Effects of glutathione S-transferase A1 (GSTA1) genotype and potential modifiers on breast cancer risk

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**Abstract**

Glutathione S-transferases (GSTs) are a family of phase II enzymes that are involved in the detoxification of carcinogens, environmental toxins and products of oxidative stress, by catalyzing conjugation with glutathione (1,2). GST alpha class is the primary hepatic GST, but is also expressed in human breast (2). Preferred substrates of GSTA1 include polycyclic aromatic hydrocarbons (e.g. benzo[α]pyrene), known tobacco smoke carcinogens (3). The GSTs, including GSTA1, are also involved, to some extent, in the metabolism of isothiocyanates (ITCs), potent anti-carcinogens that are derived from consumption of cruciferous vegetables (4), including broccoli, cabbage, cauliflower, Brussels sprouts, kale and collard greens. Notably, GSTs are also induced by these dietary components.

Glutathione S-transferases (GSTs) are phase II enzymes that are involved in the detoxification of a wide range of carcinogens. The novel GSTA1*A and GSTA1*B genetic polymorphism results in differential expression, with lower transcriptional activation of GSTA1*B (variant) than that of GSTA1*A (common) allele. Considering that cruciferous vegetables induce GSTs, which metabolize tobacco smoke carcinogens, we hypothesized that the variant GSTA1*B genotype may predispose women to breast cancer, particularly among low cruciferous vegetable consumers and among smokers. Thus, we evaluated potential relationships between GSTA1 polymorphisms and breast cancer risk, in relation to vegetable consumption and smoking status in the Long Island Breast Cancer Study Project (1996–1997), a population-based case–control study. Genotyping (1036 cases and 1089 controls) was performed, and putative breast cancer risk factors and usual dietary intakes were assessed. Having GSTA1*A/A or *B/B genotypes was not associated with increased breast cancer risk, compared to having the common *A/A genotype. However, among women in the lowest two tertiles of cruciferous vegetable consumption, *B/B genotypes were associated with increased risk (OR (95% CI) = 1.73 (1.10–2.72) for 0–1 servings/week), compared to women with *A/A genotypes. Among women with *B/B genotypes, a significant inverse trend between cruciferous vegetable consumption and breast cancer risk was observed (P for trend = 0.05), and higher consumption (4+ servings/week) ameliorated the increased risk associated with the genotype. Current smokers with *B/B genotypes had a 1.89-fold increase in risk (OR (95% CI) = 1.89 (1.09–3.25)), compared with never smokers with *A/A genotypes. These data indicate that GSTA1 genotypes related to reduced GSTA1 expression are associated with increased breast cancer primarily among women with lower consumption of cruciferous vegetables and among current smokers.

**Introduction**

Glutathione S-transferases (GSTs) are a family of phase II enzymes that are involved in the detoxification of carcinogens, environmental toxins and products of oxidative stress, by catalyzing conjugation with glutathione (1,2). GST alpha class is the primary hepatic GST, but is also expressed in human breast (2). Preferred substrates of GSTA1 include polycyclic aromatic hydrocarbons (e.g. benzo[α]pyrene), known tobacco smoke carcinogens (3). The GSTs, including GSTA1, are also involved, to some extent, in the metabolism of isothiocyanates (ITCs), potent anti-carcinogens that are derived from consumption of cruciferous vegetables (4), including broccoli, cabbage, cauliflower, Brussels sprouts, kale and collard greens. Notably, GSTs are also induced by these dietary components.

