Polymorphisms of the genes encoding the GSTM1, GSTT1 and GSTP1 in Korean women: no association with endometriosis

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Endometriosis, one of the most common gynaecologic disorders, shows significantly elevated prevalence in industrial areas and there is also a possible genetic predisposition. Glutathione-S-transferases (GSTs) are enzymes involved in the metabolism of many disease-causing carcinogens and mutagens that are present in human environments. An association between the incidence of endometriosis and the GST genotypes of patients has been suggested. The objective of the present study was to investigate whether the polymorphisms of GSTM1, GSTT1 and GSTP1 are related to endometriosis. Blood samples were available from 259 controls and 194 patients with advanced endometriosis diagnosed by both pathology and laparoscopic findings. The proportion of the GSTM1, GSTT1 and GSTP1 genotypes of the control group were comparable to other populations. There was no significant evidence that the distribution of the GSTM1 and GSTT1 genotype differed between the patients and the controls, with an allelic odds ratio (OR) = 1.074 [95% confidence interval (CI) = 0.737–1.564] and 1.239 (95% CI = 0.853–1.799), respectively. Also, there was no significant difference in the proportion of GSTP1 genotypes between the women with endometriosis and the control group with the OR = 0.823 (95% CI = 0.536–1.264). The higher risk alleles were contended as GSTM1, GSTT1 null mutation and GSTP1 Ile105lle polymorphism. There was no significant increase in the risk of endometriosis as the number of higher risk alleles of the GST family increased. In conclusion, our findings suggest that the GSTM1, GSTT1 and GSTP1 genetic polymorphisms are not associated with the development of endometriosis in Korean women.

Key words: endometriosis/GSTM1/GSTT1/GSTP1/polyorphism

Introduction

Endometriosis is one of the most common gynaecologic disorders, but its aetiology and pathogenesis remain obscure. There is increasing evidence that endometriosis is inherited as a complex genetic trait, implying that multiple gene loci interact with each other and with the environment to produce the phenotypic disease (Kennedy, 1997). A number of candidate genes have been studied to date. Associations have been reported between the endometriosis and the polymorphisms in the estrogen receptor (Georgiou et al., 1999), human arylhydrocarbon receptor repressor gene (Watanabe et al., 2001), p53 codon 72 (Chang et al., 2002), PROGS receptor gene polymorphism (Wieser et al., 2002), androgen receptor trinucleotide (Hsieh et al., 2001), galactose-1-phosphate uridyl transferase genes (Cramer et al., 1996) and detoxification enzyme genes (Baranova et al., 1997).

Endometriosis shows significantly elevated frequency in industrial areas (Nisolle et al., 1997) and there is a possible genetic predisposition (Kennedy et al., 1996). The glutathione-S-transferases (GSTs) constitute a family of xenobiotic-detoxifying phase II enzymes catalysing the conjugation of glutathione to a variety of electrophilic compounds including polycyclic aromatic hydrocarbons (PAH), which are widely present in the human environment and known to be carcinogenic (Smith et al., 1995; Strange and Fryer, 1999).

Several GSTs are polymorphic and some allelic variants causing enzyme activity impairment are suspected to increase susceptibility to malignancies associated with environmental PAH, particularly colorectal cancer (Strange and Fryer, 1999; Cotton et al., 2000). A very small portion of endometriosis develops into cancer later, but endometriosis itself is not a cancer disease. It has many similar characteristics of cancer diseases, for example progressive growth, invasive growth, estrogen-dependent growth, recurrence and a tendency to metastasis (Gorp et al., 2004).

The polymorphic genes GSTM1, GSTT1 and GSTP1 are involved in the detoxification of a variety of potential carcinogenic compounds, such as hydrocarbon diol-epoxides, steroids and genotoxic lipoperoxidation products (Ketterer, 1998). Deletion variants or null alleles exist for the GSTT1 and GSTM1 genes and these present biochemically as a failure to express protein (Hu et al., 1999).

