Glutathione Transferase Null Genotype, Broccoli, and Lower Prevalence of Colorectal Adenomas

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Abstract

Cruciferous vegetables, especially broccoli, may prevent cancer through anticarcinogenic compounds. For example, broccoli contains isothiocyanates that induce carcinogen-detoxifying enzymes. Glutathione transferase enzymes conjugate isothiocyanates, leading to excretion. We hypothesized that broccoli consumption in combination with the glutathione transferase M1 (GSTM1) null genotype would be associated with a lower prevalence of colorectal adenomas because of higher isothiocyanate levels. We used a case-control study of mainly asymptomatic subjects aged 50–74 years who underwent a screening sigmoidoscopy at either of two Southern California Kaiser Permanente Medical Centers during 1991–1993. Cases (n = 459) had a first-time diagnosis of histologically confirmed adenomas detected by flexible sigmoidoscopy. Controls (n = 507) had no polyp detected. Subjects had a 45-min in-person interview for information on various risk factors and basic demographic data and completed a 126-item, semiquantitative food frequency questionnaire. Blood samples were used for GSTM1 genotyping. Subjects with the highest quartile of broccoli intake (an average of 3.7 servings per week) had an odds ratio of 0.47 (95% confidence interval, 0.30–0.73) for colorectal adenomas, which are precursors to cancer. Subjects with the GSTM1 null genotype (P for trend, 0.001; P for interaction, 0.01). The observed broccoli-GSTM1 interaction is compatible with an isothiocyanate mechanism.

Introduction

Cruciferous vegetables are viewed as potentially protective against cancer (1, 2). These vegetables include broccoli, brussels sprouts, cabbage, cauliflower, kohlrabi, and kohlrabi, which are varieties of Brassica oleracea (3). Of early epidemiological evidence for a protective effect against colorectal cancer, Graham et al. (4) found an OR of 0.3 comparing people who ate cabbage at least once per week to those who never ate cabbage. Other studies gave similar results, based on data for: cruciferous vegetables as a group (5, 6); broccoli, cauliflower, brussels sprouts, and turnips (7); and cabbage, cauliflower, and sprouts (8). However, not all studies have found inverse associations between cruciferous vegetables and colorectal cancer (9–12).

Isothiocyanate compounds are derived from glucosinolates in various plants. Medicinal properties of glucosinolates are historically important, as seen in writings by Pythagoras and Hippocrates. At least 20 such compounds were identified by the 1980s (13). All cruciferous vegetables are believed to contain glucosinolates, but brussels sprouts (600–3900 μg/g), broccoli (450–1480 μg/g), and Savoy cabbage (470–1290 μg/g) have the highest content among Brassica oleracea. (14)

Anticarcinogens in cruciferous vegetables induce enzymes that detoxify environmental mutagens. Broccoli extract was the most potent enzyme inducer in a murine hepatoma cell assay (14), probably due to sulforaphane, an isothiocyanate compound (14, 15). Sulforaphane blocked 7,12-dimethylbenz(a)-anthracene-induced mammary tumors in rats, supporting a conclusion that isothiocyanates protect against cancer (16).

Zhang et al. (17) and Kolm et al. (18) found that GSTM1 rapidly conjugates isothiocyanates to glutathione, leading to excretion. Approximately 50% of people completely lack GSTM1 enzyme, due to homozygous deletion of the gene (i.e., the GSTM1 null genotype: Ref. 19–21). We hypothesized that a cancer preventive effect of broccoli would be stronger with the GSTM1 null genotype, due to potentially slower excretion of isothiocyanates. Here we show effects of broccoli consumption and the GSTM1 null genotype on prevalence of colorectal adenomas, which are precursors to cancer.

Materials and Methods

Cases and Controls. Subjects were from one of two Southern California Kaiser Permanente Medical Centers (Bellflower or Torrance) who had had a screening sigmoidoscopy from 1991 to 1993. Subjects were mainly asymptomatic subjects aged 50–74 years who underwent a screening sigmoidoscopy. Controls had no polyp detected.