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**GSTA1**

Glutathione S-transferase A1 (GSTA1) is a member of the GST family and is involved in the detoxification of carcinogens, environmental toxins and products of oxidative stress. GSTA1 is known to be induced by cruciferous vegetables and is involved in the metabolic activation of some carcinogens, such as benzo[α]pyrene, a known tobacco smoke carcinogen. GSTA1 is also involved in the metabolism of isothiocyanates (ITCs), which are potent anti-carcinogens derived from cruciferous vegetables. The GSTA1 genotypes, particularly the *B/B* allele, have been associated with an increased risk of breast cancer, particularly among women with lower consumption of cruciferous vegetables and among current smokers.
The novel GSTAI*A and GSTAI*B genetic polymorphism, containing three linked base substitutions in the promoter at positions –567, –69 and –52, results in differential expression (14), with lower transcriptional activation with GSTAI*B (variant) than with GSTAI*A (common) alleles in vitro (15). Individuals with GSTAI*A have T, C and G at positions –567, –69 and –52, respectively, and those with GSTAI*B have G, T and A (3). In a directed mutagenesis assay, the G→A change at position –52 (NCBI rs no. 3957356) was responsible for differential promoter activity, and this modification also altered binding of the ubiquitous transcription factor SP1 (15). We (C.B.A.) previously reported differences in breast cancer survival after therapy associated with this GSTAI* genotype (16). To our knowledge, the associations between the novel GSTAI* genotype and breast cancer risk have not been evaluated and, therefore, merit further investigation.

Considering that GSTAI* polymorphisms may affect the detoxification efficiency of carcinogens, and that cruciferous vegetable consumption and cigarette smoking are sources of exposure to anti-carcinogens and carcinogens respectively, we hypothesized that the variant GSTAI*B genotype may predispose women to breast cancer, particularly those who are low vegetable consumers or smokers. Thus, we evaluated whether GSTAI*B genotype was associated with increased breast cancer risk, and whether the association between GSTAI*B genotype and breast cancer was higher among lower vegetable consumers or smokers in the LIBCSP.

Materials and methods
Study population

The LIBCSP, a population-based case–control study of breast cancer, was described previously (17). In brief, the cases were English-speaking women >20 years of age with newly diagnosed, primary in situ or invasive breast cancer who resided in Nassau and Suffolk Counties in Long Island, New York. Incident cases were ascertained between 1 August 1996, and 31 July 1997, using a rapid reporting network, developed by the study investigators. English-speaking controls, who were residents of the same two counties as the cases but did not have a history of breast cancer, were identified using Waksberg’s method of random-digit dialling (RDD) (18) for women under the age of 65 years, and from Health Care Finance Administration (HCFA) rosters for women who were 65 years or older. Controls were frequency matched to the expected age distribution of case women by 5-year age groups. All respondents signed informed consent forms prior to the study interview.

Upon receiving physician and participant consent, 1508 cases (82.1%) and 1556 controls (62.8%) were interviewed in their homes by a trained interviewer. Among case and control respondents who completed the interviewer-administered questionnaire, 98.2% and 97.6% self-completed the food frequency questionnaire (FFQ), and 73.0% and 73.3% donated a blood sample (17). As previously published (17), an increase in breast cancer among women on Long Island was found to be associated with lower parity, late age at first pregnancy, fertility problems, age at menarche, hormone replacement therapy, family history, body mass index and suspected risk factors for breast cancer, including reproductive, hormonal, and lifestyle histories. To control for differences in breast cancer survival after therapy associated with this GSTAI* genotype (16), and to control for the expected age distribution of case women by 5-year age groups. All controls but did not have a history of breast cancer, were identified using Waksberg’s method of random-digit dialling (RDD) (18) for women under the age of 65 years, and from Health Care Finance Administration (HCFA) rosters for women who were 65 years or older. Controls were frequency matched to the expected age distribution of case women by 5-year age groups. All respondents signed informed consent forms prior to the study interview.