The GSTP1 polymorphism is the result of a base substitution (A → G), which leads to an amino acid substitution (codon 105, Isoleucine → Valine). This amino acid substitution, in the GSTP1 binding site, modifies its catalytic activity. The GSTP1 enzymes with val 105 with altered heat stability and specific activity of the val-containing isoform (Zimmick et al., 1994; Ali Osman et al., 1997) have a 7-fold higher efficiency for conjugation of PAH diol-epoxides, and in vitro this polymorphic form significantly reduces
the level of benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide-induced DNA adducts (Hu et al., 1999). These and other GST variants have been investigated as risk factors for predisposition to numerous cancers, including lung, bladder, breast, colorectal, gastric, liver, larynx and skin cancer, with conflicting results between and within cancer types (Rebeck, 1997; Hengstler et al., 1998; d’Errico et al., 1999; Dunning et al., 1999).

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. Dioxin is considered a possible contributor to the development of this disease (Rier and Yeaman, 1997), even if evidence for such a role in humans is limited (Mayani et al., 1997) and the other studies show no association (Eskenazi et al., 2002).

Current investigations are devoted to detoxification system genes, such as arylamine N-acetyltransferase 2, GSTM1 and GSTT1 in the development of endometriosis, but results are not consistent. There are very few studies reported on investigation of the effects of GSTP1 on endometriosis development.

In this study, we have investigated the potential influence of GSTM1, GSTT1 and GSTP1 polymorphism in Korean women with and without endometriosis to assess the risk of individuals or combined genotypes that alter the metabolizing capabilities for dioxin and estrogen in the development of endometriosis.

Materials and methods

Subjects

The study protocol was approved by the Institutional Review Board on the Use of Human Subjects in Research at Ewha Womans University and informed consent was obtained from each patient. The endometriosis patients had undergone laparotomy or laparoscopy at the Obstetrics and Gynecology Department of Ewha Womans University Hospital. Patients consisted of 194 unrelated Korean women and were diagnosed with advanced stage endometriosis (stages III and IV) by both pathological and laparoscopic findings according to the revised American Fertility Society classification of endometriosis (American Fertility Society, 1985). Blood samples were collected from the patient group for DNA. The 259 women undergoing laparoscopic surgery or laparotomy for non-malignant lesions, such as benign ovarian cyst, were included in the control group. Blood samples were collected for DNA extraction.

GSTM1 and GSTT1 genotyping

GSTM1 and GSTT1 genotyping for gene deletions were carried out by PCR as described by Lin et al. (1998) with minor modifications. DNA samples were amplified with the primers 5’-GAACTCCCTGAAAAGCTAAAGC-3’ and 5’-GTGGGCTCAATAATACGGTGG-3’ (Bioneer, Seoul, Korea) for GSTM1, which produced a 219 bp product; 5’-TCACCGGATCATGGCCAGCA-3’ and 5’-TTCCTACTGTCCTCACATCTC-3’ (Bioneer, Seoul, Korea) for GSTT1, which produced a 459 bp product. Amplification of beta actin gene with the primers 5’-GCCCTCTGCTAACAAGTCTTAC-3’ (Bioneer, Seoul, Korea) and 5’-GCCCTAAAAAGGAAATCCCCATAC-3’ (Bioneer, Seoul, Korea) was used as an internal control and produced a 350 bp product. PCR was performed in a final volume of 50 μl, consisting of DNA (0.1 μg), dNTP (0.2 mM each) (Perkin–Elmer), MgCl2 (2.5 mM), each primer (1.0, 0.3 and 0.2 μM for GSTM1, GSTT1 and beta actin, respectively), Taq polymerase (1.25 U) (Perkin–Elmer), reaction buffer and 2% dimethyl-sulphoxide. Amplification was performed with an initial denaturation at 95°C for 12 min, followed by 35 cycles of amplification which was performed at 94°C for 1 min, 62°C for 1 min and 72°C for 1 min and a final extension at 72°C for 10 min, using a GeneAmp 9600 thermal cycler (Perkin–Elmer). The amplified product was visualized in an ethidium bromide stained 1.5% agarose gel. If the study subject is null for the gene, no PCR product is present, but the beta actin gene fragment acts as a positive PCR control.

