect that somatic genetic factors might contribute to the development of endometriosis (Treloar et al., 1999).

The glutathione S-transferases (GSTs) are a family of enzymes responsible for the metabolism of xenobiotics and carcinogens. GSTM1, one member of the GST family, was formerly termed GST1 or GST class ‘mu’ (Mannervik et al., 1992). GSTM1 is critical in the detoxification of the oxidative stress product during ovulation (Baxter et al., 2001). Failure to detoxify these products may result in rapid accumulation of genetic damage and increase susceptibility to epithelial ovarian cancer (Baxter et al., 2001). Endometriosis is characterized by cyclical degeneration and chronic inflammation, which will result in the production of reactive oxygen species and other toxins. Because of the detoxification properties of the GST enzymes, it is logical to suspect the role of GSTM1-related gene in endometriosis patients.

Many gene polymorphisms have been reported to be associated with endometriosis, including GSTM1 gene polymorphism (Baranova et al., 1997, 1999; Arvanitis et al., 2003). The GSTM1 gene is located on chromosome 1p13 (Zhong et al., 1992). GSTM1 gene deletions might influence an individual’s enzymatic function, impair their detoxification system and further increase the risk when exposed to carcinogens and toxic chemicals (Seidegard et al., 1990). An elevated frequency of the inactive GSTM1 gene has been reported in endometriosis patients (Baranova et al., 1997, 1999). Baranova et al. (1999) observed a significant excess of the GSTM1 null genotype among women with endometriosis.

Myeloperoxidase (MPO), a 150 kDa hemoprotein secreted by activated macrophages, is involved in many pathological processes (Rutgers et al., 2003). MPO plays an important role in the oxidative pathway of neutrophils and monocytes by producing hypochlorous acid (HOCl). MPO functions not only antimicrobiotically, but also acts as a metabolic enzyme with many other substrates, which produces some reactive intermediates and consumes hydrogen peroxide (Schabath et al., 2000). Moreover, the MPO–HOCl system has been shown to oxidize low-density lipoprotein (LDL) (Winterbourn et al., 2003).
2000), activate carcinogens (Schabath et al., 2000) and reduce nitric oxide (NO) bioavailability (Eiserich et al., 1998). A functional MPO promoter polymorphism, 2463G/A, has been associated with incidence or severity of inflammatory diseases, including atherosclerosis, Alzheimer’s disease, and some cancers (Kumar et al., 2004). MPO–463G/A polymorphism could modify the binding site for the SP1 transcription factor and significantly decrease the expression of MPO as well as the severity of leukemia (Piedrafita et al., 1996).

It is generally accepted that heritable genetic factors might contribute to the development of endometriosis. Unlike mutations, polymorphisms are not directly linked to a certain disease. However, they are useful tools in the study of multifactorial disorders (Anderson et al., 1994). In our previous surveys, we observed the correlation of endometriosis and some gene polymorphisms, including p53 (Chang et al., 2002) and androgen receptor (Hsieh et al., 2001). Based on these surveys, we tried to assess the risk of endometriosis associated with GSTM1 and MPO gene polymorphism. We aimed to evaluate whether these polymorphisms are attractive markers for endometriosis susceptibility. To our knowledge, this report is the largest survey for GSTM1 polymorphisms in endometriosis. Furthermore, it is the first report about the MPO polymorphism in endometriosis.

### Materials and methods

Pre-menopausal Taiwanese women with surgically diagnosed endometriosis and non-endometriosis were included. All patients were divided into two groups: (i) endometriosis stage III/IV (n = 150); (ii) non-endometriosis (n = 159). All individuals with endometriosis accepted laparoscopy or laparotomy management and were confirmed pathologically. All patients had normal blood pressure without obvious cardiovascular diseases. There were non-significant differences between both groups in age, weight and height. All women had consented to peripheral blood sampling for genotype analyses. The studies were approved by the ethical committee and institutional review board of the China Medical University Hospital. Informed consents were signed by all women who donated their blood.

