Glutathione S-Transferase M1 Genotypes and the Risk of Squamous Carcinoma of the Cervix: A Population-based Case-Control Study

Chu Chen,1 Margaret M. Madeleine,2 Noel S. Weiss,2,3 and Janet R. Daling2,3

The authors determined the GSTM1 genotype of 190 Caucasian women with invasive squamous cell cervical cancer in western Washington State between January 1986 and June 1994 and of 206 controls. It was found that 53% of cases and 57% of controls had the GSTM1 null genotype. The age-adjusted odds ratio associated with GSTM1 null genotype was 0.8 (95% confidence interval 0.6, 1.3). The lack of association between cervical cancer risk and GSTM1 genotype was true both for smokers and nonsmokers of cigarettes and for heavy and light smokers. These data suggest that women with the GSTM1 null genotype are not at increased risk of cervical cancer. Am J Epidemiol 1999;150:568-72.

case-control studies; cervix neoplasms; genetic markers; glutathione transferase

Epidemiologic evidence suggests that the risk of squamous cervical cancer is strongly related to human papillomavirus (HPV) infection (1) and to cigarette smoking, particularly smoking in the recent past (2–4). Among the cancer-causing substances found in tobacco smoke are polynuclear aromatic hydrocarbons (PAHs), aromatic amines, and nitroso-compounds (5–8). Glutathione S-transferases (EC2.5.1.18) are a family of cytosolic dimeric enzymes which facilitates excretion of a variety of hydrophobic substances, including mutagenic metabolites of PAHs and nitroso-compounds, by catalyzing the transfer of reduced glutathione to their electrophilic sites (9, 10). Genetic polymorphisms for some of these enzymes, including GSTM1, have been described in humans (11–14). Approximately 50–60 percent of the Caucasian population lacks GSTM1 activity because they lack the GSTM1 gene (they have the null genotype). Most of the studies that have examined the potential association between GSTM1 and the risk of smoking-related cancers, such as lung and bladder cancers, have observed an increased risk among individuals who lack GSTM1 activity toward substrate trans-stilbene oxide or who lack the GSTM1 gene. The goal of the present study was to examine whether individuals who have the GSTM1 null genotype are at increased risk for squamous cancer of the cervix.

MATERIALS AND METHODS

Subject eligibility, Identification, and recruitment

Cases were women aged 18–74 years who resided in the three-county urban area that includes Seattle, were diagnosed with incident invasive cervical cancer between January 1986 and June 1994, and took part in a case-control study of cervical cancer. Previous reports from this study described the methods in more detail (4). A population-based registry (the Cancer Surveillance System) that is part of the Surveillance, Epidemiology, and End Results (SEER) program was used to identify cases with biopsy-confirmed invasive cervical cancer of all histologic types. In the present analyses, we excluded nonwhite women and 160 women whose tumor had a histology other than squamous cell carcinoma.

Population-based controls were identified using random-digit telephone dialing (15) and were frequency matched to the age distribution of the cases in 5-year age intervals. We were able to determine characteristics relevant to study eligibility (age, residence for more than 3 months in the three-county area, and presence of a telephone) for 93.4 percent of all residential telephone numbers that we contacted.

Data collection

During July 1987 to December 1995, an hour-long interview was administered by trained interviewers in a standardized way to cases and controls. Topics included
demographic information, as well as reproductive, birth control, sexual, and smoking histories prior to a woman's date of diagnosis (or a corresponding "reference date" for controls). We were able to complete an in-person interview with 64.6 percent (573/887) of the eligible cases identified. Reasons for nonparticipation included loss to follow-up and patient refusal (20.5 percent), doctor refusal (5.9 percent), death (8.8 percent), and two interviews that were not completed before this analysis (0.2 percent). Among eligible controls, 72.7 percent (1,340/1,842) of women agreed to be interviewed, for an overall response of 68.0 percent. Reasons for non-participation included refusal (26.9 percent) and seven interviews (0.4 percent) that were not completed at the time of this analysis. Of the 1,340 interviewed women, those with a history of cervical cancer (n = 23) or hysterectomy (n = 341) were excluded, as were controls who were assigned reference dates outside the dates of this study (n = 70) or who were non-white (n = 56).