GSTP1 genotyping

The GSTP1 exon 5 105 ile = val polymorphisms were determined by PCR and restriction fragment length polymorphism according to the method of Harris et al. (1997). DNA samples were amplified with the primers 5’-ACCCACGGCTCTATGGAA-3’ and 5’-TGAGGCCACAA-GAAGCCCT-3’ (Bioneer, Seoul, Korea). The PCR amplification was carried out with 50 ng DNA in 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.3 mM dextoxyribonucleotide triphosphates (Perkin–Elmer), 200 ng of each primer and 1.0 U of Taq polymerase (Perkin–Elmer) in a total volume of 40μl. Amplification was performed with an initial denaturation step at 95°C for 12 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 45 s and a final extension at 72°C for 10 min.

The amplification product (20 μl) was digested with 5 U of BsmAI (New England Biolabs, Beverly, MA) in 50 mM NaCl, 10 mM Tris–HCl, 10 mM MgCl2 and 1 mM dithiothreitol, and incubated at 55°C for 16 h. Then fragment lengths were analysed on a 3% agarose gel with ethidium bromide (0.5 mg/ml). When the BsmAI restriction site was present, the fragment of 176 bp was digested into two lengths of 91 and 85 bp. Electrophoresis of the digested PCR products showed individuals homozygous (ile/ile) for the GSTP1 BsmAI polymorphism as one band of 176 bp. Heterozygous (ile/val, val/val) for the polymorphism showed three bands of 176, 91 and 85 bp (Figure 1).

Statistical analysis

For statistical analysis, χ2-test and logistic regression analysis were used. A P value of <0.05 was considered statistically significant. The Hardy–Weinberg equilibrium analyses were performed to compare observed and expected genotype frequencies using a chi-square test. All analyses were conducted using the Statistical Package for Social Science version 11.5 (SPSS Inc., Chicago, IL).

Results

GSTM1 gene polymorphism

The proportion of control group individuals with GSTM1 null mutations was 56%, which is comparable to population controls in other studies. No significant differences were found between the endometriosis group (57.7%) and the control group (56%) for the GSTM1 null mutation. The GSTM1 null mutation did not increase the risk of endometriosis development [odds ratio (OR) = 1.074, 95% confidence interval (CI) = 0.737–1.564, P = 0.774] (Table I).

GSTT1 gene polymorphism

The proportion of control group individuals with GSTT1 null mutations was 48.3% comparable to population controls in other studies. No significant differences were found between the endometriosis group (53.6%) and the control group (48.3%) for the GSTT1 null mutation. The GSTT1 null mutation did not increase the risk of endometriosis development (OR = 1.239, 95% CI = 0.853–1.799) (Table I).
**Table I.** Allelic frequencies and ORs for *GSTM1* and *GSTT1* polymorphisms in Korean women with and without endometriosis

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Endometriosis (%)</th>
<th>Control (%)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>GSTM1</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>112 (57.7)</td>
<td>145 (56.0)</td>
<td>1.074 (0.737–1.564)</td>
</tr>
<tr>
<td>Present</td>
<td>82 (42.3)</td>
<td>114 (44.0)</td>
<td>(P = 0.774)</td>
</tr>
<tr>
<td>Total</td>
<td>194</td>
<td>259</td>
<td></td>
</tr>
<tr>
<td><em>GSTT1</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>104 (53.6)</td>
<td>125 (48.3)</td>
<td>1.239 (0.853–1.799)</td>
</tr>
<tr>
<td>Present</td>
<td>90 (46.4)</td>
<td>134 (51.7)</td>
<td>(P = 0.296)</td>
</tr>
<tr>
<td>Total</td>
<td>194</td>
<td>259</td>
<td></td>
</tr>
</tbody>
</table>

OR = odds ratio; CI = confidence interval.