The GSTM1*wild/null and MPO–463*G/A gene polymorphisms were determined according to previously described methods (Baxter et al., 2001; Table I. The primer sequences and PCR conditions for GSTM1 and MPO–463 G/A gene polymorphisms

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Primer sequences (5’ → 3’)*</th>
<th>PCR conditions (°C/s)</th>
<th>Restriction enzyme digestion</th>
<th>Allele</th>
<th>DNA fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1</td>
<td>F-AGCTGCCCTACTTGATTGATGGG</td>
<td>93/30 62/30 72/20</td>
<td>–</td>
<td>Wild-type&lt;sup&gt;a&lt;/sup&gt;</td>
<td>271</td>
</tr>
<tr>
<td></td>
<td>R-CTGGGGACACTCAAATTTCTTG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1A1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>F-GCATGGAGCTTGCGATGCTTG</td>
<td>93/30 62/30 72/20</td>
<td>–</td>
<td>Wild-type</td>
<td>583</td>
</tr>
<tr>
<td></td>
<td>R-TAGGAGCTCTTGCTCATGCTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPO–463</td>
<td>F-CGGTATAGGCCACAAATGGTGGA</td>
<td>93/30 60/30 72/45</td>
<td>AciI (37°C/30 min)</td>
<td>A allele</td>
<td>289 + 61</td>
</tr>
<tr>
<td></td>
<td>R-GCAATGGTTCAAGCGATTCCTC</td>
<td></td>
<td></td>
<td>G allele</td>
<td>169 + 120 + 61</td>
</tr>
</tbody>
</table>

* <sup>F</sup> and <sup>R</sup> indicate forward and reverse primers.
<sup>a</sup>Homozygotes or heterozygotes could not be specified.
<sup>b</sup>Concomitant PCR with GSTM1 gene for internal controls.

---

**Figure 1.** Genotyping of (A) GSTM1*wild/null and (B) MPO–463*G/A gene polymorphisms (M: Marker).
**Reynolds et al., 2002.** The genomic DNA was prepared from peripheral blood leukocytes by use of a genomic DNA isolation kit (Blossom, Taipei, Taiwan). A total of 50 ng genomic DNA was mixed with 20 pmol of each polymerase chain reaction (PCR) primer in a total volume of 25 μl containing 10 mM Tris- HCl pH 8.3, 50 mM potassium chloride, 2.0 mM magnesium chloride, 0.2 mM each deoxyribonucleotide triphosphate and 1 U DNA polymerase (Amplitag; Perkin-Elmer, Foster City, CA). A total of two gene polymorphisms were surveyed, including GSTM1* wild/null and MPO–463*G/A. The SNP information for the genes involved was obtained via the NCBI website (http://www.ncbi.nlm.nih.gov/LocusLink/).

The PCR primer sequences and condition of each primer are listed in Table I. To confirm the successful amplification, an internal control was included in the PCR reaction of GSTM1. It consisted of a 583 bp amplion from the CYP1A1 gene (Nicholl et al., 1999). The PCR amplification was performed in a programmable thermal cycler GenAmp PCR system 2400 (Perkin Elmer Applied Biosystems, Foster City, CA). After PCR amplification, the individual gene polymorphisms were analyzed after restriction digestion (New England Biolabs Inc., Beverly, MA). The base pairs for their wild-type, null type and SNP type are listed in Table I.

The PCR products were mixed together and 10 μl of this solution was loaded into 3% agarose gel containing ethidium bromide for electrophoresis. Each allele was recognized according to its size (Figure 1). Genotypes and allelic frequencies for GSTM1 and MPO gene polymorphisms in both groups were compared. Correlation of the GSTM1 and MPO genotype and endometriosis might also contribute to an imbalance of sex hormones or alter susceptibility. Endometriosis, a multifactorial disease, involves complex interactions between hormones and cytokines activation, immunoinflammatory processes and genetic factors (Vigano et al., 1998). Recent experimental studies indicated that dioxin may be involved in the pathogenesis of endometriosis (Gibbons, 1993). Dioxin is widely present in the environment; most people absorb traces of dioxin by exposure to pesticides in their diet. These toxins might also contribute to an imbalance of sex hormones or alter growth factors and the immune response (Mayani et al., 1997).

