Linkage and association studies of the relationship between endometriosis and genes encoding the detoxification enzymes \textit{GSTM1}, \textit{GSTT1} and \textit{CYP1A1}

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An association between endometriosis and the glutathione \textit{S}-transferase (\textit{GST}) \textit{M1} null mutation has been reported in French and Slavic populations. We aimed to replicate this association of endometriosis in a UK population, and to test for association with the \textit{GSTT1} null mutation or the cytochrome \textit{P450 (CYP) 1A1 MspI} polymorphism. We genotyped 148 women each with endometriosis (sporadic cases, \(n = 91\); familial cases, \(n = 57\)), a population control of 95 male blood donors, and a control group of 53 women with a normal pelvis at hysterectomy. No significant differences were found between cases and controls in the frequencies of the \textit{GSTM1} and \textit{GSTT1} null mutations, or the \textit{CYP1A1 MspI} polymorphism. However, the combination of the \textit{GSTM1} null genotype and the \textit{CYP1A1 MspI} polymorphism was associated with a small increased risk of endometriosis, and this warrants further investigation. We also tested for linkage to the chromosome 1p13 region, to which \textit{GSTM1} has been mapped, in 52 sister-pairs with stage III–IV disease using three highly polymorphic microsatellite markers. However, there was no evidence of linkage, suggesting that this region may not be implicated in disease susceptibility.

\textit{Key words: CYP1A1/endometriosis/genetics/GSTM1/GSTT1}

\textbf{Introduction}

Studies investigating the relationship between endometriosis and polymorphisms in several candidate genes involved in xenobiotic detoxification have been reported (Baranov et al., 1996; Baranova et al., 1997a, 1999). The genes encoding the enzymes glutathione \textit{S}-transferase (\textit{GST}) \textit{M1} and \textit{T1} and arylamine \textit{N}-acetyltransferase (\textit{NAT2}) have been studied because of data suggesting that exposure to environmental pollutants, in particular dioxins, may be implicated in the aetiology of the disease (Rier et al., 1993).

Enzymes belonging to the \textit{GST} and cytochrome \textit{P450 (CYP)} families are involved in the two-stage detoxification process of a number of pro-carcinogens. The genes for these enzymes are part of the aryl hydrocarbon (Ah) gene battery and are under Ah receptor control (Nebert and Gonzalez, 1987). The Ah receptor binds a number of different classes of chemicals, including halogenated aromatics such as dioxin, polycyclic aromatic hydrocarbons and plant metabolites, such as \(\beta\)-naphthoflavone, all of which induce transcription of the genes in this battery (Safe, 1995). Dioxin and the other compounds act by binding to and activating the Ah receptor. The receptor–dioxin complex is translocated to the nucleus where it binds to DNA in the 5'-flanking region and activates transcription of \textit{CYP} genes, such as \textit{CYP1A1} and \textit{CYP1A2}, which code for phase I detoxification enzymes. These enzymes act by metabolizing pro-carcinogens into reactive, carcinogenic intermediates which are subsequently catalysed with enzymes such as the glutathione-\textit{S}-transferases (\textit{GST}), in the phase II detoxification process.

The gene encoding the enzyme \textit{CYP1A1} gene (15q22–q24) has a polymorphism which is 264 bases downstream from the polyadenylation site at the 3' end of exon 7. The thymine to cytosine substitution introduces a new \textit{MspI} restriction site, facilitating genotyping. This \textit{MspI} polymorphism has been associated with an increased risk for smoking-induced lung cancer (Xu et al., 1996). It has been demonstrated that high-inducibility of the \textit{CYP1A1} gene is related to the presence of
the Mspl polymorphism (Petersen et al., 1991), although the exact mechanism of this relationship is unknown.

The glutathione S-transferases (GST) catalyse the conjugation of reactive hydrophobic and electrophilic compounds to reduced glutathione thereby deactivating their toxicity. Many of the known GST substrates are xenobiotic, and different classes of enzymes are specific for different substrates (Board et al., 1990). Two of the genes for GST isoenzymes, GSTM1 (1p13) and GSTT1 (2q11.2), have deletions which result in null alleles, for which homozygosity confers a complete lack of enzyme activity. The enzymes appear to be important in the detoxification of products of oxidative stress, for example lipid hydroperoxides, alkenals and DNA hydroperoxides, as well as potential carcinogens such as methyl halides, benz[a]pyrene epoxides and the polycyclic aromatic hydrocarbons (PAH) present in diet and in tobacco smoke (Sarhanis et al., 1996). The null mutation of GSTM1 has been associated with colon cancer (Zhong et al., 1995), chronic bronchitis in heavy smokers (Baranova et al., 1997b) and prostate cancer (Murata et al., 1998).

