**GSTM1 null polymorphism and susceptibility to endometriosis and ovarian cancer**

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It is likely that heritable genetic factors contribute to the development of endometriosis, which is a putative precursor of the endometrioid and clear cell histological subtypes of ovarian cancer. The phase II glutathione-S-transferases (GSTs) are a family of enzymes responsible for metabolism of a broad range of xenobiotics and carcinogens. Allelic variants of GSTs that have impaired detoxification function may increase the rate of genetic damage and thereby increase the susceptibility to cancer. The null genetic polymorphism in the gene encoding the GST class μ (GSTM1) enzyme has been reported to be significantly elevated in endometriosis patients and may represent an endometriosis susceptibility allele. In this study the frequency of the GSTM1 null genotype was investigated in 84 cases of endometriosis, 293 cases of ovarian cancer and 219 controls. All cases and controls were derived from women resident in the south east of England. The frequency of the GSTM1 null allele was not over-represented in the endometriosis patients (47.6%) compared with the controls (48.9%) (P = 0.898). In the ovarian cancer group the GSTM1 null genotype was significantly elevated compared with controls (59.0 versus 48.9%, P = 0.025). When stratified according to histological subtype a significantly increased GSTM1 null genotype was only observed for the endometrioid (65.4%, P = 0.013) and the combined endometrioid/clear cell ovarian cancers (67.0%, P = 0.004). We conclude 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.

**Introduction**

Endometriosis is defined as a condition in which tissue histologically similar to endometrium is found at sites outside the uterine cavity. The aetiology of endometriosis is uncertain, but implantation of viable endometrium refluxed into the peritoneal cavity during menstruation is the most widely invoked theory to explain its origin (1). Very little is known about the underlying mechanisms that lead to the development of endometriosis, which is remarkable given that it is one of the most common gynaecological diseases that may affect more than 10% of all pre-menopausal women (2). Although endometriosis is generally considered a benign disease there is good evidence to suggest that it may undergo malignant transformation and is the precursor of endometrioid and clear cell ovarian cancer (3–6).

Somatic genetic alterations have been identified in endometriotic lesions and these may contribute to its initiation and progression (5,6). It is likely that heritable genetic factors also contribute to the development of endometriosis (7–9). In the cancer field many genes are now being investigated as potential low penetrance predisposing genes (10) and some of these are now being investigated in endometriosis. The phase II glutathione-S-transferases (GSTs) are a family of enzymes responsible for the metabolism of a broad range of xenobiotics and carcinogens. It has been proposed that GSTM1 is critical in the detoxification of the products of oxidative stress produced during repair of the ovarian epithelium following follicle rupture. Failure to detoxify these products may result in rapid accumulation of genetic damage and increase susceptibility to epithelial ovarian cancer. A similar model could be proposed for endometriosis, since it is characterized by cyclical degeneration and chronic inflammation, conditions which will result in production of reactive oxygen species. An elevated frequency of the inactive variant of the GSTM1 gene has recently been reported in endometriosis patients from Russia, Ukraine and France (11–13). In particular, Baranova et al. (12) observed a highly significant excess of the GSTM1 null genotype among French women with endometriosis compared with controls, suggesting that defects in carcinogen detoxification may be involved in the pathogenesis of this disease. Although very interesting, this conclusion was based on a small number of cases and needs to be interpreted cautiously.

In ovarian cancer only two studies have investigated the GSTM1 null polymorphism and neither observed any significant associations, but again the size of each study was small (14,15). In addition, neither study investigated with any rigour potential differences in GSTM1 genotype frequencies with respect to histological subtype. This is an important consideration, as there is good evidence to suggest that the different histological subtypes of ovarian cancer arise via distinct pathways (16,17). In particular, the endometrioid and clear cell subtypes probably arise via malignant transformation of endometriosis and not the ovarian surface epithelium (6). Consequently, if the GSTM1 null allele represents an endometriosis predisposing allele then it should also be over-represented among endometrioid and clear cell ovarian cancers.

The aim of this study was to assess the risk of endometriosis and ovarian cancer associated with the GSTM1 null polymorphism and to investigate possible associations with histological subtypes of ovarian cancer.