**Discussion**

Numerous chronic disorders, such as endometriosis, osteoporosis, hypertension, diabetes and asthma, have been attributed to genetic susceptibility. Endometriosis, a multifactorial disease, involves complex interactions between hormones and cytokines activation, immunoinflammatory processes and genetic factors (Vigano et al., 1998). Recent experimental studies indicated that dioxin may be involved in the pathogenesis of endometriosis (Gibbons, 1993).

GSTM1 functions both as a detoxification enzyme and an intracellular drug- and hormone-binding protein (Chasseaud, 1979). GSTM1 catalyzes the detoxification of genotoxic chemicals, including the products of chronic oxidative stress such as cytotoxic lipid and DNA species (Hayes et al., 1995). GSTM1 enhances the conjugation of glutathione with several alkylating agents (Dulik et al., 1990). The GSTM1 gene is specific for certain carcinogens, including trans-stilbene oxide and a metabolite of benzopyrene contained in smoke fumes (Seidegard et al., 1990). The GSTM1 gene might influence the related isoenzyme expression as well as the host susceptibility to lung cancer among smokers (Seidegard et al., 1990).

Impaired GSTM1 function might result in increased risk to DNA damage and malignant transformation. The null condition of GSTM1 (0/0 genotype) represented an expanded deletion (~10 kb) of the gene, which might impair the further production of mRNA and protein (Seidegard et al., 1988).

GSTM1 gene deletion (0/0 genotype) is a useful marker for the early detection of many diseases, including endometriosis, ovarian cancer (Baxter et al., 2001), cystic fibrosis (Baranov et al., 1996), bladder (Brockmoller et al., 1984), lung (Nakachi et al., 1993) and stomach cancers (Harada et al., 1992). Baranov and colleagues (1999) reported a highly significant excess of the GSTM1 null genotype in women with endometriosis versus controls (76.9 versus 45.8%). Recently, Arvanitis et al. (2003) also demonstrated that the GSTM1 null deletion adds to this risk of endometriosis. In contrast, Baxter et al. (2001) demonstrated that the GSTM1 null allele is not an endometriosis susceptibility allele; however, it may predispose endometriotic lesions to malignant transformation to endometrioid and clear cell ovarian cancer. Some investigators demonstrated the non-association between the individual diseases with GSTM1, including cancers of the ovary (Cehisselbauer et al., 1992), bladder (Brockmoller et al., 1994), lung (Brockmoller et al., 1993) and stomach (Harada et al., 1992). These discrepancies might be due to different illness classification, racial and disease variation.

---

**Table II. Distributions of GSTM1 genotypes in women with and without endometriosis**

<table>
<thead>
<tr>
<th>Genotype Endometriosis</th>
<th>Group (i) n = 150 (%)</th>
<th>Non-endometriosis Group (ii) n = 159 (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type (+/+ homozygote; +/+ heterozygote)</td>
<td>55 (36.7)</td>
<td>151 (95)</td>
<td>0.001</td>
</tr>
<tr>
<td>Null type (0/0 homozygote)</td>
<td>95 (63.3)</td>
<td>8 (5)</td>
<td></td>
</tr>
</tbody>
</table>

**Table III. Genotypes and allelic frequencies for MPO*G/A polymorphism in women with and without endometriosis**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Endometriosis Group (i) n = 150 (%)</th>
<th>Non-endometriosis Group (ii) n = 159 (%)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>4 (2.7)</td>
<td>3 (1.9)</td>
<td>NS</td>
</tr>
<tr>
<td>A/G</td>
<td>26 (17.4)</td>
<td>27 (17)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>119 (79.9)</td>
<td>129 (81.1)</td>
<td></td>
</tr>
</tbody>
</table>

**Allele frequencies**

| A         | 34 (11.4) | 33 (10.4) | NS      |
| G         | 264 (88.6)| 285 (89.6)|         |

NS, not significant. *P-value was calculated by χ² tests.
In this study, we observed that the genotype distribution for GSTM1 gene polymorphism was significantly different between the individuals with and without endometriosis. The null genotype is related with higher susceptibility of endometriosis; whereas the wild-type is related with lower risk of endometriosis development. Our data strongly suggest that the lack of GSTM1 gene products might substantially contribute to the pathogenesis of endometriosis. However, our finding only suggested their connection as well as possibility. The related scientific proof for the underlying mechanisms is still warranted.