An association between endometriosis and the GSTM1 null mutation was first observed in a Slavic population: 34/42 (81%) of affected women were homozygous compared to 26/67 (39%) of controls (Baranova et al., 1996). The same group (Baranova et al., 1997a) then found in a French population that 43/50 (86%) women with all stages of endometriosis had the GSTM1 null mutation, compared to 33/73 (46%) of a control group consisting of women having a termination of pregnancy (P < 0.0001). If only women with stage III–IV disease were included, then 19/21 (90%) had the GSTM1 null mutation. More recently, no association was found between endometriosis and the GSTT1 null mutation (Baranova et al., 1999).

In view of the previously reported association between endometriosis and the GSTM1 null mutation, we aimed to investigate this association in a UK population, and to test in sister-pair families for linkage to the chromosomal region, 1p13, to which GSTM1 has been mapped. An analysis of the combination of CYP1A1 Mspl and the GSTM1 null mutation was also conducted to determine whether women with endometriosis were more likely to carry both mutations. This strategy was justified because the presence of the CYP1A1 Mspl polymorphism, which may enhance the up-regulation of transcription by compounds such as dioxin, in combination with the absence of activity in a phase II enzyme, such as GSTM1, could result in an excess of reactive intermediates which may in turn be involved in disease aetiology.

Materials and methods

Association studies

Study participants

Blood or tissue was obtained from the following four groups, and DNA was extracted for genotyping: (i) male blood donors (n = 100), (ii) pre-menopausal women aged 40–50 with a normal pelvis at hysterectomy (n = 54), (iii) sporadic cases with histologically confirmed endometriosis (n = 101) and (iv) unrelated women with surgically confirmed moderate–severe (stage III–IV) (American Fertility Society, 1995) endometriosis and a family history of the disease (n = 57). Fifty-five of the affected familial cases were Caucasian; the ethnicity of the remaining two was unknown. The ethnicity of the sporadic cases and controls was also unknown because of the need to maintain anonymity. However, non-Caucasian, male blood donors in the Oxford region are very rare and the overwhelming majority of women undergoing surgery at the John Radcliffe are Caucasian. Ethical approval was obtained from the Oxford Research Ethics Committee for the use of samples from known individuals.

Population controls. Samples from males who had donated blood in the Oxford region were supplied anonymously by the National Blood Service.

Normal controls. Pre-menopausal women, aged 40–50 years, with a normal pelvis at hysterectomy for benign menstrual disorders were identified through the records of the John Radcliffe Hospital, Oxford. Women from this age group were chosen to maximize the probability that they were unaffected by endometriosis, i.e. to avoid including younger women who might develop the disease in later life. Women with adenomyosis were excluded from the study. Normal tissue, fixed in formalin and paraffin-embedded, from these patients was provided anonymously from the archives of the Cellular Pathology Department at the John Radcliffe Hospital.

Sporadic cases. Women with histologically confirmed endometriosis were identified from histopathology reports. Samples of normal, formalin-fixed, paraffin-embedded tissue from these patients were provided anonymously as for group normal controls.

Familial cases. Families with endometriosis, including sisters, mother-daughter pairs and other pedigrees with surgically confirmed disease were recruited for the Oxford Endometriosis Gene (OXEGENE) study (Kennedy, 1997). The aim of the study is to identify genes conferring susceptibility to endometriosis. Blood samples were obtained from family members of pedigrees with surgically confirmed stage III–IV disease. A sample from only one individual with stage III–IV disease from each UK family was used in the association studies.

Genotyping

DNA was extracted from 9 ml EDTA anti-coagulated blood samples using either the standard proteinase K phenol–chloroform method (for familial cases), the Q1Amp (Qiagen, UK) DNA extraction kit (for population controls) or the Q1Amp tissue kit (for normal controls and sporadic cases).

