Single Nucleotide Polymorphism of Pi-Class Glutathione S-Transferase and Susceptibility to Endometrial Carcinoma

Queeny K.Y. Chan,1 Ui-Soon Khoo,1 Hextan Y.S. Ngan,2 Chong-Qing Yang,1 Wei-Cheng Xue,1 Kelvin Y.K. Chan,1,2 Pui-Man Chiu,1 Philip P.C. Ip,1 and Annie N.Y. Cheung1

Abstract

Purpose: Endometrial carcinoma is the most common gynecologic cancer in developed countries. Prolonged unopposed estrogen exposure has been identified as the major risk factor. The pi-class glutathione S-transferase (GSTP1) is a phase II metabolic enzyme that is important in the detoxification of a wide range of electrophiles including carcinogenic steroid-hormone intermediates generated through oxidative metabolism. In this study, we aimed at determining the association between the GSTP1 polymorphism and the risk of endometrial carcinoma in a Chinese population.

Experimental Design: Genotyping of 180 cases and 200 age-matched controls were assessed by PCR-RFLP approach and confirmed by direct sequencing.

Results: Statistical analysis showed that patients of valine allele carriers had 2.03-fold of increased risk of developing endometrial carcinoma ($P < 0.01$). The allele frequencies for the Ile and Val variants between the cancer cases and controls were also significantly different ($P < 0.01$; odds ratio, 1.59; 95% confidence interval, 1.13-2.23). Such association was shown in endometrial cancers as a group and in type I endometrioid adenocarcinoma but not the type II nonendometrioid adenocarcinoma. In addition, the Val allele was found significantly associated with high-grade endometrial cancer and/or endometrial cancer of deep myometrial invasion ($P < 0.01$). Interestingly, the relatively low frequency of Val/Val genotype in both the cancer cases and controls, in parallel with the lower incidence of endometrial cancer in Chinese, was observed when compared with those in Caucasians.

Conclusions: Our findings suggested that the GSTP1 Ile$^{105}$Val polymorphism was associated with an increased risk of endometrial cancer. Further studies may be required to explore the possible significance of these polymorphisms on GSTP1-related metabolism that may affect the susceptibility of Asians to endometrial carcinoma.

Endometrial carcinoma is the most common gynecologic cancer worldwide, although marked variation in the incidence exists among countries. The incidence in the United States, England, and Hong Kong varies from 25, 13.8, to 10 per 100,000 women, respectively (1–5). Such geographic variation may be related to different genetic susceptibility and environmental factors including dietary patterns (1, 2). Prolonged unopposed estrogen stimulation, including use of tamoxifen, late menopause, and obesity, has been identified as the major risk factor for endometrial cancer (6). Individual genetic variation due to polymorphism in genes involved in metabolism of steroid hormones may also be critical in determining the susceptibility of developing endometrial carcinoma (7).

The glutathione S-transferases (GST, EC 2.5.1.18), are a family of phase II metabolic enzymes known to play a role in detoxification of reactive oxidative species (8). In addition, they are capable of binding steroid hormones noncovalently and minimize the effects of short-term fluctuations in extracellular hormone levels (9). There are at least five gene superfamilies (alpha, pi, mu, theta, and zeta) coding for soluble GSTs in human (10). Among all, the pi-class (also called placental GST) is the major isoform expressed consistently in a wide range of tissues, including prostate, placenta, breast, colon, esophagus, and lung (11, 12). We have shown reduced expression of GSTP1, partly due to the promoter hypermethylation, in endometrial cancer (13). Reduced expression of the gene has also been observed in prostate cancer (14), another cancer sensitive to steroid hormone. GSTP1 may thus play a role in hormone-related carcinogenesis.

There are all together 10 single nucleotide polymorphisms published in GSTP1 coding region. Among them, two were nonsynonymous with minor allele frequencies of >0.05 and may be suitable for further study. Ile$^{105}$Val polymorphism is
of particular interest due to its possible functional significance that the altered amino acid composition may be involved in the xenobiotic substrate binding site (H site). Previous studies have shown the significant association of GSTP1 Val/Val genotype with the increased risk of prostate cancer (15, 16). No studies on the association between the GSTP1 polymorphisms and the risk of endometrial cancer have been reported. We therefore designed a retrospective case control study to evaluate the role of the GSTP1 variants as risk factors for endometrial cancer and to investigate the association between these variants and clinicopathologic variables of endometrial cancers.