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Measurements

GSTAI* genotyping. Genomic DNA was extracted from mononuclear cells in whole blood separated by Ficoll (Sigma Chemical, St Louis, MO) and washed twice with phosphate-buffered saline (PBS). Pelleted cells were frozen at –80°C until DNA was isolated from them by standard phenol and chloroform isoamyl alcohol extraction and RNase treatment (19). Genotyping was performed by BioServe Biotechnologies (Laurel, MD) using Sequenom’s high-throughput matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (20). PCR was performed in a total volume of 5 μl, containing 10x Buffer B (Solis Biodyne), 0.5 μl; DNA (diluted to 2.5 ng/μl); 2 μl; primers (5’-ACGTGTTGATGTTAACGCTTGCGCT-3’ and 5’-ACGTGTTGATGTTGCCCTAATCTG-3’), at 2 pmol/μl and 0.5 μl each; MgCl2 (25 mM, 0.5 μl); dNTPs (2.5 mM each, 0.25 μl); Taq polymerase (Qiagen), 0.02 μl; and water 0.73 μl.

Gene–vegetation interactions were evaluated by joint categories of GSTAI* genotype and vegetable intake. For vegetable consumption data, participants with daily energy intakes >3500 kcal or <400 kcal (cases = 36 and controls = 42) were dropped from the analyses. Total caloric intake was included in the multivariate model to control for confounding by total energy intake (23). In the fully adjusted model, we further adjusted associations with each category by the other specific categories of vegetables. For example, models assessing cruciferous vegetables were adjusted for yellow, leafy and total vegetables. Because there is concern about multi-colinearity when vegetable variables are adjusted together in the fully adjusted model, multi-colinearity was tested by calculating variance inflation factor (VIF) (24). However, because the fully adjusted model did not substantially change the estimates of effect, only the age- and total calorie-adjusted results are shown in the Table II.

Gene–smoking interactions were evaluated by joint categories of GSTAI* genotype and smoking (i.e. active smoking status and pack years of smoking). To test statistical interaction, we created a multiplicative scale, a cross-product term of the ordinal score for each genotype and the risk factor variables (e.g. genotype × vegetables or smoking) was included in multivariate models. The log-likelihood statistic for models that included a multiplicative interaction term was compared to those that did not. Tests for trend were conducted using the ordinal values for GSTAI* genotype.
Results
Among those with DNA available, 94% of cases and 93% of controls were Caucasian; 4% of cases and 4% of controls were African-Americans. Age range of cases and controls were 25.1–98.1 years (mean = 58.7, median = 57.8) and 20.3–95.5 years (mean = 56.1, median = 55.6), respectively. Thirty two percent of cases and 34% of controls were pre-menopausal women. Among case women with information on hormone receptor status and GSTA1 genotype (n = 667), 407 (61.0%) were diagnosed with an ER+PR+ tumor, 95 (14.2%) had an ER+PR– tumor, 31 (4.7%) had an ER–PR+ tumor and 134 (20.1%) had an ER–PR– tumor. Overall median and interquartile distribution of total fruit and vegetable consumption were 14 (range, 9–21) servings per week for cases and 15 (range, 10–22) servings per week for controls. Current smokers often tended to smoke more than former smokers in both mean duration and pack years (35.6 ± 11.7 years and 32.7 ± 29.8 pack years per day for current smokers, and 21.2 ± 13.7 years and 18.7 ± 23.5 for former smokers). In these data, there were 739 former smokers (34.4% of cases and 35.8% of controls) with a mean of 19.05 ± 12.45 (range, 1.08–62.64) years since quitting smoking.

GSTA1 genotypes and breast cancer risk
Associations between GSTA1 genotypes and breast cancer risk are shown in Table I. *A/A*, *A/B*, and *B/B* genotypes were present in 35, 48 and 17% of controls, and the genotype distribution followed HWE (P = 0.84) among controls. *A/A* and *B/B* genotypes were not associated with breast cancer risk. Risk associated with *B/B* genotypes was slightly higher in pre-menopausal women; however, there was no statistical interaction by menopausal status (P = 0.44).