Blood collection and GSTM1 genotyping

Beginning in February 1993, we attempted to collect a peripheral blood sample from the women with whom we were conducting interviews (103 cases and 206 controls, representing 60.3 percent and 61.9 percent, respectively, of interviewed subjects). We also recontacted cases who had previously been interviewed and who still lived in the area, and we obtained a sample of peripheral blood (n = 87, or 45.3 percent of interviewed cases from the earlier period). In all, peripheral blood specimens were available from 190 cases and 206 controls. Detailed descriptions for the preparation of genomic DNA from these specimens and for the determination of GSTM1 genotypes by polymerase chain reaction (PCR) were given in a previous report (16).

Data analysis

The relative risk of cancer associated with the GSTM1 null genotype was estimated using the odds ratio obtained from a multiple logistic regression analysis. Subjects with missing values for any variables in a model were excluded from that model. Age at reference date (linear, continuous) was controlled in estimating some of the relative risks presented.

RESULTS

The characteristics of the study participants are presented in table 1. The age distributions of cases and controls were similar. Cases tended to have less education and lower income than controls. Cases were more likely to be unmarried, to have had their first intercourse before age 17 years, and to have had a larger number of sex partners and term pregnancies. Cigarette smoking at diagnosis/reference date was considerably more common among cases than among controls (41.6 percent vs. 25.7 percent).

The frequency of the GSTM1 null genotype was 53.2 percent among cases and 57.3 percent among controls. The age-adjusted odds ratio associated with the GSTM1 null genotype was 0.8 (95 percent confidence interval 0.6, 1.3) (table 2). Further adjustment for the other characteristics that differed between cases and controls did not materially influence this result. The lack of an association between cervical cancer and GSTM1 genotype was true both for smokers and nonsmokers of cigarettes and for heavy and light smokers. However, there was a suggestion of an elevated risk associated with the null genotype among women who had smoked for 30 years or more.

Among the cervical cancer cases interviewed prior to February 1993, the period during which blood samples were obtained on a relatively low percent of eligible women, we observed a similar proportion of women with the GSTM1 null genotype (52.9 percent) to that among the cases interviewed subsequently (53.4 percent).

DISCUSSION

Our study had several limitations. First, blood samples for genotyping were not obtained on all potentially eligible study subjects. This was a particular problem for women with cervical cancer diagnosed in the 1980s, since they were not even asked for a blood specimen until at least several years after their initial interview, and at that time a sizable number of them could not be contacted. Also, because we made an effort to characterize the genotype of cases who had been interviewed in the earlier years of the parent study, but not controls from those earlier years, on average the diagnosis dates of the cases were earlier in time than the corresponding reference dates of controls. For these reasons, the size of the case-control difference for exposures assessed in the interview, particularly those that vary with calendar time (cigarette smoking), may have been misestimated. On the other hand, the size of the case-control difference for a genetically determined characteristic such as GSTM1 genotype was probably little affected by any of the above. Finally, the number of subjects included in our study was not large enough to rule out chance as a plausible explanation for the observed negative association between the GSTM1 null genotype and cervical cancer, or as a plausible explanation for the variation in the size of that association across various subgroups of women.