CYP1A1 Mspl polymorphism. A 340 bp fragment of the 3′-end of CYP1A1 containing the polymorphic region of interest was amplified using PCR, as described previously (Sivaraman et al., 1994). Primers were synthesized by Gibco Life Technologies, Rockville, USA (5′-CAGTGAAGAGG-TGATAGCGCT; 3′-TGGGAGTCTTGTCT-CATOCCT). The 15 μl polymerase chain reaction (PCR) reaction contained 0.1 μg DNA, 0.2 μg each primer, 1× PCR buffer, 2.5 mmol/l MgCl₂, 0.2 mmol/l each dNTP and 0.3 units Taq DNA polymerase (all purchased from Qiagen). Reactions were amplified using the following thermal profile: initial denaturation of 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 63°C for 30 s and elongation at 72°C for 30 s, and a final elongation of 72°C for 5 min (ABI 9700 thermocycler; PE Applied Biosystems). The PCR products were digested with Mspl (Boehringer Mannheim) for 3 h at 37°C. The products of digestion were visualized under UV light after electrophoresis on a 1.5% agarose gel containing ethidium bromide (0.1 μg/ml). Genotyping was successful in 98/100 (98%) male controls, 49/54 (91%) normal controls, 76/101 (75%) sporadic cases and 53/57 (93%) familial cases. The PCR was successful less often when DNA was extracted from paraffin-embedded, formalin-fixed archival tissue (i.e. in the sporadic cases). As a result
of the fixation in formalin, the extracted DNA fragment sizes were <600 bp, and the likelihood of successful amplification was diminished.

**Multiplex PCR for genotyping of GSTM1 and GSTT1.** A multiplex PCR was conducted to genotype for GSTM1 and GSTT1 simultaneously, as previously described (Arand et al., 1996). For this method, primers for fragments of the GSTM1 and GSTT1 genes, and primers for an omnipresent albumin gene control were placed into the same PCR reaction tube. If the study subject is null for the gene, no PCR product is present, but the albumin gene fragment acts as a positive PCR control. Primers were synthesized by Gibco Life Technologies. The 15 µl PCR reaction contained 0.1 µg of the DNA sample to be genotyped, 4.5 ng GSTM1 primers, 1.5 ng GSTT1 primers and 9 ng albumin primers, 1× PCR buffer, 2.5 mmol/l MgCl2, 0.2 mmol/l each dNTP and 0.3 U Taq DNA polymerase (all purchased from Qiagen). Reactions were amplified using the following thermal profile: initial denaturation of 95°C for 2 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 64°C for 1 min and elongation at 72°C for 1 min, followed by a final elongation of 72°C for 5 min. Other procedures were identical to the CYP1A1 genotyping.

Genotyping for GSTM1 was successful in 100/100 (100%) male controls, 75/101 (74%) sporadic cases and 57/57 (100%) familial cases. Genotyping for GSTT1 was successful in 98/100 (98%) male controls, 75/101 (74%) sporadic cases and 57/57 (100%) familial cases. As for CYP1A1, genotyping had a lower success rate in the sporadic cases, because the DNA was extracted from formalin-fixed tissue.

**Statistical analysis.** Statistical comparisons of proportions of genotypes were made using a two-sided Fisher’s exact test at the 95% level, unless otherwise stated.

**Sib-pair analysis**

**Familial DNA samples for linkage analysis**

Families with endometriosis were recruited by a number of methods, as previously described (Kennedy, 1997). DNA was extracted from EDTA anti-coagulated blood samples using the standard proteinase K, phenol/chloroform method. In total, 192 individuals from 43 nuclear families, involving in total 52 sister-pairs with stage III–IV endometriosis, were genotyped for these three markers described below. The families were from the UK (n = 17), the USA (n = 18), Norway (n = 3), Germany (n = 3), Canada (n = 1) and Belgium (n = 1).

**Genotyping**

From the Généthon microsatellite maps (Dib et al., 1996), three markers (D1S2635-10cM-D1S2844-5cM-D1S2762) were selected from the region of chromosome 1 to which GSTM1 has been mapped (Zhong et al., 1992). They were chosen for high heterozygosity to maximize the information content. Primers (Table I) were synthesized by PE Applied Biosystems and the forward primer was fluorescence-labelled. A separate PCR reaction mixture was prepared for each primer pair, containing 0.33 µmol/l of each primer, 1×PCR buffer, 250 µmol/l each dNTP, 0.04 U/µl AmpliTaq Gold™ Taq polymerase and 2.5 µmol/l MgCl2 and 0.1 µg target DNA in a final PCR volume of 7.5 µl. All reagents were purchased from PE Applied Biosystems. PCR amplification was performed without mineral oil, using an ABI 9700 thermocycler (PE Biosystems) with heated lid. Reactions were amplified using the following thermal profile: initial denaturation of 95°C for 12 min, followed by 10 cycles of denaturation at 94°C for 15 s, annealing at 55°C for D1S2844 and D1S2635 or 50°C for D1S2762 for 15 s and elongation at 72°C for 30 s, followed by 20 cycles of denaturation at 90°C for 15 s, with other conditions the same as for the first 10 cycles.