GSTA1 genotypes, vegetable consumption and breast cancer risk
The ORs for breast cancer risk by GSTA1 genotypes and vegetable consumption are shown in Table II. We observed a significant 70% increase in risk among women with the *B/B* genotypes and the lowest tertiles of consumption of cruciferous vegetables [OR (95% CI) = 1.73 (1.10–2.72)] for 0–1 servings/week (tertile 1); OR (95% CI) = 1.77 (1.15–2.77) for 2–3 servings/week (tertile 2), compared with those with *A/A* genotypes and the lowest tertiles of intake. These estimates changed little when we adjusted for other vegetables in the multivariate model [data not shown, further adjusted yellow, leafy, and total vegetables, OR (95% CI) = 1.72 (1.10–2.72) for 0–1 servings/week (tertile 1); OR (95% CI) = 1.84 (1.18–2.88) for 2–3 servings/week (tertile 2)].

Although the *B/B* homozygotes comprised only 17% of the population (controls), a significant inverse trend between cruciferous vegetable consumption and breast cancer risk was observed in these women (P for trend = 0.05). This trend was not detected among women with the *A/A* or *A/B* genotypes (P for trend = 0.35 and 0.94, respectively). Among women with *B/B* genotypes, higher cruciferous vegetable (4+ servings/week, tertile 3) consumption ameliorated the expected increased risk associated with the *B/B* genotypes.

In an analysis restricted to the *B/B* genotypes, the OR for the highest tertile (4+ servings/week) versus the lowest tertile of cruciferous vegetable consumption (0–1 serving/week) was 0.57 (95% CI, 0.33–0.98). Conversely, associations between GSTA1 genotypes and breast cancer risk were most pronounced among women whose consumption was in the lowest tertile of cruciferous vegetables (P for trend = 0.01). However, statistically significant associations between GSTA1 *B/B* genotype and risk were not detected among women in the third tertile of cruciferous vegetable consumption (*B/B* genotype and 4+ servings/week, OR (95% CI) = 1.00 (0.62–1.62), P for trend = 0.89). Multiplicative interactions between GSTA1 genotypes and cruciferous vegetable consumption in relation to breast cancer risk were not statistically significant (P for multiplicative interaction = 0.10).

Lower consumption of yellow or leafy vegetables with *B/B* genotypes was not associated with an increased risk of breast cancer. We observed a 54% increase in risk (OR (95% CI) = 1.54 (1.08–2.38)) among the lowest consumers of total vegetables with *B/B* genotypes. However, this association was not significant and somewhat attenuated when we adjusted for other vegetables, including cruciferous vegetables [OR (95% CI) = 1.44 (0.93–2.23)]. When we tested for multi-collinearity among the vegetable intake variables by calculating the VIF, the values were <10; thus the vegetable categories were not collinear (cruciferous vegetables: 1.05, yellow vegetables: 1.59, leafy vegetables: 1.79 and total vegetables: 2.65). Inverse trends between breast cancer risk and yellow, leafy, and total vegetable consumption were not statistically significant among women with *B/B* genotypes, as well as *A/A* and *A/A* genotypes (data not shown).

The associations between GSTA1, vegetable consumption and breast cancer risk did not differ by menopausal status. Associations between risk and *B/B* genotypes among the lowest tertile of consumers were similar for pre-menopausal [OR (95% CI) = 1.66 (0.88–3.11)] and post-menopausal [OR (95% CI) = 1.56 (0.91–2.71)] women, although cell sizes were small and estimates somewhat unstable.

GSTA1 genotypes, smoking status and breast cancer risk
Current smokers with GSTA1*B/B* genotypes had an 89% increase in breast cancer risk [OR (95% CI) = 1.89 (1.09–3.25)], compared to those with *A/A* genotypes who never smoked (Table III). The joint effects of smoking and GSTA1 genotypes in relation to breast cancer risk were somewhat more pronounced among pre-menopausal women. Current

Table I. Breast cancer risk associated with GSTA1 polymorphisms: Long Island Breast Cancer Study Project, 1996–1997