In this survey, the GSTM1 null genotype frequency (63.3%) in individuals with endometriosis was significantly lower than that observed by Baranova et al. (1997, 1999; 75–86%) and higher than that observed by Arvanitis et al. (2003; 58.5%). The discrepancies between Baranova et al. (1997, 1999) and Arvanitis et al. (2003) suggested racial differences within the French and Greek populations, even though they are both Caucasian populations. However, the conclusions of Baranova et al. (1997, 1999) were based on fewer case numbers (<100). Our study of 150 endometriosis cases and 159 controls provides the stronger support for the claim that the GSTM1 null allele is a predisposing factor for endometriosis. Furthermore, our finding is the first indication that GSTM1 gene deficiency predisposes to endometriosis in an Asian population.

However, we also observed a different distribution of null GSTM1 genotype between normal Asians and Caucasians (Board, 1990; Groppi et al., 1991; Harada et al., 1992). The fluctuation of GSTM1 deficiency found in most Caucasians is in the range of 40–52% (Baranova et al., 1996; Arvanitis et al., 2003). In contrast, in our study we found the null genotype in 5% of the normal controls. The discrepancy might be mainly due to the ethnic differences between Asians and Caucasians. Furthermore, whether the GSTM1 0/0 genotype in different populations results from identical deletion or from other alterations of the GSTM1 gene remains unknown.

MPO is a 150 kDa hemoprotein stored exclusively in the azurophilic granules of monocytes and neutrophils (PMNs). MPO produces not only the strong oxidant bleach (hypochlorous acid) from hydroperoxide and chloride ions but also oxidizes LDL into a macrophage high-uptake form, inactivates protease inhibitors, and consumes nitric oxide (Rutgers, 2003). MPO produces between Asians and Caucasians. Furthermore, whether the GSTM1 null allele is a predisposing factor for endometriosis. In this study, we observed the non-association between the MPO promoter –463 gene polymorphisms and the susceptibility to endometriosis. We also observed the frequency of the A allele (10–11%) in our population was significantly lower than the reported frequencies in Caucasian populations (23.4% (London et al., 1997), 25.7% (Le Marchand et al., 2000), 21.2% (Cascorbi et al., 2000), 29.8% (Schabath et al., 2000) and 30.6% (Misra et al., 2001)). These discrepancies might be due to racial variation.

In conclusion, an association between endometriosis and GSTM1 gene polymorphism exists. The GSTM1 null genotype is related to an increased susceptibility to endometriosis. The GSTM1 gene polymorphism likely contributes to the pathogenesis of endometriosis. It also suggests the defects in carcinogen detoxification may be involved in the pathogenesis of endometriosis. In contrast, the MPO*–463G/A gene polymorphisms are not related to the susceptibility of endometriosis. Although the real roles of GSTM1 and MPO gene polymorphism have not yet been clarified, these polymorphisms deserve more attention to realize their roles upon endometriosis development. This study could be extended to investigate whether and how the detoxification affects the endometriosis formation. Furthermore, after the clarification of these issues, GSTM1 gene polymorphism may become a useful marker to predict the future development of endometriosis and to permit early therapeutic intervention in women at high risk for endometriosis.

References


Baranova VS, Ivashenko T, Bakay B et al. (1996) Proportion of the GSTM1 0/0 genotype in some Slavic populations and its correlation with cystic fibrosis and some multifactorial diseases. Hum Genet 97,516–520.