Am J Epidemiol Vol. 150, No. 6, 1999

GSTM1 Genotype and Risk of Cervical Cancer 569

Downloaded from https://academic.oup.com/aje/article/150/6/568/95426 by guest on 21 March 2019
Glutathione S-transferase *M1* has been hypothesized to play an important role in the detoxification of PAHs in tobacco smoke. The results of several (although not all) studies suggest that the *GSTM1* null genotype or phenotype is associated with an increased risk of cancers of the lung (17–23), bladder (24, 25), and colon (26). Data on the relation of *GSTM1* genotypes to the occurrence of cancers of the anogenital tract are limited. A hospital-based British study obtained results similar to ours, i.e., a somewhat smaller proportion of squamous cervical cancer cases than controls with menorrhagia had the null genotype (50.7 percent vs. 58.7 percent) (27). The same group of investigators also found the null genotype to be present in a smaller proportion of patients with high grade cervical intraepithelial neoplasia than controls (42 percent vs. 53 percent) (28). Our prior study of anal cancer (another smoking-related tumor) (16) observed an even stronger negative association (*GSTM1* null genotype present in 39.4 percent of cases vs. 56.7 percent of controls). Possible explanations for the lack of a positive association of *GSTM1* null genotype and the risk of anogenital cancers are: 1) The PAHs in tobacco smoke are not involved in the development of anal and cervical cancer. Thus, there is no reason to believe that variation in the genotypes that determine the activity of enzymes that detoxify PAHs, such as GSTM1, should be correlated with risks of these cancers. Other carcinogens in tobacco smoke and their metabolizing enzymes influence risk instead. 2) PAHs are important for the etiology of cervical and anal cancer, but another cellular factor(s) makes variation in *GSTM1* genotype unimportant. 3) Those PAHs that play a role in the etiology of anogenital cancers are not detoxified by GSTM1, but by other enzymes. These possibilities need to be explored further.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Squamous cell carcinoma cases (n=190)</th>
<th>Controls (n=206)</th>
<th>OR* 95% CI</th>
<th>OR* 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. with null genotype</td>
<td>% Null genotype</td>
<td>No. with null genotype</td>
<td>% Null genotype</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>53.2</td>
<td>118</td>
<td>57.3</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>61</td>
<td>47.5</td>
<td>58</td>
<td>63.8</td>
</tr>
<tr>
<td>35-49</td>
<td>71</td>
<td>50.7</td>
<td>95</td>
<td>56.8</td>
</tr>
<tr>
<td>≥50</td>
<td>58</td>
<td>62.1</td>
<td>53</td>
<td>50.9</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>59</td>
<td>49.2</td>
<td>94</td>
<td>57.4</td>
</tr>
<tr>
<td>Ever</td>
<td>131</td>
<td>55.0</td>
<td>112</td>
<td>57.1</td>
</tr>
<tr>
<td>Former</td>
<td>52</td>
<td>57.7</td>
<td>59</td>
<td>57.6</td>
</tr>
<tr>
<td>Current</td>
<td>79</td>
<td>53.2</td>
<td>53</td>
<td>56.6</td>
</tr>
<tr>
<td>Years smoked (current smokers)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;15</td>
<td>13</td>
<td>46.2</td>
<td>19</td>
<td>68.4</td>
</tr>
<tr>
<td>15-29</td>
<td>37</td>
<td>51.4</td>
<td>21</td>
<td>61.9</td>
</tr>
<tr>
<td>≥30</td>
<td>29</td>
<td>58.6</td>
<td>13</td>
<td>30.8</td>
</tr>
<tr>
<td>Average daily no. of cigarettes smoked</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>smoked (current smokers)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>30</td>
<td>56.7</td>
<td>32</td>
<td>65.6</td>
</tr>
<tr>
<td>≥20</td>
<td>49</td>
<td>51.0</td>
<td>21</td>
<td>42.9</td>
</tr>
</tbody>
</table>

* OR, odds ratio associated with the null genotype, relative to non-null genotypes. OR, crude odds ratio; CI, confidence interval; ORa, age-adjusted odds ratio.

ACKNOWLEDGMENTS

This work was supported by grant no. RO1 ES06728 from the National Institute of Environmental Health Sciences and grant nos. PO1 CA42792 and R35 CA39779 from the National Cancer Institute, National Institutes of Health. It was also supported by the Cancer Surveillance System of the Fred Hutchinson Cancer Research Center, which is funded by contract no. N01-CN-05230 from the Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute with additional support from the Fred Hutchinson Cancer Research Center.

We thank Christen Lubinski for technical support in conducting the GSTM1 assays; Anita C. Randle for technical assistance in preparation of the manuscript; Elizabeth Tickman for coordinating the blood drawing; Lisa A. Clayton, Andrea A. English, Marion R. Knudson, Shirley Opsted, and Diane E. Woodford, Wayne K. Fritschle, Jason E. Love, Neill A. Wiegand, and Lisa M. Szmyrma for processing the specimens.

REFERENCES


10. Robertson IG, Guthenberg C, Mannervik B, et al. Differences in stereoselectivity and catalytic efficiency of human glutathione transferase in the conjugation of glutathione with 7b,8a-dihydroxy-9a,10a-oxo-7,8,9,10-tetrahy-