<table>
<thead>
<tr>
<th></th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>ORa</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total participants</td>
<td>1036</td>
<td>1089</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td><em>A/A</em></td>
<td>342</td>
<td>336</td>
<td>1.00</td>
<td>0.89–1.30</td>
</tr>
<tr>
<td><em>A/B</em></td>
<td>498</td>
<td>522</td>
<td>1.08</td>
<td>0.89–1.30</td>
</tr>
<tr>
<td><em>B/B</em></td>
<td>196</td>
<td>181</td>
<td>1.20</td>
<td>0.94–1.54</td>
</tr>
<tr>
<td>Pre-menopausal womenb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A/A</em></td>
<td>110</td>
<td>113</td>
<td>1.00</td>
<td>0.75–1.46</td>
</tr>
<tr>
<td><em>A/B</em></td>
<td>153</td>
<td>179</td>
<td>1.04</td>
<td>0.94–2.28</td>
</tr>
<tr>
<td><em>B/B</em></td>
<td>68</td>
<td>53</td>
<td>1.46</td>
<td>0.94–2.28</td>
</tr>
<tr>
<td>Post-menopausal womenb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A/A</em></td>
<td>223</td>
<td>229</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td><em>A/B</em></td>
<td>332</td>
<td>324</td>
<td>1.07</td>
<td>0.84–1.36</td>
</tr>
<tr>
<td><em>B/B</em></td>
<td>127</td>
<td>124</td>
<td>1.06</td>
<td>0.78–1.45</td>
</tr>
</tbody>
</table>

aUnconditional logistic regression adjusted for age.
bExcluding 23 cases and 45 controls with missing information on menopausal status.
P for multiplicative interaction by menopausal status = 0.44.
Table II. Breast cancer risk associated with GSTA1 polymorphisms by consumption of vegetables: Long Island Breast Cancer Study Project, 1996–1997

<table>
<thead>
<tr>
<th>Vegetable Type</th>
<th>Low vegetable intake (tertile 1)</th>
<th>Intermediate vegetable intake (tertile 2)</th>
<th>High vegetable intake (tertile 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases^a</td>
<td>Controls^a</td>
<td>OR (95% CI)^b</td>
</tr>
<tr>
<td>Cruciferous vegetable</td>
<td>0–1 servings/week</td>
<td>290</td>
<td>327</td>
</tr>
<tr>
<td>A/A</td>
<td>87</td>
<td>122</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>A/B</td>
<td>144</td>
<td>151</td>
<td>1.31 (0.92–1.88)</td>
</tr>
<tr>
<td>B/B</td>
<td>68</td>
<td>54</td>
<td>1.73 (1.10–2.72)</td>
</tr>
<tr>
<td>Yellow vegetable</td>
<td>0–1 servings/week</td>
<td>325</td>
<td>330</td>
</tr>
<tr>
<td>A/A</td>
<td>99</td>
<td>118</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>A/B</td>
<td>164</td>
<td>155</td>
<td>1.22 (0.86–1.73)</td>
</tr>
<tr>
<td>B/B</td>
<td>62</td>
<td>57</td>
<td>1.30 (0.80–1.98)</td>
</tr>
<tr>
<td>Leafy vegetable</td>
<td>0–2 servings/week</td>
<td>349</td>
<td>352</td>
</tr>
<tr>
<td>A/A</td>
<td>115</td>
<td>132</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>A/B</td>
<td>157</td>
<td>161</td>
<td>1.10 (0.79–1.54)</td>
</tr>
<tr>
<td>B/B</td>
<td>77</td>
<td>59</td>
<td>1.43 (0.93–2.18)</td>
</tr>
<tr>
<td>All vegetable</td>
<td>0–11 servings/week</td>
<td>328</td>
<td>350</td>
</tr>
<tr>
<td>A/A</td>
<td>106</td>
<td>130</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>A/B</td>
<td>152</td>
<td>162</td>
<td>1.12 (0.80–1.58)</td>
</tr>
<tr>
<td>B/B</td>
<td>70</td>
<td>58</td>
<td>1.54 (1.08–2.38)</td>
</tr>
</tbody>
</table>

^aVegetable consumption based on tertiles (low, intermediate, high, respectively) of control group.
^bORs and 95% CIs calculated by unconditional logistic regression, adjusted for age and total calorie.
^cExcluding 52 cases and 55 controls with missing or unreliable (daily total caloric intake <400 kcal or >3500) information on diet.
^dP for multiplicative interaction: 0.10 (cruciferous vegetable), 0.50 (yellow vegetable), 0.19 (leafy vegetable) and 0.17 (total vegetable).