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3 The abbreviations used are: OR, odds ratio; CI, confidence interval; GSTM1, glutathione transferase M1.
Sunset) and had a sigmoidoscopy during the period from January 1, 1991, through August 25, 1993. Eligible men and women were ages 50–74 years, residents of Los Angeles or Orange counties, and fluent in English. None had a history of invasive cancer, inflammatory bowel disease, familial polyposis, previous bowel surgery, symptoms suggestive of gastrointestinal disease, or a physical or mental problem that would preclude an interview. Cases had a first-time diagnosis of one or more histologically confirmed colorectal adenomas. Controls were selected from subjects with no polyps and were individually matched to cases by gender, age (within a 5-year category), sigmoidoscopy date (within a 3-month category), and center where examined.

There were 628 potentially eligible cases and 689 potentially eligible controls during the accrual period. Seventy cases and 94 controls refused an interview, and 29 cases and 32 controls could not be contacted. Response rates (number initially eligible controls during the accrual period. Seventy cases and 94 controls refused an interview, and 29 cases and 32 controls could not be contacted. Response rates (number interviewed/number eligible) were 84% for cases and 82% for controls. A replacement was identified when the control subject originally matched to a case could not be interviewed.

Indications for sigmoidoscopy among interviewed subjects were: "routine" for 45% of cases and 44% of controls; "minor symptoms" for 16% of cases and 13% of controls; and "not specified" for 39% of cases and 43% of controls. Average depth of the flexible sigmoidoscope was 55 ± 11 (SD) cm for cases and 59 ± 5 cm for controls. Fifteen cases had carcinoma in situ. Size and number of polyps were recorded by the sigmoidoscopist.

Subjects gave information on smoking, therapeutic drug use, physical activity, height, weight, family history of cancer, and other factors during a 45-min, in-person interview. The interview was 5 months after sigmoidoscopy, on average, and exposure data referred to the period up to the sigmoidoscopy. Interviewers were unaware of case or control status for 70% of cases and 87% of controls.

**Diet Data.** A 126-item, semiquantitative food frequency questionnaire (22), asking about diet during the year before sigmoidoscopy, was filled-out by 519 cases and 556 controls. The questionnaire provides information on intake of the following cruciferous vegetables: broccoli; cabbage or cole slaw; cauliflower; brussels sprouts; and kale, mustard, or chard greens. Standard methods were used to compute nutrient intakes. Specific foods corresponding to items on the questionnaire were selected based on the relative frequency of consumption among participants of the 1988–1989 Nationwide Food Consumption Survey, southwest region. The Nutrient Data System (NDB version 2.4) was used as a nutrient database for foods. Data on nutrient content of supplements were from the Harvard School of Public Health.

**Genotyping.** GSTM1 genotyping was done on 977 subjects who provided a blood specimen. Dried blood spots on blotter paper (Schleicher and Schuell no. 903) were used for DNA templates for 936 specimens. Liquid DNA was used for the remaining 41 specimens. PCR assays were as described (23). Duplicate assays were done for all specimens, without knowledge of case or control status.

**Data Analysis.** The analysis included 966 subjects, because 11 of the 977 genotyped subjects did not complete a food frequency questionnaire and had to be excluded. We assumed that persons who did not answer a specific item on the questionnaire (including cruciferous vegetables) never ate that item, having found that missing responses most commonly turn out to be zero.

We used unconditional logistic regression to estimate ORs and controlled for the matching factors—date of sigmoidoscopy (6-month intervals), age (5-year intervals), gender, and center attended—as indicator variables in the model. This allowed us to include information on unmatched subjects in the analysis. Unmatched controls occurred when, for example, their matched cases did not speak English or were found to have invasive cancer at follow-up colonoscopy. Unmatched cases occurred when we were unable to interview an eligible control. Unmatched subjects also occurred if blood, and therefore GSTM1 genotypes, was obtainable from only one subject of a pair. Categories of vegetable intake as presented in the tables were entered as indicator variables in the model.

Covariates considered for possible inclusion in the multivariate models were: race; body mass index; vigorous leisure time activity; intake of total energy, saturated fat, and fruits and vegetables; family history of colorectal cancer; intake of nonsteroidal anti-inflammatory drugs; and smoking. None of the covariates changed the size of the point estimates of interest by more than 10%. We therefore adjusted only for matching variables, smoking, and intake of total energy, saturated fat, and all other fruits and vegetables (i.e., vegetables not specified by the model).