DNA from a particular patient was included in every run to control for gel-to-gel variation. The three PCR products from each patient were pooled and run on an ABI 377 (PE Biosystems). Analysis was conducted using Genescan® and Genotyper® software (PE Biosystems).

**Statistical analysis.** Statistical multipoint analysis of the data was conducted using GeneHunter-Plus version 1.2 (Kruglyak et al., 1996; Kong and Cox, 1997). The non-parametric linkage (NPL) function used was the ‘all’ function which examines all individuals simultaneously, assigning a higher score when more affected individuals share the same allele identical by descent (IBD). The corresponding P values and marker information content values were also determined using this package. We also applied the transmission disequilibrium test (TDT) (Spielman et al., 1993; Sham and Curtis, 1995) to the sib-pair genotype data. The TDT tests for both linkage and linkage disequilibrium and is based on the unequal probability of transmission of two different marker alleles from parents to affected offspring.

**Results**

**CYP1A1 MspI association study**

The CYP1A1 MspI variant allele frequencies in male blood donors (0.10) and in women with a normal pelvis (0.14) were comparable to the control group frequency of 0.09 observed in two other studies (Sivaraman et al., 1994; Lucas et al., 1996).
A typical gel is shown in Figure 1 and the results are summarized in Table II. No significant differences were found between the polymorphism frequency in sporadic cases compared to that in male controls or women with a normal pelvis at hysterectomy. Likewise, no significant differences were observed between the frequency in familial cases and the two different control groups.

**GSTM1 and GSTT1 association studies**
The proportion of control group individuals with null mutations (20 and 28% for GSTT1 and 45 and 52% for GSTM1) was comparable to population controls in other studies. For example, amongst female controls recruited in the UK, the prevalence of the GSTT1 null mutation has been 61/325 (19%) (Sarhanis et al., 1996) and the prevalence of the GSTM1 null mutation among controls has been 94/225 (42%) in a UK-based study (Zhong et al., 1993).

A typical gel is shown in Figure 2 and the results are summarized in Table III. No significant differences were found between the four case groups and the two control groups for either the GSTM1 or the GSTT1 null mutations.

**CYP1A1 MspI polymorphism/GSTM1 null mutation combination study**
An analysis of the proportion of patients who carried the GSTM1 null mutation and the CYP1A1 MspI polymorphism was conducted. Among male controls who had the GSTM1 null mutation, 5/44 (11%) carried at least one MspI variant CYP1A1 allele. Among women with a normal pelvis, 4/26 (15%) had the same combination of genotypes. In comparison, 8/29 (28%) of sporadic cases and 9/24 (38%) of familial cases had both the GSTM1 null mutation and carried at least one MspI variant CYP1A1 allele. The difference between the proportion of male controls and familial cases with the genotype combination was statistically significant \( P = 0.025 \), relative risk \( = 4.68 \), 95% confidence interval \( (CI) = 1.17–19.6 \). Comparisons between the other groups of cases and controls were not significantly different (for male controls versus sporadic cases, \( P = 0.11 \); normal controls versus familial cases, \( P = 0.11 \); sporadic cases, \( P = 0.34 \)).

The proportion of individuals in each group who had both the GSTM1 and GSTT1 null mutations was not significantly different. Combining the frequencies of the GSTT1 null mutation and CYP1A1 MspI polymorphism resulted in sample sizes that were too low for valid comparison.

**Linkage studies**
The proportion of individuals successfully genotyped was 99% for D1S2635, 91% for D1S2844 and 93% for D1S2762. The accuracy of the genotyping data was checked by comparing the calculated heterozygosity of each marker to published values from Génethon (Dib et al., 1996). The observed and expected values were not significantly different for any of the three markers. The observed heterozygosity was 85% for D1S2635 (published value 87%), 82% for D1S2844 (published value 81%) and 83% D1S2762 (published value 81%).