Table III. Breast cancer risk associated with GSTA1 polymorphisms by smoking status: Long Island Breast Cancer Study Project, 1996–1997

<table>
<thead>
<tr>
<th>Smoking Status</th>
<th>Cases^a</th>
<th>Controls^a</th>
<th>OR (95% CI)^b</th>
<th>Cases^a</th>
<th>Controls^a</th>
<th>OR (95% CI)^b</th>
<th>Cases^a</th>
<th>Controls^a</th>
<th>OR (95% CI)^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total participants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Active smoking status</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>145</td>
<td>167</td>
<td>1.00 (ref)</td>
<td>125</td>
<td>132</td>
<td>1.06 (0.76–1.48)</td>
<td>69</td>
<td>81</td>
<td>1.05 (0.75–1.58)</td>
</tr>
<tr>
<td>A/B</td>
<td>238</td>
<td>249</td>
<td>1.09 (0.82–1.45)</td>
<td>171</td>
<td>177</td>
<td>1.10 (0.81–1.50)</td>
<td>85</td>
<td>91</td>
<td>1.15 (0.79–1.67)</td>
</tr>
<tr>
<td>B/B</td>
<td>96</td>
<td>78</td>
<td>1.39 (0.95–2.02)</td>
<td>57</td>
<td>77</td>
<td>0.82 (0.55–1.24)</td>
<td>40</td>
<td>59</td>
<td>0.87 (0.54–1.42)</td>
</tr>
<tr>
<td>Pack years^d</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>145</td>
<td>167</td>
<td>1.00 (ref)</td>
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<td>91</td>
<td>0.91 (0.63–1.31)</td>
<td>122</td>
<td>121</td>
<td>1.20 (0.85–1.71)</td>
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<tr>
<td>A/B</td>
<td>238</td>
<td>250</td>
<td>1.09 (0.82–1.45)</td>
<td>105</td>
<td>117</td>
<td>1.07 (0.77–1.48)</td>
<td>149</td>
<td>148</td>
<td>1.18 (0.85–1.66)</td>
</tr>
<tr>
<td>B/B</td>
<td>96</td>
<td>78</td>
<td>1.39 (0.95–2.02)</td>
<td>34</td>
<td>50</td>
<td>0.83 (0.53–1.31)</td>
<td>62</td>
<td>51</td>
<td>1.45 (0.92–2.31)</td>
</tr>
</tbody>
</table>

^aExcluding 10 cases and 11 controls with missing information on smoking.
^bORs and 95% CIs calculated by unconditional logistic regression, adjusted for age.
^cActive smoking status: a current cigarette smoker was defined as a smoker within the 12 months prior to the reference date (defined as date of diagnosis for cases and date of identification for controls); a former smoker was defined as a smoker who reported quitting >12 months prior to the reference date.
^dPack years was divided by on median of current or former smokers of controls (16.35 pack years).
^P for multiplicative interaction: 0.12 (active smoking, total participants), 0.51 (packyears, total participants), 0.25 (active smoking, pre-menopausal women), 0.32 (packyears, pre-menopausal women), 0.18 (active smoking, post-menopausal women), and 0.14 (packyears, post-menopausal women).

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pre-menopausal smokers with the GSTA1*B/*B genotypes had a 2.69-fold increase in the risk of getting breast cancer [OR (95% CI) = 2.69 (1.09–6.63)], compared with those with *A/*A genotypes, who never smoked. We stratified by dose (pack-years of smoking, median). Heavier smokers (≥16.35 pack-years) with *B/*B genotypes, experienced the most pronounced increase in their risk of breast cancer, compared to lighter smokers (<16.35 pack-years) or nonsmokers with *A/*A genotypes. Yet, cell sizes were small, and risk estimates were unstable (Table III). Among post-menopausal women, smoking status did not modify the association between the GSTA1 genotype and breast cancer risk, although women with *B/*B genotypes who had never smoked had a somewhat increased risk of breast cancer.

