Research Article

GSTM1 and GSTT1 Copy Number Variation in Population-based Studies of Endometrial Cancer Risk

Stalo Karageorgi1,3,4, Jennifer Prescott1,3,4, Jason Y.Y. Wong1,3,4, I-Min Lee2,3, Julie E. Buring2,3, and Immaculata De Vivo1,3,4

Abstract

Background: Glutathione S-transferases (GST) detoxify a broad range of carcinogenic metabolites and lack of GSTM1 and GSTT1 activity due to gene deletions are prevalent. The associations of GSTM1 and GSTT1 polymorphisms with endometrial cancer risk have been inconsistent.

Methods: We investigated gene dosage effects of GSTM1 and GSTT1 copy number in 441 endometrial cancer cases and 1,237 matched controls selected from the Nurses’ Health Study and Women’s Health Study, as well as gene–environment interactions.

Results: Carriers of at least 2 GSTT1 genes had an increased risk of endometrial cancer (OR = 1.51, 95% CI = 1.04–2.19; \( P_{\text{trend}} = 0.04 \)) compared with women who were GSTT1 null. GSTM1 was not associated with endometrial cancer risk (OR2/3 vs. 0 copies = 0.82, 95% CI = 0.52–1.27; \( P_{\text{trend}} = 0.41 \)). We did not observe effect modification of either the GSTM1 or GSTT1 association with cancer risk by smoking status, postmenopausal hormone use, or body mass index.

Conclusions: Our results suggested GSTM1 copy number does not influence endometrial cancer risk, whereas higher GSTT1 copy number may be associated with increased risk. Our findings supported that GSTT1 differs in its substrate specificity from GSTM1 and may generate intermediates more genotoxic to endometrial cells than the parent chemical. Future studies are needed to clarify this relationship.

Impact: We hypothesized risk associated with GST enzymes may differ depending on environmental and/or occupational exposures. Our assessment of gene–environment interactions suggested GSTM1 and GSTT1 are not involved in the \textit{in vivo} human metabolism of estrogen and its metabolites. Cancer Epidemiol Biomarkers Prev; 20(7); 1447–52. ©2011 AACR.

Introduction

Glutathione S-transferases (GST) are enzymes that conjugate a broad range of electrophilic xenobiotic and carcinogenic compounds to glutathione. Conjugated metabolites are generally less genotoxic and more efficiently excreted (1, 2). Among individuals of European descent, \(~50\%\) completely lack GSTM1 activity (3, 4) and \(~25\%\) lack GSTT1 activity (5) as a result of homozygous gene deletions (6, 7). Experimental evidence suggests enzyme activity is directly proportional to the number of gene copies present (6, 8, 9). As reduced GST activity may lower an individual’s ability to metabolize carcinogens, individuals with fewer GST genes may be at higher risk for tumor formation (10).

On the basis of current experimental data, a clear relationship between GST function and endometrial cancer risk cannot be inferred. \textit{In vitro} and rat experiments suggest GSTs metabolize estradiol and deactivate estrogen-derived metabolites (1, 11), which play a significant role in endometrial carcinogenesis. In contrast, estrogen-glutathione conjugates were not found among 2 women dosed i.v. with radiolabeled 2-hydroxyestradiol (12). In addition, GSTT1 has been shown to differ in substrate specificity from most other GST enzymes. For some substrates, GSTT1 may generate intermediates that are more genotoxic than the parent chemical (13). In the presence of such exposures, increased GSTT1 activity could theoretically augment rather than reduce cancer risk.

Three case–control and a cohort study of endometrial cancer risk have found inconsistent associations with GSTM1 and GSTT1 homozygous gene deletions (14–17). All but 1 study used methodology that could not distinguish heterozygote carriers from homozygote carriers.

Authors' Affiliations: 1Channing Laboratory, Department of Medicine; 2Division of Preventive Medicine, Brigham and Women’s Hospital/Harvard Medical School; 3Department of Epidemiology; and 4Program in Molecular and Genetic Epidemiology, Harvard School of Public Health, Boston, Massachusetts

Note: S. Karageorgi and J. Prescott contributed equally to this work.