**Materials and methods**

**Subjects**

Details of cases and controls have been described previously (18–20). Incident cases of ovarian tumours and endometriosis were ascertained from women...
undergoing primary surgery for these diseases in hospitals from south east England between 1993 and 1998. The control group consisted of 219 healthy Caucasian female volunteers, also from south east England. Only malignant ovarian tumours (n = 293) and ovarian endometriosis (n = 84) were included in the study. In each case the histological diagnosis was confirmed by a specialist gynaecological pathologist. The ovarian cancers studied consisted of 127 serous, 40 mucinous, 78 endometrioid, 13 clear cell, 29 undifferentiated and 6 mixed Müllerian tumours.

**GSTM1 genotyping**

DNA was prepared from blood lymphocytes as described previously (21). The *GSTM1* gene deletion polymorphism was identified by amplification of a part of exon 4 which lies within the 10 kb deletion in the null genotype. The primers used for *GSTM1* were G1 (5’-ctgccactgtagatggg-3’) and G2 (5’-ctggattgtagcagatcatgc-3’), which amplify a 271 bp product in non-null homozygotes and non-null heterozygotes but not in null homozygotes (11). To confirm successful amplification an internal control was included in each PCR reaction, which consisted of a primer set which amplifies a 201 bp polymorphic exonic CAG microsatellite in the *AIB1* gene (22). PCR was carried out in a reaction volume of 10 μl containing 10–200 ng genomic DNA, 2.5 mmol/nl, 1X reaction buffer (Applied Biotechnologies, UK), 200 nM dATP, dTTP, dGTP and dCTP (Promega, UK) and 0.2 U Taq DNA polymerase (Red Hot Taq; Applied Biotechnologies, UK). PCR consisted of an initial denaturation at 94°C for 5 min followed by 35 cycles of 94°C for 40 s, 56°C for 60 s and 72°C for 60 s, with one cycle of 72°C for 10 min. Reaction products were resolved on 2% agarose gels.

**Statistical analysis**

Comparisons of frequency were analysed using Fisher’s exact test.

**Results**

**GSTM1** null genotyping performed by multiplex PCR enabled the precise discrimination of individuals homozygous for the null allele. In order to reduce the possibility of recording a false *GSTM1* null genotype the PCR conditions were optimized so that amplification was biased towards the *GSTM1* gene fragment as illustrated in Figure 1. Over 100 samples were genotyped twice in independent experiments to assess the consistency of the assay and in no case was a discrepancy observed. Table I shows the *GSTM1* null genotype frequency among 84 cases of endometriosis, 293 cases of ovarian cancer and 219 controls. The *GSTM1* null genotype frequency of 48.9% among the controls is consistent with previous reported Caucasian control populations (10). The *GSTM1* null genotype among the endometriosis cases was not significantly different from the controls (P = 0.898). However, because of the relatively small number of cases we had only a 30% power to detect an odds ratio (OR) of 1.5 at a significance level of 0.05. Among the ovarian cancers as a whole the *GSTM1* null genotype was significantly higher than the controls (59.0 versus 48.6%, P = 0.025). Stratifying the ovarian cancers with respect to histological subtype revealed that only the endometrioid subtype had a significantly increased frequency of the *GSTM1* null genotype (65.4%, P = 0.013). The *GSTM1* null genotype frequency was strikingly high among the clear cell types (76.9%), but this did not reach statistical significance because of the low number of cases studied. Clear cell ovarian tumours are generally considered to be a variant of the endometrioid type and are likely to have a common aetiology (23). The frequency of the *GSTM1* null genotype among the combined clear cell and endometrioid types was 67%, which was highly statistically significant (P = 0.004). The frequency of the null genotype among the serous subgroups was also high compared with the controls (57.5 versus 48.9%), but this failed to reach statistical significance (P = 0.147).

**Discussion**

Baranova et al. (12) reported a highly significant excess of the *GSTM1* null genotype in French women with endometriosis versus controls (76.9 versus 45.8%, P = 0.0001) and concluded that the *GSTM1* null allele represents a predisposing factor for endometriosis. However, this conclusion was based on only 65 cases and 72 controls. Our study of 84 endometriosis cases and 219 controls does not provide any support for the assertion that the *GSTM1* null allele is a predisposing factor for endometriosis. Although our study is only slightly larger than that reported by Baranova et al., we did not observe any excess of the *GSTM1* null genotype among the endometriosis cases and, indeed, the frequency was lower than in the controls. Consequently, we believe that the *GSTM1* null genotype is unlikely to represent a significant risk factor for endometriosis. Nevertheless, this will need verification from additional independent studies.