**GSTP1 gene polymorphism**

The frequencies of *GSTP1* Ile/Ile genotype were 72.7% (141/194) in patients and 67.6% (175/259) in controls. The frequencies of *GSTP1* Ile/Val were 23.7% (46/194) in patients and 27.4% (71/259) in controls. The frequencies of *GSTP1* Val/Val were 3.6% (7/194) in patients and 5% (13/259) in controls. No significant differences were found between the endometriosis group and the control group.

Using the *GSTP1* homozygote (Ile/Ile) as a reference group, the OR of *GSTP1* heterozygotes (Ile/Val) and *GSTP1* homozygotes (Val/Val) was 0.823 (95% CI = 0.536–1.264, P = 0.388). It did not reach statistical significance (Table II).

**Risk of endometriosis with allele combination of GST genotypes**

We contend that the *GSTM1*, *GSTT1* null mutation and *GSTP1* Ile105Ile polymorphisms are putative high-risk alleles. In order to assess the existence of any interaction between the polymorphism of *GSTM1*, *GSTT1* and *GSTP1*, we calculated the frequency of the simultaneous presence of the three putative ‘high-risk’ genotypes. Individuals carrying all three presumptive low-risk genotypes—*GSTM1* and *GSTT1* non-deleted (present) and *GSTP1* val/val and Ile/val genotypes—were used as the reference group. Table III shows the OR of endometriosis associated with each combination of genotypes. The figures (0, 1, 2 and 3) are the number of putative high-risk genotypes. There was no statistically significant difference with the number of high-risk GST alleles (1, 0.995 (95% CI = 0.602–1.644), 1.462 (95% CI = 0.853–2.507), 1.367 (95% CI = 0.559–3.339) (Table III) and no trend according to the number of putative risk genotypes (by linear association, df = 1, P = 0.11; data were not shown).

**Discussion**

Endometriosis is a multifactorial disease with significantly elevated frequency in industrial areas (Nisolle et al., 1997). The lack of detoxification, which is genetically determined, might be a risk factor for endometriosis development. So, analysis of GST gene status, particularly detection of *GSTM1* and *GSTT1* null mutation, and *GSTP1* polymorphism could have a prognostic and pathologic importance. One of the strengths of our study may be a relatively larger number of endometriosis cases. Although it is at the lower end of what is required for studies of complex diseases, it is larger than those that have been commonly used in endometriosis studies. Also, the population of our study consisted of all Korean women and they are thought to be genetically homogeneous.

*GSTM1* activity is absent in about 50% of Caucasians as a consequence of the inheritance of two null alleles (deletion of the gene). A Japanese control population showed a 47.5% frequency of the *GSTM1* null genotype, whereas Caucasian control populations showed higher frequencies (53.1%), e.g. 54.3% in USA and 50.4% in The Netherlands (Garte et al., 2001). In our study, the Korean control population showed a 56% frequency of the *GSTM1* null genotype. It is comparable to another Korean control’s frequency of 53.7% (Choi et al., 2003).

Similarly, the *GSTT1* activity is deficient in about 20% of Caucasians, resulting from homozygous deletion. A Japanese control population showed a 35.3% frequency of the *GSTT1* null genotype, whereas Caucasian control populations showed lower frequencies, e.g. 27.6% in USA and 22.9% in The Netherlands (Garte et al., 2001). In our study, the control population showed a 48.3% frequency of the *GSTT1* null genotype and it was comparable to Asian control population average frequencies (47%) from the report of Garte et al. (2001).

The *GSTP* subfamily comprises only *GSTP1*. The A1578G substitution in *GSTP1* creates the Ile105Val polymorphism that leads to the expression of an enzyme with reduced activity. In our study, the proportion of the *GSTP1* genotype of the control group was comparable to other Korean populations (allele frequency of genotype*Ile*; our study/other Korean study = 0.8145/0.8230; data not shown) (Pae et al., 2003). These are similar to the allele frequency in the Chinese population (0.8705) (Lewis et al., 2002), whereas Caucasian populations showed lower frequencies, e.g. 0.6689 in the UK population (Seiawan et al., 2001).