Corresponding Author: Immaculata De Vivo, Brigham and Women’s Hospital/Harvard Medical School, 181 Longwood Ave, Boston, MA 02115. Phone: 617-926-2094; Fax: 617-525-2008. E-mail: devivo@channing.harvard.edu

doi: 10.1158/1055-9965.EPI-11-0190

©2011 American Association for Cancer Research.

www.aacnjournals.org

Published OnlineFirst May 10, 2011; DOI: 10.1158/1055-9965.EPI-11-0190
carriers. Thus, the studies could not assess potential dose-response relationships with endometrial cancer risk. In addition, none of these studies explored putative interactions between \textit{GSTM1} and \textit{GSTT1} and established endometrial cancer risk factors.

We therefore set out to examine whether we could better understand the relationship between \textit{GSTM1} and \textit{GSTT1} genes and endometrial cancer risk by assessing potential gene dosage effects and gene-environment interactions. We genotyped copy number variation in the largest number of endometrial cancer cases and controls to date from 2 case-control studies nested within the prospective Nurses’ Health Study (NHS) and Women’s Health Study (WHS) cohorts. We used commercially available assays to quantify copy number of \textit{GSTM1} and \textit{GSTT1} in each participant.

Materials and Methods

\textbf{Study populations}

Details of these study populations have been previously described (18). Briefly, 304 NHS cases with pathologically confirmed invasive endometrial cancer were selected from a subcohort of women who donated a blood sample. Eligible cases consisted of women who were diagnosed any time after cohort inception (1976) up to June 1, 2004 with no previous cancer diagnosis except nonmelanoma skin cancer. We randomly selected 826 controls from the NHS blood subcohort who had not had a hysterectomy and were free of cancer (except nonmelanoma skin cancer) up to and including the questionnaire cycle in which the case was diagnosed. Controls were matched to cases according to age, menopausal status and postmenopausal hormone (PMH) use (current vs. not current) at blood collection. From the WHS, we selected 137 cases and 411 random controls by using similar inclusion and exclusion criteria as in the NHS. WHS cases were diagnosed after blood collection (1993–1995) and before June 1, 2002. For the NHS, completion of the self-administered questionnaire and submission of the biospecimen was considered to imply informed consent. Written informed consent was obtained from all women before entry into the WHS trial. The study protocols were approved by the Human Research Committee of Brigham and Women’s Hospital, Boston, MA.

\textbf{GSTM1 and GSTT1 copy number assays}

Genomic DNA was extracted from peripheral blood leukocytes by the QIAmp (Qiagen) 96-spin blood protocol. \textit{GSTM1} and \textit{GSTT1} copy number was determined by using TaqMan Gene Copy Number Assays designed by Applied Biosystems. Duplex real-time PCR reactions were run on the Applied Biosystems 7900HT Real-Time PCR system by using gene-specific primers and a gene-specific FAM-MGB probe along with primers for the RNase \textit{P} gene and VIC/Tamra probe as the reference gene. Samples were run in triplicate in a 384-well format by using 10 ng of genomic DNA per reaction. We used Sequence Detection Software (Applied Biosystems) to quantify the gene copy number of each sample. We included 5\% QC samples, as well as Coriell Transformed Lymphocyte cell lines and an endometrial cancer cell line (RL952) with known \textit{GSTM1} and \textit{GSTT1} copy number as external controls. We did not test and authenticate these cell lines. Concordance for blinded samples was more than 90\%. Expected genotypes for external controls were observed for 100\% of replicates.

\textbf{Statistical analyses}

Conditional logistic regression was used to estimate ORs and 95\% CIs for \textit{GSTM1} and \textit{GSTT1} copy number associations with endometrial cancer risk. Genotypes were coded as categorical variables (0, 1, and 2 or 3 gene copies). Individuals missing genotype information or with a copy number more than 3 were excluded from analyses (n = 23 for \textit{GSTM1}; n = 41 for \textit{GSTT1}). Factors adjusted for in regression models are listed in Table 1. Analyses were conducted by using the pooled NHS and WHS data and adjusted for study, a method equivalent to a fixed-effects model meta-analysis (19).

To assess gene-environment interaction, we conducted stratified analyses of \textit{GSTM1} copy number by established endometrial cancer factors (smoking status: never, past, current; PMH use: never, past, current; body mass index (BMI): <25, 25–30, >30) using unconditional logistic regression (20). We used the likelihood ratio test to determine significance. Analyses were restricted to women of European ancestry (98\% of participants). \textit{P} values are 2-sided; \textit{P} values less than 0.05 were considered statistically significant. We used SAS Version 9.1 software (SAS Institute).