We observed a significant elevation in the *GSTM1* null genotype among 293 ovarian cancers, which is in contrast to two previous studies (14,15) which failed to detect any significant association. However, both of these studies were lacking in statistical power since they involved only 84 cases and 325 controls (14) and 103 patients and 115 controls (15). In addition, our analysis with respect to histological subtype revealed that the high frequency of *GSTM1* null genotypes was predominantly due to a highly significant excess among the endometrioid and clear cell subtypes [P = 0.004, OR 2.14, 95% confidence interval (CI) 1.28–3.55]. The two previous studies did not specifically address histological subtype frequencies of *GSTM1* null genotypes, although Hengstler et al. (15) did not observe a difference between serous

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**Table I. GSTM1 null frequency among endometriosis and ovarian cancer cases**

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>GSTM1 null</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OR (95% CI)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>219</td>
<td>107 (48.9%)</td>
<td>0.898</td>
<td>0.95 (0.58–1.58)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>84</td>
<td>40 (47.6%)</td>
<td>0.393</td>
<td>0.93 (0.53–1.63)</td>
</tr>
<tr>
<td>All ovarian cancers&lt;sup&gt;c&lt;/sup&gt;</td>
<td>293</td>
<td>173 (59.0%)</td>
<td>0.025</td>
<td>1.54 (1.06–2.14)</td>
</tr>
<tr>
<td>EC</td>
<td>78</td>
<td>51 (65.4%)</td>
<td>0.013</td>
<td>1.98 (1.16–3.38)</td>
</tr>
<tr>
<td>CC</td>
<td>13</td>
<td>10 (76.9%)</td>
<td>0.083</td>
<td>3.49 (0.93–13.03)</td>
</tr>
<tr>
<td>EC/CC</td>
<td>91</td>
<td>61 (67.0%)</td>
<td>0.004</td>
<td>2.14 (1.28–3.55)</td>
</tr>
<tr>
<td>S</td>
<td>127</td>
<td>73 (57.5%)</td>
<td>0.147</td>
<td>1.42 (0.91–2.20)</td>
</tr>
<tr>
<td>MU</td>
<td>40</td>
<td>21 (52.5%)</td>
<td>0.732</td>
<td>1.16 (0.59–2.27)</td>
</tr>
<tr>
<td>UD</td>
<td>29</td>
<td>16 (55.2%)</td>
<td>0.558</td>
<td>1.29 (0.59–2.81)</td>
</tr>
<tr>
<td>MMT</td>
<td>6</td>
<td>2 (33%)</td>
<td>0.684</td>
<td>0.52 (0.09–2.92)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Fisher’s exact test.

<sup>b</sup>Odds ratio with 95% confidence interval in parentheses.

<sup>c</sup>Histological subtype abbreviations: EC, endometrioid; CC, clear cell; S, serous; MU, mucinous; UD, undifferentiated adenocarcinoma; MMT, mixed Müllerian tumour.
and non-serous subtypes, which is in fact consistent with our findings. It is becoming increasingly clear that analysis of epithelial ovarian cancer as a single disease is inappropriate as there is abundant evidence to show that the major histological subtypes develop via different pathways. This is particularly true for the endometrioid and clear cell ovarian cancers, which are believed to arise via malignant transformation of endometriotic foci which are endometrial in origin (6). We hypothesize that endometrioid and clear cell ovarian cancers are likely to be more closely associated at the molecular genetic level with endometrial cancers, rather than with the serous or mucinous subtypes. In this respect it is interesting that a small study of endometrial cancers also demonstrated a significant excess of GSTM1 null genotypes among 80 endometrial cancers and 60 controls (24).

Isoforms of GSTM1 catalyse the detoxification of genotoxic chemicals, including the products of chronic oxidative stress such as cytotoxic lipid and DNA species (25). The GSTM1 null allele is an attractive candidate as an endometrioid ovarian cancer susceptibility allele since endometriosis is characterized by cyclical degeneration and chronic inflammation, conditions which will result in the production of reactive oxygen species. Consequently, impairment of GSTM1 function within an endometriotic lesion is likely to result in increased susceptibility to DNA damage and a propensity to malignant transformation.

In summary, our data suggest that the GSTM1 null allele does not increase susceptibility to endometriosis. The significant excess of GSTM1 null alleles among the ovarian cancers may be accounted for by a frequent increase among the endometriotic foci in individuals who are GSTM1 null may be prone to malignant transformation due to inefficient removal of the products of oxidative stress. Although this model is biologically plausible, we recognize that our conclusions are based on relatively small numbers and will require verification from additional independent studies.

References


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GSTM1, endometriosis and ovarian cancer