Our study showed no significant difference in the frequencies of the *GSTM1* null mutations between the cases and the controls (57.7 versus 56%). These data conflict with previously published findings reporting an association between the *GSTM1* null mutation and endometriosis (Baranova et al., 1996; Baranova et al., 1997). However, according to more recent reports, there were similar results to our study: no significant differences in the frequency of the *GSTM1* null mutation in control and endometriosis cases in south-east England (Baxter et al., 2001) and the OXEGENE collaborative group report (Hadfield et al., 2001) were found. Moreover, in the Japanese population, there is no association of endometriosis with the *GSTM1* mutations (Morizane et al., 2004).

In our study, no significant difference in the frequencies of the *GSTT1* null mutations between the endometriosis patients and the controls was observed (53.6 versus 48.3%). The lack of an association between the *GSTT1* null mutation and the endometriosis is consistent with the previous reports of Baranova et al. (1999), the OXEGENE collaborative group (Hadfield et al., 2001) and of the Japanese population (Morizane et al., 2004).

There was no previously reported data about endometriosis associated with the *GSTP1* polymorphism. We report here that *GSTP1* polymorphism does not influence the overall risk of advanced stage endometriosis.

When the effect of the combined genotypes is different from the sum of the independent effects of each genotype, there is a possible
interaction or synergic effect of each genotype. Some studies have reported the association of combined gene polymorphisms with endometriosis and cancer risk. But in our study, the combination of GSTM1 null and GSTT1 null did not increase the risk of endometriosis. Kihara and Noda (1999) reported an indication of a potential interaction between the GSTP1 and GSTM1 genes in a population of Japanese male smokers aged 50–69 years, in which a higher risk of lung cancer was associated with the combination of the variant allele for the GSTP1 and GSTM1 null genotype. We found that risk of endometriosis was not associated with the combination of the GSTP1 and GSTM1 null genotypes. The increased risk for women with GSTM1 null and GSTT1 null genotypes together with GSTP1 val/val genotype (OR = 3.77, 95% CI = 1.10–12.88) was reported in breast cancer (Charrier et al., 1999), and chronic lymphocytic leukaemia (Yuille et al., 2002). In this present study, we think there is no possibility of a trend of an increasing risk of endometriosis with the number of putative ‘high-risk’ alleles of the GST family.

The hypothesis of various GST polymorphisms possibly being associated with pathophysiology of endometriosis is not proven in this study.

In conclusion, an association between endometriosis with GSTM1, GSTT1 and GSTP1 gene polymorphisms did not exist. The genotypes and allele frequencies of these polymorphisms are not useful markers for the prediction of endometriosis susceptibility.

References
Hu X, Herzog C, Zimmnack P and Singh S (1999) Differential protection against benzo(a)pyrene-7,8-dihydriodiol-9,10-epoxide-induced DNA damage in

<table>
<thead>
<tr>
<th>No. of high-risk genotypes</th>
<th>GST status</th>
<th>GSTT1</th>
<th>GSTP1</th>
<th>Cases</th>
<th>Controls</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Present</td>
<td>Present</td>
<td>val/val, val/val</td>
<td>10</td>
<td>16</td>
<td>1.0*</td>
<td>(0.493)</td>
</tr>
<tr>
<td>1</td>
<td>Present</td>
<td>Null</td>
<td>ile/val, val/val</td>
<td>52</td>
<td>89</td>
<td>0.995</td>
<td>(0.464)</td>
</tr>
<tr>
<td>2</td>
<td>Null</td>
<td>Present</td>
<td>ile/ile</td>
<td>91</td>
<td>106</td>
<td>1.462</td>
<td>(0.167)</td>
</tr>
<tr>
<td>3</td>
<td>Null</td>
<td>Null</td>
<td>ile/ile</td>
<td>41</td>
<td>48</td>
<td>1.367</td>
<td>(0.493)</td>
</tr>
</tbody>
</table>

*GSTM1 and GSTT1 non-deleted (present) and GSTP1 val/val and ile/ile-genotypes used as the reference group.
OR = odds ratio; CI = confidence interval.


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