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Variant allele frequency</th>
<th>CYP1A1 MspI (+/-) or (+/+</th>
<th>95% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male controls</td>
<td>98</td>
<td>0.10</td>
<td>18 (18)</td>
<td>9.8–25.6</td>
</tr>
<tr>
<td>Women with a normal pelvis at hysterectomy</td>
<td>49</td>
<td>0.14</td>
<td>12 (24)</td>
<td>13.3–38.9</td>
</tr>
<tr>
<td>Cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All casesb</td>
<td>129</td>
<td>0.11</td>
<td>27 (21)</td>
<td>14.3–29.0</td>
</tr>
<tr>
<td>Stage III-IV disease only</td>
<td>98</td>
<td>0.11</td>
<td>21 (21)</td>
<td>13.8–30.9</td>
</tr>
<tr>
<td>Sporadic cases only</td>
<td>76</td>
<td>0.10</td>
<td>14 (18)</td>
<td>10.5–29.0</td>
</tr>
<tr>
<td>Familial cases only</td>
<td>53</td>
<td>0.12</td>
<td>13 (25)</td>
<td>13.8–38.3</td>
</tr>
</tbody>
</table>

Results are given as both the overall polymorphic allele frequency and the number of subjects carrying either one (+/-) or two (+/+ ) polymorphic alleles. Values in parentheses are percentages.
*95% confidence interval for the percentage of +/- or +/- subjects.

**Figure 2.** Electrophoresis of the products of multiplex polymerase chain reaction (PCR). The 480 bp band shows the presence of the GSTT1 gene (upper lanes 1–3, 5–8, 10; lower lanes 1, 2, 4–6, 8, 9) and the 215 bp band shows the presence of the GSTM1 gene (upper lanes 1, 3, 6, 9; lower lanes 2, 4, 6–7). The 350 bp band is an albumin gene fragment, which gives a positive PCR control. The lower lane 10 shows a negative PCR control. The methodology cannot identify individuals hemizygous for the null mutations and consequently the cases with bands present above are either non-null/non-null homozygotes or null/non-null heterozygotes.

Table II. Prevalence of the CYP1A1 MspI polymorphism in women with endometriosis and controls
Presented here was designed according to the frequencies studies (Lander and Schork, 1994). The association study sampled may be heterogeneous. Finally, it is possible that the aetiologies, as has long been suggested. Thirdly, the populations endometriosis may be a heterogeneous condition with different exposure to environmental risk factors. This implies that frequency of the polymorphism in the population and the genes may in ...

Discussion

No significant differences in the frequencies of the GSTM1 and GSTT1 null mutations between cases and controls were observed. These data conflict with previously published findings reporting an association between the GSTM1 null mutation and endometriosis (Baranov et al., 1996; Baranova et al., 1997a). However, they are entirely consistent with a more recent report which found no significant difference in the frequency of the GSTM1 null mutation in 84 women with endometriosis and 219 healthy female volunteers from south east England (47.6 versus 48.9%) (Baxter et al., 2001). The lack of an association between the GSTT1 null mutation and endometriosis is consistent with the findings of Baranova et al. (1999).

The different findings can be explained in a number of ways. Firstly, it is important to choose appropriate controls in endometriosis association studies given that the patients are usually selected on the basis of having infertility and/or pain. The control group in one study (Baranova et al., 1997a), consisting of women undergoing termination of pregnancy, may not be representative of the general population. Secondly, genes may influence disease susceptibility relative to the frequency of the polymorphism in the population and the exposure to environmental risk factors. This implies that endometriosis may be a heterogeneous condition with different aetiologies, as has long been suggested. Thirdly, the populations sampled may be heterogeneous. Finally, it is possible that the findings conflict because one or more of the studies was not sufficiently powered, a potential problem with all association studies (Lander and Schork, 1994). The association study presented here was designed according to the frequencies previously observed: using 106 cases and 106 controls, the study had 99% power (α = 0.0001) to detect the odds ratio (OR) of 7.2 reported by Baranova et al. (1997a). Similar numbers (113 cases and 113 controls) would have detected an OR of 3 with 90% power (α = 0.01). It is therefore unlikely that the present study lacked the power to find a similar association. One drawback of the study is that individuals were not genotyped for heterozygosity of the GSTM1 and GSTT1 null mutations. It is possible that the presence of one null allele may be sufficient to confer susceptibility for endometriosis. However, the same approach was adopted by Baxter et al. (2001) and, given the high frequency of GSTM1 homozygotes, any significant association should have been detected by genotyping in this way.