Subjects homozygous for the GSTM1 gene deletion were coded as having the GSTM1 null genotype. Interaction between vegetable intake and GSTM1 genotype was assessed by models containing interaction terms of vegetable intake with GSTM1 genotype. One hypothesis is that the null genotype may be associated with increased risk of colorectal cancer in smokers, so that smoking might modify a vegetable-GSTM1 interaction. We did not stratify analyses by smoking status, however, because no interaction between smoking and GSTM1 was found in our original analyses (23). We did restrict the analysis to people who never smoked to corroborate that any vegetable-
Table 2  ORs for colorectal adenomas by intake of cruciferous vegetables

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Intake frequency (servings)</th>
<th>OR</th>
<th>CI</th>
<th>P_trend</th>
<th>P_trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>1.00</td>
<td>0.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1–3/month</td>
<td>0.65</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1/week</td>
<td>0.65</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;1/week</td>
<td>0.47</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cabbage/cole slaw</td>
<td>95% CI</td>
<td>0.20–0.93</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls/Cases</td>
<td>72/105</td>
<td>0.43–0.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean servings/week</td>
<td>0</td>
<td>1</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cauliflower</td>
<td>95% CI</td>
<td>0.96–1.86</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls/Cases</td>
<td>134/108</td>
<td>0.75–1.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean servings/week</td>
<td>0</td>
<td>1</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>95% CI</td>
<td>0.58–1.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls/Cases</td>
<td>204/212</td>
<td>0.68–1.51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean servings/week</td>
<td>0</td>
<td>1</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kale, mustard, chard greens</td>
<td>95% CI</td>
<td>0.55–1.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls/Cases</td>
<td>357/332</td>
<td>0.75–2.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean servings/week</td>
<td>0</td>
<td>1</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The effect of single vegetables (as indicator variables for categories) were adjusted for smoking (indicator variables for current smoking and ex-smoking): matching variables (age in 5-year categories; date of sigmoidoscopy in five categories total): clinic; gender; saturated fat (indicator variables for quartiles of saturated fat intake in the total population); energy intake (calories as continuous variable); intake of fruits and vegetables (indicator variables for quartiles of fruit and vegetable intake minus the specific vegetable(s) of interest in the model).

* *P* for trend was calculated by entering the mean number of servings/week in each group as a categorical variable in the model.

* *P* for trend was calculated by entering the intake of the respective vegetable as a continuous (ordered) variable in the model.

**GSTM1** interaction was not different in this subgroup. Cigarette smoking data were abstracted from in-person interviews, and results of the main effect of smoking are presented elsewhere (24). Individuals who had never smoked 100 cigarettes in their life were coded as never smokers. Subjects who had smoked more than 100 cigarettes were coded as ex-smokers if they quit smoking before the sigmoidoscopy and as current smokers if they were smoking at the time of sigmoidoscopy.

Restriction of analyses to subjects with an energy intake between 800 and 4200 kcal did not substantially alter results and was thus omitted.

**Results**

Cases and controls are described in Table 1. Male:female distribution, ethnic mix, and average ages were fairly comparable for the two groups. There was a lower percentage of smokers among controls. On average, controls ate more fruits and vegetables per week, including cruciferous vegetables and broccoli.

Main effects of broccoli and of cruciferous vegetables on the prevalence of colorectal adenomas are shown in Table 2. Earlier we presented protective effects of cruciferous vegetables and broccoli in our study population (25). Because we now focus on interaction between cruciferous vegetables and **GSTM1**, we again show the main effects of cruciferous vegetables and broccoli, but among subjects with known **GSTM1** genotype.

We estimated ORs for single cruciferous vegetables across four levels of serving intake: never; 1–3/month; 1/week; and >1/week. Increased consumption of broccoli and kale, but not of other cruciferous vegetables, were associated with lower prevalence of colorectal adenomas (broccoli: *P* for trend, 0.007; kale: *P* for trend, 0.04). The OR comparing the highest to lowest category of broccoli intake was 0.47 (95% CI, 0.30–0.73). The corresponding OR for kale was 0.41 (95% CI, 0.16–1.06).