Materials and Methods

Subjects. One hundred eighty cases of endometrial carcinoma from Chinese populations were retrieved from the archives of Department of Pathology, Queen Mary Hospital, Hong Kong from 1986 to 2002. All patients with endometrial cancer operated and managed at Queen Mary Hospital were recruited for study. Clinical and pathology records were reviewed to ascertain the diagnosis. Patients who were referred from other hospitals where hysterectomy was done were not included in this study because paraffin-embedded tissue blocks were not available for study. The patients' age ranged from 31 to 86 years (mean = 52.93 ± 11.54 years). The cancer cases were histologically reviewed using established criteria (17, 18). Histologically, there were 154 pure endometrioid carcinomas, 16 endometrioid carcinomas with focal squamous differentiation, one mucinous carcinoma as well as seven cases of high-grade papillary serous carcinoma, and two clear cell carcinoma. Two hundred age-matched control subjects ages 27 to 82 years (mean = 54.20 ± 11.23 years), were recruited from patients who had undergone hysterectomy for benign gynecologic conditions, including prolapse (n = 30), leiomyoma (n = 159), endometrial polyp (n = 5), adenomyosis (n = 5), and pyometra (n = 1). Patients with endometrial hyperplasia were excluded as control subjects.

Genotype detection. Before DNA extraction, 5-μm-thick section of formalin-fixed paraffin-embedded tissue block was cut and H&E stained to confirm histologic diagnosis. For the cancer cases, only the non tumor blocks were recruited. Microdissection was done in eight of the cases to remove small foci of tumor tissue. Ten consecutive 10-μm-thick sections from each tissue block were deparaffinized and genomic DNA was extracted using the conventional phenol/chloroform method following the proteinase K digestion (19).

The GSTP1 A<sup>114</sup>G (Ile<sup>105</sup>Val) substitution was detected using the PCR-RFLP approach as the substitution by guanine introduced a restriction site that could be recognized by an endonuclease Alw26I. The primers for the PCR were forward 5’-CAGCCCAACCC-CAGGGCTCTA-3’ and reverse 5’-GGTGCCAGATGCTCACATAGTTGG-3’. The PCR reaction was carried out in a 40-μL mixture containing 100 ng of sample DNA, 1× PCR buffer, 2.0 mmol/L MgCl<sub>2</sub>, 200 mmol/L deoxynucleotide triphosphate, 10 pmol of each primers, and 1.5 units of FastStart Taq polymerase (Roche Applied Science, Indianapolis, IN). Amplification, which resulted in a 132-bp fragment, was achieved by 40 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 1 minute at 72°C. At the end, the reactions were extended for 7 minutes at 72°C. Each PCR product (5 μL) was subjected to Alw26I digestion and electrophoresed on 3% agarose gel. Direct sequencing was done in 50 cases to confirm results of RFLP.

Statistical analysis. Two-sided Pearson’s χ<sup>2</sup> test, or Fisher’s exact test if appropriate was used to evaluate differences with regard to the distribution of alleles or genotypes between the cancer and the control groups. The strength of the associations between the GSTP1 polymorphism and risk of endometrial carcinoma were estimated using odds ratios (OR) and 95% confidence intervals (95% CI). The associations between the single nucleotide polymorphisms and clinicopathologic variables when available were also analyzed. Stratified analyses to separate subgroups with different levels of tumor behavior were further done in the case that there were significant differences among the tested cases and controls. The probability of Hardy-Weinberg equilibrium was assessed by χ<sup>2</sup> test. Ps < 0.05 were considered statistically significant. All statistical analyses were done with SPSS 11.0 (SPSS, Inc., Chicago, IL).

Results

Figure 1 showed representative samples of genotyping for Ile<sup>105</sup>Val of the GSTP1 gene in endometrial carcinoma. The size of amplicon was 132 bp. After digestion by Alw26I, two fragments, 94 and 38 bp, were produced for homozygous Val/Val genotype whereas the homozygous Ile/Ile remained as the size of 132 bp. The heterozygous genotype was represented by a combination of the fragments found in either allele. Results of direct sequencing on 20 of the heterozygous cases, as well as 15 of the homozygous Ile/Ile and Val/Val cases, respectively, were consistent with the results reflected on agarose gel of RFLP and confirmed genotypes of the samples. The genotypes of control subjects were in Hardy-Weinburg equilibrium (P = 0.86, χ<sup>2</sup> test).

The distribution of the three GSTP1 genotypes and Val allele frequencies, with their respective ORs for endometrial cancers and controls were presented in Table 1. Endometrial cancers of two major histologic types have also been separately analyzed. Analysis showed that results of type I endometrioid endometrial cancers were very similar to the findings of endometrial cancers as a group. The allele frequencies for the Ile and Val variants were found to be 0.722 and 0.278 among the endometrial carcinoma cases, compared with 0.805 and 0.195 among the control population (P = 0.01). The relative OR of 1.59 (95% CI, 1.13-2.23) showed that the valine allele was correlated with a higher risk of developing endometrial carcinoma comparing with the isoleucine allele. There were also significant differences (P < 0.01) in genotype frequencies between cancer and control groups (Ile/Ile, 63%; Ile/Val, 31%; Val/Val, 4% for controls; Ile/Ile, 48.3%; Ile/Val, 47.8%; Val/Val, 3.9% for cases). By considering the Val<sup>105</sup> Val as a disease-associated allele, further analysis on valine carriers, including the patients of Ile/Val or Val/Val genotypes, showed
that Val carriers had a significantly higher ($P < 0.01$), 2.03-fold risk of developing endometrial cancers. Such correlations were still shown when patients who developed cancers at a younger age ($<35$ or $<45$) were excluded from the study (statistical data were not shown).