\textbf{Results}

Descriptive characteristics of the study populations have been published (18). Among our controls, 53\% of participants were homozygous \textit{GSTM1} null and 21\% were homozygous \textit{GSTT1} null. \textit{GSTM1} carriers were not at increased risk of endometrial cancer (OR = 0.93, 95\% CI = 0.73–1.19; Table 1) compared with women with the \textit{GSTM1} null genotype. Women with at least 2 \textit{GSTM1} genes were not at increased risk of endometrial cancer (OR = 0.82, 95\% CI = 0.52–1.27) compared with individuals with 0 copies, and a gene dosage effect of \textit{GSTM1} was not observed (\textit{P}_{\text{trend}} = 0.41). In contrast, \textit{GSTT1} carriers were at an increased risk of endometrial cancer compared with women with the \textit{GSTT1} null genotype. Women with at least 2 \textit{GSTT1} genes were ~50\% more likely to develop endometrial cancer than women who carried 0 copies (\textit{P}_{\text{trend}} = 0.04).

Lifestyle and reproductive factors that expose women to higher levels of estrogens, particularly, if unopposed by progesterone, are established risk factors for endometrial cancer development (21). For example, PMH users in the NHS study with current long-term (5 or
more years) estrogen only use are at ~11-fold increased risk of endometrial cancer (21). Women in the highest tertile of BMI have ~2-fold increased risk compared with women in the bottom tertile (unpublished NHS data). Whereas, we found about a 30% reduced risk of endometrial cancer associated with both past and current smoking, which is hypothesized to have an anti-estrogenic effect (22). Because GSTs presumably deactivate estrogen-derived metabolites (1, 2), we investigated whether the association of\textit{GSTM1} and \textit{GSTT1} with endometrial cancer risk is modified by these factors that alter estrogen exposure. As the decrease in endometrial cancer risk associated with smoking seems limited to postmenopausal women (23, 24), we limited the smoking–GST interaction analyses to postmenopausal women. We did not observe effect modification of endometrial cancer risk associated with \textit{GSTM1} or \textit{GSTT1} carrier status by smoking status (\textit{P}_\text{trend} \geq 0.92; Table 2), PMH use (\textit{P}_\text{interaction} \geq 0.14; Table 2), or BMI (\textit{P}_\text{interaction} \geq 0.13; Table 2). Endometrial cancer risk associated with \textit{GSTT1} carrier status appeared somewhat more pronounced among past and current PMH users. When stratified further by PMH type (never, ever estrogen, ever estrogen plus progesterone users), we still did not observe an interaction with \textit{GSTM1} or \textit{GSTT1} (data not shown). Some suggestion of a reduced risk of endometrial cancer associated with \textit{GSTM1} carrier status was seen only among women with a BMI less than 25.

### Table 1. Association between copy number variants of the \textit{GSTM1} and \textit{GSTT1} and endometrial cancer risk in the pooled NHS and WHS data

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
<th>OR(^a) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{GSTM1}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>232</td>
<td>621</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>156</td>
<td>442</td>
<td>0.96 (0.74–1.24)</td>
</tr>
<tr>
<td>2/3</td>
<td>39</td>
<td>114</td>
<td>0.82 (0.52–1.27)</td>
</tr>
<tr>
<td>\textit{P}_\text{trend}</td>
<td></td>
<td></td>
<td>0.41</td>
</tr>
<tr>
<td>\textit{Carrier}</td>
<td>195</td>
<td>556</td>
<td>0.93 (0.73–1.19)</td>
</tr>
<tr>
<td>\textit{GSTT1}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>64</td>
<td>239</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>199</td>
<td>526</td>
<td>1.33 (0.92–1.92)</td>
</tr>
<tr>
<td>2/3</td>
<td>157</td>
<td>401</td>
<td>1.51 (1.04–2.19)</td>
</tr>
<tr>
<td>\textit{P}_\text{trend}</td>
<td></td>
<td></td>
<td>0.04</td>
</tr>
<tr>
<td>\textit{Carrier}</td>
<td>356</td>
<td>927</td>
<td>1.41 (1.00–1.99)</td>
</tr>
<tr>
<td>\textit{GSTM1/GSTT1}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null/null</td>
<td>36</td>
<td>125</td>
<td>1.00</td>
</tr>
<tr>
<td>Null/carrier</td>
<td>193</td>
<td>482</td>
<td>1.33 (0.85–2.08)</td>
</tr>
<tr>
<td>Carrier/null</td>
<td>28</td>
<td>108</td>
<td>0.83 (0.46–1.52)</td>
</tr>
<tr>
<td>Carrier/carrier</td>
<td>161</td>
<td>431</td>
<td>1.23 (0.78–1.94)</td>
</tr>
</tbody>
</table>

\(^a\)Conditional logistic regression adjusted for matching factors and smoking at diagnosis, age at menarche, age at first birth and parity, BMI (kg/m\(^2\)) at diagnosis, postmenopausal hormone use at diagnosis, menopausal status, and age at menopause at diagnosis, ever oral contraceptive use.