Results from a model with vegetable intake as a continuous variable (instead of indicator variables) agreed well with the results from the model shown in Table 2. The ORs (and 95% CIs) per additional serving/week and the *Ps* for trend were: broccoli, 0.91 (0.83–0.99), *P* = 0.03; cabbage, 1.02 (0.91–1.15), *P* = 0.74; cauliflower, 0.91 (0.79–1.05), *P* = 0.20; Brussels sprouts, 1.00 (0.80–1.26), *P* = 0.97; and kale, 0.78 (0.62–0.99), *P* = 0.04.

A main effect of the **GSTM1** null genotype was reported earlier (23). The overall OR for adenomas with the **GSTM1** null genotype was 0.85 (95% CI, 0.65–1.10), consistent with no **GSTM1** effect.

We used quartiles of vegetable intake to assess interaction between **GSTM1** and vegetables, because there were too few subjects in the reference category of “never” for all cruciferous vegetables combined. For broccoli, quartiles produced the same groupings as in Table 2. The overall risk of adenomas decreased with increasing intake of cruciferous vegetables (Table 3), but the effect was somewhat weaker than for broccoli (Table 2). Analysis of quartiles was not possible for Brussels sprouts and kale, because they were rarely eaten.

A protective effect of broccoli and cruciferous vegetables...
Effects of broccoli and cruciferous vegetables

<table>
<thead>
<tr>
<th>Broccoli</th>
<th>GSTM1 null</th>
<th>GSTM1 non-null</th>
<th>P*</th>
<th>Without GSTM1 stratification</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR</td>
<td>1.00</td>
<td>0.20</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.65-1.00</td>
<td>0.10-0.71</td>
<td>0.53</td>
<td>0.04</td>
</tr>
<tr>
<td>Controls</td>
<td>36/24</td>
<td>85/56</td>
<td>77/33</td>
<td>70/64</td>
</tr>
<tr>
<td>Cases</td>
<td>0.5</td>
<td>1</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Serving/week</td>
<td>0.5</td>
<td>1.0</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Cruciferous vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OR</td>
<td>1.00</td>
<td>0.20</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.65-1.00</td>
<td>0.10-0.71</td>
<td>0.53</td>
<td>0.04</td>
</tr>
<tr>
<td>Controls</td>
<td>68/65</td>
<td>56/58</td>
<td>59/49</td>
<td>66/32</td>
</tr>
<tr>
<td>Cases</td>
<td>0.6</td>
<td>1.3</td>
<td>7.3</td>
<td></td>
</tr>
<tr>
<td>Serving/week</td>
<td>0.6</td>
<td>1.3</td>
<td>7.3</td>
<td></td>
</tr>
</tbody>
</table>

* Effects of broccoli and cruciferous vegetables per servings/week: sum of broccoli, cabbage/cole slaw, cauliflower, brussels sprouts, kale/mustard/chard greens (as indicator variables for quartiles) were adjusted for smoking (indicator variables for current smoking and ex-smoking); matching variables (age in 5-year categories; date of sigmoidoscopy in five categories total); clinic; gender; saturated fat (indicator variables for quartiles of saturated fat intake in the total population); energy intake (calories as a continuous variable); intake of fruits and vegetables (indicator variables for quartiles of fruit and vegetable intake minus the specific vegetable(s) of interest in the model). For broccoli, the Ps for trend was calculated from entering the mean number of servings/week in each quartile (calculated separately for GSTM1 null and non-null genotypes) as a categorical variable into the model. For cruciferous vegetables, the Ps for trend was calculated from entering the mean number of servings/week in each quartile (calculated separately for GSTM1 null and non-null genotypes) as a categorical variable into the model. For each vegetable, the Ps for trend were 0.04 for cruciferous vegetables and 0.02 for broccoli.