No significant differences were observed in this study between the frequency of the MspI CYP1A1 polymorphism in endometriosis patients compared to that in the controls. These results almost certainly exclude an association in this population for the polymorphism, given that the study was well powered. Sample sizes of 100 alleles (i.e. 50 individuals) in the case and control groups were required to detect with >80% power an increase of 0.15 over the normal allele frequency of 0.10.

The combination of homozygosity for the GSTM1 null mutation and carrying at least one MspI variant CYP1A1 allele appeared to increase the risk of endometriosis, with 11 and 15% of controls having both genotypes compared to 28 and 38% of cases. The investigation of the combination of genotypes is supported by findings in lung cancer. Benzo[a]pyrene diol-epoxide DNA adduct levels in the lung tissue of lung cancer patients were found to be significantly higher in those patients homozygous for both the CYP1A1 MspI polymorphism and the GSTM1 null mutation, compared to patients with other genotype combinations (Rojas et al., 1998). Other studies have found that Japanese patients with prostate cancer are at increased risk if they have variant CYP1A1 in combination with the GSTM1 null mutation (Murata et al., 1998). Patients with both genotypes also have an increased risk of acute lymphoblastic leukaemia (OR = 3.3) when compared with the risk of either genotype alone (Krajnovic et al., 1999). A third study investigating lung cancer found that Table III. Prevalence of homozygosity for the GSTM1 and GSTT1 null mutations in women with endometriosis and controls without the disease

<table>
<thead>
<tr>
<th>Subjects</th>
<th>GSTM1</th>
<th>GSTT1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% null</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male controls</td>
<td>100</td>
<td>45 (45)</td>
</tr>
<tr>
<td>Women with a normal pelvis at hysterectomy</td>
<td>52</td>
<td>27 (52)</td>
</tr>
<tr>
<td>Cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All casesb</td>
<td>132</td>
<td>59 (45)</td>
</tr>
<tr>
<td>Stage III–IV disease only</td>
<td>96</td>
<td>46 (45)</td>
</tr>
<tr>
<td>Sporadic cases only</td>
<td>75</td>
<td>33 (44)</td>
</tr>
<tr>
<td>Familial cases only</td>
<td>57</td>
<td>26 (46)</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

a95% confidence interval for the percentage of homozygous null subjects.
bSporadic plus familial cases.
neither the GSTM1 null mutation nor the CYP1A1 MspI genotype alone confers an increased risk for lung cancer; however, the presence of both genotypes is associated with increased risk (OR = 2.0, 95% CI 1.0–3.4) (García-Closas et al., 1997).

The observed association between endometriosis and the GSTM1 null mutation with the CYP1A1 MspI polymorphism may justify further investigation in a larger study, taking into account epidemiological factors such as caffeine intake, smoking and region of residence. Interestingly, the GSTM1 null mutation has been associated with high inducibility of CYP1A1 gene transcription by dioxin in in-vitro studies (Vaury et al., 1995). It was demonstrated that the levels of CYP1A1 mRNA induced by dioxin were low when at least one GSTM1 allele was present, whereas the GSTM1 null mutation resulted in the induction of high CYP1A1 mRNA levels by dioxin (Vaury et al., 1995). The possibility of conducting association studies of other genes in the aryl hydrocarbon gene battery, including CYP1A2, NADPH:quinone oxidoreductase, aldehyde dehydrogenase, and UDP-glucuronosyltransferase in relation to endometriosis should also be investigated.

In view of evidence for the relationship between the GSTM1 null mutation, the CYP1A1 MspI polymorphism and endometriosis we conducted linkage analysis in 52 sib-pairs. There was no evidence for linkage to three markers in the chromosome 1p13 region, to which GSTM1 maps, suggesting that this region may not be implicated in disease susceptibility. However, the families studied were drawn from a number of different populations and this may have reduced the power of detecting linkage. The TDT analysis of the three markers also showed no statistically significant evidence for linkage and, as the non-transmitted parental alleles are used as control alleles, this analysis should not be sensitive to population stratification.

In summary, we have found no significant differences between cases and controls in the frequencies of the GSTM1 and GSTT1 null mutations, or of the CYP1A1 MspI polymorphism. There was also no evidence for linkage in 52 sister-pairs with stage III–IV disease using markers in the 1p13 region, to which GSTM1 has been mapped. The combination of the GSTM1 null genotype and the CYP1A1 MspI polymorphism was, however, associated with a small increased risk of endometriosis, and this needs to be investigated further in a larger, independent sample.

Acknowledgements

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References