The associations between the single nucleotide polymorphisms and clinicopathologic variables were also analyzed (Table 2). Statistical analysis showed that histologic grading of endometrial cancer was significantly associated with overall genotypes ($P < 0.01$), Val carriers ($P < 0.01$), as well as allele frequencies ($P < 0.01$). Similar associations were shown when the extent of myometrial invasion of the cancer was correlated with genotypes ($P = 0.02$), Val carriers ($P = 0.02$), and allele frequencies ($P = 0.02$). The valine variant was associated with high-grade cancer and deep myometrial invasion. On the other hand, other clinicopathologic variables such as staging and the extent of cervical invasion did not statistically correlate with the overall genotypes ($P = 0.29$ and $0.23$, respectively), Val carriers ($P = 0.48$ and $0.65$, respectively) nor the allele frequencies ($P = 0.29$ and $0.14$, respectively; data not shown in tables).

**Discussion**

To our best knowledge, this is the first study reporting an association between $GSTP1$ Ile$^{105}$ Val polymorphism and endometrial carcinoma. Our study showed that there were significant differences in genotypes and allelic frequencies between patients with endometrial cancers and controls. Individuals who were valine allele carriers, besides being found to have higher risk of endometrial cancer, were also more likely to develop high-grade endometrial cancer and/or cancer with deep myometrial invasion.

Table 1. Genotype distributions and allele frequencies of $GSTP1$ among endometrial cancer cases and controls and their respective contributions to the risk of the cancer

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Controls, $n$ (%)</th>
<th>Cancer cases</th>
<th>Genotypes</th>
<th>Total</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Genotypes</td>
<td>OR (95% CI)</td>
<td>$P$</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Ile/Ile</td>
<td>130 (65.0)</td>
<td>87 (48.3)</td>
<td>Reference (1)</td>
<td>83 (48.5)</td>
<td>0.01</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>Ile/Val</td>
<td>62 (31.0)</td>
<td>86 (47.8)</td>
<td>2.07 (1.36-3.17)</td>
<td>82 (48.0)</td>
<td>0.01</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td>Val/Val</td>
<td>8 (4.0)</td>
<td>7 (3.9)</td>
<td>1.31 (0.46-3.74)</td>
<td>6 (3.5)</td>
<td>0.18 (0.39-5.51)</td>
<td>1 (11.2)</td>
</tr>
<tr>
<td>Val carriers</td>
<td>70 (35.0)</td>
<td>93 (51.7)</td>
<td>2.03 (1.34-3.07)</td>
<td>88 (51.5)</td>
<td>0.01</td>
<td>5 (55.6)</td>
</tr>
</tbody>
</table>

**NOTE:** Statistically significant data are highlighted in bold. Reference value of 1 was assigned to the homozygous wild-type genotype Ile/Ile against which the ORs for other genotypes were calculated.

Table 2. Genotype distributions and allele frequencies of $GSTP1$ in endometrial cancer cases stratified by grading and extent of myometrial invasion and their respective contributions to the risk of the cancer

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Overall grading</th>
<th>High grade</th>
<th>Low grade</th>
<th>Overall myometrial invasion</th>
<th>Deep myometrial invasion</th>
<th>Superficial myometrial invasion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$ (%)</td>
<td>OR (95% CI)</td>
<td>$P$</td>
<td>$n$ (%)</td>
<td>OR (95% CI)</td>
<td>$P$</td>
</tr>
<tr>
<td>Ile/Ile</td>
<td>87 (49.7)</td>
<td>Reference (1)</td>
<td>0.01</td>
<td>29 (32.6)</td>
<td>Reference (1)</td>
<td>0.01</td>
</tr>
<tr>
<td>Ile/Val</td>
<td>81 (46.3)</td>
<td>4.23 (2.22-8.07)</td>
<td>0.01</td>
<td>55 (61.8)</td>
<td>3.98 (2.31-6.84)</td>
<td>0.01</td>
</tr>
<tr>
<td>Val/Val</td>
<td>7 (4.0)</td>
<td>5.00 (0.91-27.35)</td>
<td>0.01</td>
<td>5 (5.6)</td>
<td>2.80 (0.85-9.19)</td>
<td>0.73</td>
</tr>
<tr>
<td>Val carriers</td>
<td>88 (50.3)</td>
<td>4.51 (2.39-8.52)</td>
<td>0.01</td>
<td>60 (67.4)</td>
<td>4.05 (2.37-6.90)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**NOTE:** Statistically significant data are highlighted in bold. Number of cases involved in stratification did not sum up to total because of incomplete clinicohistologic information for some cases.
of this region, lower thermal stability of the enzyme, and differential catalytic activity (20, 21). Estrogen through oxidative metabolism, is well known to form carcinogenic intermediates such as quinones and semiquinones, which could be removed by conjugation with glutathione catalyzed by GSTs. The GST enzymes, therefore prevent DNA from damage by directly preventing the formation of depurinated DNA and indirectly by preventing DNA to react with reactive oxidative species. Structural and functional alteration of the gene product due to polymorphism at Ile105Val may affect these tumor suppression effects.