Discussion

Our aim is to identify mechanisms by which cruciferous vegetables protect against colorectal cancer. Although fiber and folate may contribute (26–29), these vegetables also contain nonnutrient compounds that block cancers in laboratory rats. Examples are 3,3'-diindolylmethane, indole-3-carbolin, and brassinin, found in brussels sprouts, cabbage, and cauliflower (30, 31). Here we focused mainly on broccoli, because it contains the isothiocyanate sulforaphane, a potent inducer of carcinogen-detoxifying enzymes (14).

People consume several milligrams of isothiocyanates or glucosinolates per day. Protection against cancer by these compounds is attributed to inhibition of phase 1 enzymes (32) and induction of phase 2 enzymes (Fig. 1). Induction of detoxifying, phase 2 enzymes has been called the electrophile counterattack response (33, 34). However, glutathione transferases GSTM1 and GSTP1 conjugate isothiocyanates and divert them from the enzyme induction pathway to excretion (17, 18). Lack of GSTM1 is hypothesized to favor the pathway that leads to enzyme induction, thereby protecting against neoplasms. GSTs, glutathione transferases; NQO1, NADPH:quinone oxidoreductase; mEH, microsomal epoxide hydrolase; UGTs, UDP-glucuronosyltransferases.
indirectly assessing isothiocyanate levels in relation to colorectal adenoma prevalence.

Subjects with high broccoli intake and the GSTM I null genotype had the lowest adenoma prevalence. These subjects could have higher isothiocyanate levels, due to high intake and slow excretion. A GSTM I interaction was observed only for broccoli, perhaps due to the larger range of broccoli intakes compared with other vegetables. Average broccoli intake was 1.7 servings per week, compared with 0.97 for cabbage or cole slaw; 0.70 for cauliflower; 0.27 for brussels sprouts; and 0.24 for kale, mustard, or chard greens.

The protective effect of broccoli could be due to other compounds whose levels are higher in broccoli than in other cruciferous vegetables. For example, broccoli has a higher content of carotenoids. However, adjustment for carotenoids, folate, vitamin C, and fiber in our previous analysis did not substantially change protective effects of broccoli or cruciferous vegetables (25).

The GSTM I null genotype conceivably could have raised the risk of adenomas, instead of lowering the risk, through production of less GSTM I for mutagen removal. However, GSTM I is only one of several enzymes that detoxify electrophiles. Lack of GSTM I may not matter for mutagen removal if these compounds can be quickly cleared by other enzymes. Also, GSTM I is not inducible in the colon (36) or in hepatocytes (32).

Earlier reports showed increased risks of bladder and lung cancer in smokers with the GSTM I null genotype (37–39). However, our previous work did not find a higher risk for colorectal adenomas in smokers with the null genotype (23). Analyses of broccoli and GSTM I effects in the subgroup of people who never smoked did not change results, again supporting an interpretation of no interaction between GSTM I and smoking for colorectal adenomas.

Strengths of the study were screening asymptomatic subjects for a first-time diagnosis of adenomas, a fairly large sample size, and a response rate greater than 80% for both cases and controls. Diet effects from our study population (24, 29, 40, 41) agree with other published studies, supporting the validity of our results. Weaknesses of the study were reliance on diet questionnaires, the potential for recall bias, detection of prevalent rather than incident adenomas, and assessment of adenomas in only the left colon. Some 50% of colon cancers occur in the proximal segment (42). Up to 15–17% of controls who have left-sided adenomas and controls. Diet effects from our study population (24, 29, 40, 41) agree with other published studies, supporting the validity of our results. Weaknesses of the study were reliance on diet questionnaires, the potential for recall bias, detection of prevalent rather than incident adenomas, and assessment of adenomas in only the left colon. Some 50% of colon cancers occur in the proximal segment (42). Up to 15–17% of controls who have left-sided adenomas.

In summary, prevalence of colorectal adenomas was lowest among people with high broccoli intake and the GSTM I null genotype. Slower conjugation and excretion of protective isothiocyanates, due to lack of GSTM I enzyme, is a plausible mechanism. Studies on enzyme variants and cancer risk usually focus on carcinogens, like those in cigarette smoke. In contrast, our work suggests a novel gene-environment interaction involving an anticarcinogen. If confirmed, the result suggests that people with the GSTM I null genotype may benefit more from broccoli.

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References
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