### Discussion

Of all candidate gene cancer susceptibility studies, associations with genes encoding metabolizing enzymes are among the most significant across studies. Genes encoding phase II enzymes, such as \textit{GSTM1} and \textit{GSTT1}, were the most noteworthy of these associations (25). In this study, we determined copy number of \textit{GSTM1} and \textit{GSTT1} in an endometrial case–control study with the largest sample size to date. Carriers of the \textit{GSTT1} gene (OR = 1.41, 95% CI = 1.00–1.99; Table 1) were at increased risk of endometrial cancer compared with women with the \textit{GSTT1} null genotype. Moreover, increasing \textit{GSTT1} copy number showed a positive dose response relationship with endometrial cancer risk (\textit{P}_\text{trend} = 0.04). Suggestion of a \(~20\%\) reduced risk was observed among carriers of at least 2 \textit{GSTM1} genes compared with women with the null genotype.

Previous association studies of \textit{GSTM1} and \textit{GSTT1} with endometrial cancer risk have been inconsistent. One study observed a marginal increased risk of endometrial cancer among women with the \textit{GSTM1} null genotype compared with \textit{GSTM1} gene carriers (14), another found a statistically significant decreased risk (16), whereas 2 others found no significant association (15, 17), consistent with our findings. Two of these studies did not find an association with \textit{GSTT1} (14, 16), whereas the others observed statistically significant increased risk among women with the \textit{GSTT1} null genotype compared...
with GSTT1 gene carriers (15, 17), contrary to our findings. Several possibilities could account for the discrepancy between studies. Inconsistent associations may be a consequence of small sample size (14, 17), the use of controls with a previous personal history of cancer (16), underlying differences in the exposure to GST substrates between populations, or no true association exists between the GST genes and endometrial cancer risk so that all observed associations have been due to chance.

As a class, GST enzymes are generally thought to neutralize reactive compounds (1) and therefore are hypothesized to decrease cancer risk. In our study, we found some suggestion of reduced endometrial cancer risk, which was in the expected direction, among women who possessed at least 2 GSTM1 genes. However, the observed increased risk associated with GSTT1 copy number counters this hypothesis and is more in line with the observation that GSTT1 substrates may generate genotoxic intermediates (13). It is conceivable that the women in our study, all of which are female health professionals, may be exposed to chemicals that are activated by GSTT1 rather than detoxified. In such a scenario, carriers with greater GSTT1 copy number would generate and expose cells to higher concentrations of genotoxic intermediates and thus increase the likelihood of acquiring somatic mutations that promote

### Table 2. Association of GSTM1 and GSTT1 copy number with endometrial cancer risk by smoking status, postmenopausal hormone use, and BMI in the pooled NHS and WHS data

<table>
<thead>
<tr>
<th></th>
<th>Never smokers</th>
<th>Past smokers</th>
<th>Current smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>OR^a (95% CI)</td>
</tr>
<tr>
<td><strong>GSTM1^c</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>92</td>
<td>207</td>
<td>1.00</td>
</tr>
<tr>
<td>Carrier</td>
<td>74</td>
<td>191</td>
<td>0.87 (0.59–1.28)</td>
</tr>
<tr>
<td>(P_{\text{interaction}})</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GSTT1^c</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>21</td>
<td>74</td>
<td>1.00</td>
</tr>
<tr>
<td>Carrier</td>
<td>144</td>
<td>322</td>
<td>1.69 (0.97–2.94)</td>
</tr>
<tr>
<td>(P_{\text{interaction}})</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Never PMH users</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Past PMH users</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI &lt;25</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>25 ≤ BMI &lt;30</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>BMI &gt;30</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GSTM1^c</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>90</td>
<td>305</td>
<td>1.00</td>
</tr>
<tr>
<td>Carrier</td>
<td>59</td>
<td>286</td>
<td>0.69 (0.47–1.00)</td>
</tr>
<tr>
<td>(P_{\text{interaction}})</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GSTT1^c</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>20</td>
<td>121</td>
<td>1.00</td>
</tr>
<tr>
<td>Carrier</td>
<td>125</td>
<td>461</td>
<td>1.60 (0.95–2.71)</td>
</tr>
</tbody>
</table>

^aUnconditional logistic regression adjusted for matching factors and smoking at diagnosis (except in smoking analysis), age at menarche, age at first birth and parity, BMI at diagnosis (except in BMI analysis), PMH use at diagnosis (except in PMH analysis), menopausal status, and age at menopause at diagnosis, ever oral contraceptive use.