Although possible association between endometrial cancer and GSTP1 polymorphisms has not been reported, similar studies have been done in other hormone-related cancers, although the results were conflicting. Studies done on Japanese and Caucasian populations found an increased risk of prostate cancer in association with Val/Val homozygous genotype (15, 16). Egan’s study on Chinese population also reported that women with the less common Val/Val genotype had almost twice the risk for breast cancer (22). However, other studies based predominately on Caucasian women did not show an association between the Val allele and breast cancer (23, 24) or ovarian cancer (25).

Our current GSTP1 polymorphism study done in Chinese population of Hong Kong, when compared with data from other studies, showed differences in genotype distributions and allelic frequencies among different populations and ethnic groups. Studies on European descendants in Australia, Caucasi- ans in the United States, Portugal, France, and Spain found consistent genotype distributions of around 45%, 45%, and 10% for Ile/Ile, Ile/Val, and Val/Val, respectively (25–29). On the other hand, our study and studies on Chinese populations in Shanghai and North China, as well as Korean population showed another pattern of genotype distribution, the frequencies of Ile/Ile, Ile/Val, and Val/Val were about 65%, 30%, and 5%, respectively (22, 30, 31).

Concurring with the observations made by Nerurkar et al., we found that Asians have more GSTP1 Ile/Ile genotype and less Val/Val genotype when compared with Caucasians (32). Geographic difference in genotypes has been reported in endometrial carcinoma. For example, the frequency of Ki-ras mutations in endometrial cancer is different in Americans and Japanese (33). In fact, genetic epidemiologic difference could exist even within different parts of a country, as shown in our earlier study on BRCA mutations in ovarian carcinoma (34).

The geographic variation in GSTP1 genotype frequencies is of particular importance because different GSTP1 variants would determine the binding affinity for different chemicals including the known carcinogens diol epoxides and benzo(a)pyrenes and the chemotherapeutic drug thiotaepa (21, 35). These may therefore have effect on the susceptibility of different populations to particular types of cancer when exposed to certain carcinogen. Moreover, effect of the different genotypes on drug metabolism may also affect the response of patients in different populations.

The genotype frequencies are also useful quality control variables in genetic study because consistency between the observed and reported data in genotype/allele frequencies among related ethnic control groups would reflect how representative the data are when compared with the true populations. The close proximity of genotype frequencies found in our controls when compared with other Asian suggested that the population we have selected for study resembled the randomized Asian populations.

Another interesting observation from our study was the unexpected low frequency of Val/Val genotype in both the controls and endometrial cancer cases. In fact, there were no significant differences in Val/Val frequency between the control and cancer cases, although there were much more Ile/Val heterozygotes in the cancer population. The relatively low frequency of Val/Val genotype in Chinese populations, even among endometrial cancer patients, may be the result of natural selection following long-term effect of different cultures and environmental factors on the population so that the Val/Val individuals were unfavorably selected against (36). It may be possible that the Asian population was more often contacting carcinogens in pollutants or pickled food. Because the Val/Val isofrom is less efficient in the binding and metabolism of some chemicals, a relatively low frequency of Val/Val genotype in the Chinese population may be a naturally selected protective mechanism. The low proportion of the Val/Val genotype may also explain the relatively low incidence of endometrial cancer when compared with the Caucasians.

Whereas a statistically significant association was shown between GSTP1 polymorphism and type I endometrioid carcinoma of endometrium as well as endometrial carcinoma as a group, no significant association between GSTP1 polymorphism and nonendometrioid type II cancer could be established. The latter findings may be limited by the small number of nonendometrioid carcinomas in this series although such relatively low frequency is expected as has been observed in different populations. On the other hand, such observation may also reflect a basic difference in carcinogenesis between the type I and II endometrial cancers (37). Further study involving larger number of the relative uncommon type II endometrial cancer or other genes that are related to detoxification process or steroid-hormone metabolism would help to clarify this the pathogenesis of these two main types of endometrial cancers.

References

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