Discussion

In this large population-based study, we found that associations between a polymorphism adversely affecting expression of GSTA1 and breast cancer risk were primarily observed among women in the lowest two tertiles of cruciferous vegetable consumption, and among smokers. A significant inverse trend was observed between cruciferous vegetable consumption and breast cancer risk (P for trend = 0.05) among women with the *B/*B genotype. Higher consumption (4+ servings/week) ameliorated the observed increased risk associated with the genotype. This finding is consistent with the hypothesis that GSTA1 polymorphisms may increase breast cancer risk in environments with significant exposure to carcinogens (cigarette smoke), or where there is little chemopreventive protection from specific components in cruciferous vegetables. These findings of gene–environment interactions emphasize the importance of cruciferous vegetable consumption and, perhaps smoking cessation, to reduce breast cancer risk, particularly among women with variant GSTA1 *B/*B genotypes.

GSTA1 may be particularly important in relation to breast cancer because of its interactions with sex hormones, although the evidence supporting this possibility is limited. For example, follicular maturation and luteinization increase GSTA1 expression in the bovine and porcine ovary, which also may be induced by pituitary gonadotropins (i.e. follicle-stimulating hormone and luteinizing hormone) (25,26). In addition, 350 and 250 nM estradiol was shown to inhibit human GSTA1 and GSTM1 expression respectively, but not GSTP1 and GSTT1, in the rat liver (27,28). GSTA1 is present in mammary tissue, although the enzyme is expressed most abundantly in the liver (15). Furthermore, the expression of GSTA1 does not correlate with either GSTM1 or GSTP1 expression in human mammary tissue (29), although GSTM1 and GSTP1 expressions are correlated (30), indicating an important role of GSTA1 enzyme in the breast.

Observed associations between GSTA1 polymorphisms and breast cancer risk only among low cruciferous vegetable consumers indicate that specific components in these vegetables may be important in reduction of breast cancer risk. ITCs, contained in cruciferous vegetables, are known to induce GSTA1 gene transcription in cultured human cells (31) and in animal models (32). Thus, it is possible that induction of GSTA1 gene expression by ITCs among higher cruciferous vegetable consumers could override the reduced GSTA1 gene expression due to variant GSTA1 genotype, resulting in increased risk among women with GSTA1 low activity genotypes and low consumption of cruciferous vegetables. Alternatively, ITCs may have anti-carcinogenic properties via independent mechanisms of GSTA1 induction, (i.e. leading to apoptosis by activating caspases 3, 8, 9 or 12, or controlling cell cycle by modulating cell cycle regulators) (4). Thus, since ITCs could provide additional protection from DNA damage, high consumption may ameliorate risk associated with reduced expression of GSTA1 by the polymorphism. However, the biological basis for increased risk with the *A/*A genotype with the second tertile of cruciferous vegetable consumption are unclear, and it is possible that this result is due to chance.

Although our study is the first to evaluate the association between GSTA1 genotype and breast cancer, there have been previous reports on associations between other GST polymorphisms (i.e. GSTM1, GSTP1 and GSTT1), ITCs and cancer risk. Spitz et al. (33) found that lung cancer risk was greatest among those with GST null genotypes who were low consumers of ITCs, which is consistent with our findings. They suggested that since ITCs induce GST expression, the greatest cancer risk was observed among those of a GST null genotype and low ITC intakes. Wark et al. (34) demonstrated that consumption of cruciferous vegetables was associated positively with GST enzyme activity among those with the GSTM1-positive genotype, but not those with the GSTM1-null genotype, suggesting that ITCs may be primarily responsible for this GST inducing capacity. Similarly, another study found that among individuals with low urinary ITC level, GSTM1 (OR, 2.35, 95% CI, 1.02–5.41) and GSTT1 (OR, 1.53, 95% CI, 0.68–3.44) null genotypes were associated with increased lung cancer risk. Furthermore, when the population was stratified by GSTM1 or GSTT1 genotypes, associations between risk and urinary ITCs were more pronounced among those with GST null genotypes (35,36). Those authors also suggest that GSTs may influence associations between risk and ITCs through their role in the metabolism and excretion of chemopreventive agents, such as ITCs. We (C.B.A.) recently investigated associations between breast cancer risk, consumption of cruciferous vegetables, and GSTM1 and GSTT1 genotypes in the Western New York Diet Study, and observed no significant interaction effects of GST genotype on breast risk, although sample size for women with genotype data available was small (5).