^bLikelihood ratio test of interaction with 2 degrees of freedom (df).

^cAnalysis restricted to postmenopausal women.
carcinogenesis. However, it is also possible that our result is due to chance. Selection bias is an unlikely explanation as both population-based case–control studies used in this analysis were nested within 2 large homogeneous prospective cohort studies. Furthermore, the frequency of the homozygous null genotype for GSTT1 and GSTM1 among our controls is in agreement with the frequencies reported in the literature for individuals of European descent.

If GSTs deactivate estradiol and estrogen-derived metabolites, we would have expected effect modification of the GST associations by strata of established risk factors, which serve as surrogates of estrogen exposure. As we did not observe any interactions between GSTM1, GSTT1, and established endometrial cancer risk factors, our results do not support a role for GSTs in the in vivo human metabolism of estrogen and estrogen metabolites.

We used commercially available quantitative PCR-based assays to determine GSTM1 and GSTT1 copy number. The ability to distinguish copy number allowed us to observe a gene dosage effect with GSTT1, which strengthens the evidence for a causal relationship. This is an improvement over previous PCR and RFLP methodologies that were only capable of identifying null or non-null genotypes, which may have contributed to inconsistent and contradictory results (26). In addition, we observed 3 copies of GSTM1 and GSTT1 among a very small percentage of control participants (<2% for GSTM1; <2% for GSTT1). Although this could be because of genotyping error, evidence for more than 2 copies of GSTM1 has been reported. In a Saudi Arabian population, 3% of individuals had very high GSTM1 activity, potentially due to the inheritance of a chromosome with a tandem duplication of the GSTM1 gene (8). A similar tandem duplication may exist for the GSTT1 gene.

Due to the prevalence of GST gene deletions, particularly GSTM1, the main effect of reduced GST activity on endometrial cancer risk is likely to be small, whereas the population attributable risk may be high. We did not find a main effect of GSTM1 on endometrial cancer risk but only had 14% power to detect a trend of such small effect. The direction of our results indicates that a higher copy number of GSTM1 may be associated with a reduced risk of endometrial cancer whereas higher GSTT1 copy number may increase risk among women of European ancestry. Larger, carefully designed population-based studies are needed to confirm our findings.

Disclosure of Potential Conflicts of Interest

The authors declare that there are no conflicts of interest.

Grant Support

This work was supported by the National Institute of Health (grant numbers: CA87969, CA49449, CA82838, CA407988, HL043851, and 5T32CA09001) and the HSPH-Cyprus Initiative for the Environment and Public Health funded by the Republic of Cyprus.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received February 22, 2011; revised April 24, 2011; accepted April 30, 2011; published OnlineFirst May 10, 2011.

References

17. Norskov MS, Frikke-Schmidt R, Bojesen SE, Nordestgaard BG, Loft S, Tybjaerg-Hansen A. Copy number variation in glutathione-S-transferase

www.aacajournals.org Cancer Epidemiol Biomarkers Prev; 20(7) July 2011 1451

Downloaded from cebp.aacajournals.org on October 13, 2017. © 2011 American Association for Cancer Research.
Ferase T1 and M1 predicts incidence and 5-year survival from prostate and bladder cancer, and incidence of corpus uteri cancer in the general population. Pharmacogenomics J 2010.


Cancer Epidemiology, Biomarkers & Prevention

GSTM1 and GSTT1 Copy Number Variation in Population-based Studies of Endometrial Cancer Risk

Stalo Karageorgi, Jennifer Prescott, Jason Y.Y. Wong, et al.

Cancer Epidemiol Biomarkers Prev 2011;20:1447-1452. Published OnlineFirst May 10, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/1055-9965.EPI-11-0190

Cited articles
This article cites 25 articles, 5 of which you can access for free at:
http://cebp.aacrjournals.org/content/20/7/1447.full#ref-list-1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.