Tobacco smoke contains numerous carcinogens, including nitrosamines, polycyclic aromatic hydrocarbons and aromatic amines. Thus, it is possible that genotypes resulting in reduced expression of GSTA1 are associated with increased breast cancer risk in the presence of tobacco smoke carcinogens, although a single GST polymorphism may not be enough to elevate risk in the absence of carcinogenic exposures. Results from several other studies are consistent with our findings. As reviewed by Rebeck (37), several studies have shown that individuals with GSTM1 and GSTT1 null alleles are at increased risk of lung and bladder cancer, both of which are associated with exposure to chemical carcinogens (e.g. cigarette smoking). Zheng et al. (38) also reported that GSTM1 and GSTT1 null genotypes were associated with a 60% increased risk of breast cancer, particularly among smokers.

Although our findings on GSTA1 variant alleles, when stratified by menopausal status, are based on relatively small numbers, the increased risk among pre-menopausal smokers could imply that the effects of GSTA1 polymorphisms would be pronounced in a high carcinogen environment. Although it is not clear why associations between risk, GSTA1 genotypes and smoking are more pronounced among pre-menopausal smokers, this result is due to chance.
women, it is possible that estrogens may play a role, particularly in induction of GSTA1 expression (27). However, biochemical studies to identify and elucidate possible mechanisms are needed.

These results could also be affected by sources of bias that are common to case–control studies (e.g., recall bias) (39), or to misclassification related to genotyping. Although genotyping data is not susceptible to the problem of recall bias, diet and other interview data may be susceptible to recall biases, which may have an impact on the interaction observed. One shortcoming of the present study was that we were not able to measure biomarkers of urinary excretion of ITCs; such measurements may be less susceptible to bias resulting from dietary measurement, although they have other potential biases related to case–control status. In addition, study participants were limited to English-speaking women (>97% of Long Island residents spoke English at the time this study was undertaken) (35). If English speakers differ from non-English speakers in cruciferous vegetable consumption, smoking status and genotype frequency, results based on LIBCSP data may not be generalizable to all the women. Because other GST genotype data (i.e. GSTM1 and GSTP1) were not available, we were unable to conduct combined analyses. However, GSTA1 genotype may play an independent role in the breast, since the expression of GSTA1 did not correlate with either GSTM1 or GSTP1 in human mammary tissue (29). Finally, since several stratified analyses were conducted, the results may be attributable to chance.

In summary, breast cancer risk was elevated among women with the GSTA1 *B/B* genotype who consumed lower amounts of cruciferous vegetables or who were current smokers. A significant inverse trend was observed between increasing cruciferous vegetable consumption and decreasing breast cancer risk among women with the *B/B* genotype. Higher consumption (4+ servings/week) ameliorated the observed increased risk associated with the genotype. To our knowledge, this is the first study to evaluate GSTA1 genotypes and breast cancer risk. It is based on data from a large population-based case–control study with adequate statistical power and in-depth interview assessments to be able to assess potential associations. Although genotype cannot be changed, consumption of diets rich in these vegetables and, perhaps avoidance of smoking, can be undertaken for breast cancer risk reduction.

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**References**

matrix assisted laser desorption/ionization time of flight (MALDI-TOF).


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