Green tea intake, ACE gene polymorphism and breast cancer risk among Chinese women in Singapore

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Experimental and epidemiological data have implicated a potential chemoprotective role of green tea polyphenols and a potential enhancing role of angiotensin II in the development of breast cancer in humans. Angiotensin II is converted from its precursor by angiotensin-converting enzyme (ACE). Women with low-activity genotype of the ACE gene had a reduced risk of breast cancer compared with those possessing high-activity ACE genotype. Experimental data showed that green tea polyphenols could inhibit angiotensin II-induced reactive oxygen species production. We reasoned that if this is one of the mechanisms by which green tea polyphenols protect against human breast cancer, then their effect should be more prominent among women possessing high-activity ACE genotype than women with low-activity ACE genotype. In other words, we predict a stronger inverse green tea–breast cancer association among the former versus the latter subgroup of women. To test this hypothesis, we conducted a nested case–control study involving 297 incident breast cancer cases and 665 control subjects within the Singapore Chinese Health Study. There was no association between intake frequencies of green tea and risk of breast cancer among all women or those with low-activity ACE genotype. Among women with high-activity ACE genotype, however, intake frequency of green tea was associated with a statistically significant decrease in risk of breast cancer (P for trend = 0.039); the odds ratio (95% confidence interval) was 0.33 (0.13–0.82) for women drinking green tea at least monthly and 0.29 (0.10–0.79) for those drinking green tea at least weekly compared with non-drinkers. There was a statistically significant interaction effect between green tea intake and ACE genotype on risk of breast cancer (P for interaction = 0.01). Black tea intake was unrelated to breast cancer risk irrespective of the ACE genotype. The findings of the present study highlight the importance of genetically determined factors in evaluating the role of green tea intake in the development of breast cancer.

Introduction

Tea is derived from the leaf of the plant Camellia sinensis. Although black tea is consumed mainly in USA and Europe, green tea is the main tea beverage in Asian countries, such as Japan and China. Green tea polyphenols consist primarily of catechins and gallocatechins, including epigallocatechin-3 gallate (EGCG), which has been shown to exhibit antiproliferative and antiangiogenic effects in breast cancer cell lines (1–3). In rodent models, green tea extracts or catechins in the diet can inhibit the size and multiplicity of carcinogen-induced mammary tumors and significantly increase the survival rate of carcinogen-treated animals (1,4). In human studies, consumption of green tea was inversely associated with breast cancer recurrence among Japanese women (5). A recent case-control study showed that increased consumption of green tea was significantly associated with decreasing risk of breast cancer among Asian women in Los Angeles; regular tea drinkers had a ~40% reduction in risk of breast cancer compared with non-drinkers (6). This green tea protective effect is seen primarily among women possessing the low-activity genotype of catechol-O-methyltransferase (COMT) (7). The authors hypothesized that this subgroup of women might be benefiting from their relative inability to metabolize tea catechins since COMT catalyzes one of three major elimination pathways for catechins (8).

The exact mechanism by which green tea polyphenols may exert a protective effect in carcinogenesis is not completely understood. Although antioxidative properties of green tea polyphenols may play a role (9,10), other mechanism(s) may also be involved. Since in vitro studies showed that green tea polyphenols can inhibit angiotensin II-induced reactive oxygen species (ROS) production (11) and the recent epidemiological evidence suggests a direct role of angiotensin II in human breast cancer development (12,13), we reasoned that green tea polyphenols may protect against human breast cancer through their inhibitory role in ROS production induced by angiotensin II. Angiotensin II is converted from its precursor by the catalytic action of angiotensin I-converting enzyme, which can exhibit up to a 2-fold variation in plasma levels between individuals (14). We recently reported that women possessing a functional low-activity ACE genotype exhibited a 50% reduction in breast cancer risk compared with their high-activity genotype counterparts (13). If the angiotensin II pathway is indeed involved in the anticarcinogenic actions of green tea on breast tissue, then women with higher levels of circulating angiotensin II may show a more prominent effect of green tea protection compared with women with lower levels of angiotensin II. In other words, one would expect a stronger tea–cancer association among women possessing the high-activity ACE genotype compared with their counterparts with the low-activity genotype. This report describes the results of an association study between green tea intake in combination with ACE genotypes and risk of breast cancer among
Singapore Chinese women who are participants of the Singapore Chinese Health Study, a prospective cohort study of diet and cancer.

Materials and methods

Study subjects

The study design and subject recruitment of the Singapore Chinese Health Study have been described (15). Briefly, 63,257 Chinese women and men aged 45–74 years belonging to the Hokkien or Cantonese dialect group were enrolled in the study between April 1993 and December 1998. At recruitment, information on lifestyle factors, usual diet and reproductive history (for women only) was obtained through in-person interviews. The dietary component of the questionnaire was validated through a series of 24 h food recalls (15). Study subjects were asked to choose the intake frequency of green tea and black tea, from nine predefined categories (never or hardly ever, 1–3 times a month, once a week, 2–3 times a week, 4–6 times a week, once a day, 2–3 times a day, 4–5 times a day and 6 or more times a day).

The Institutional Review Boards at the University of Southern California and the National University of Singapore had approved this study.

Between April 1994 and July 1999, we attempted to collect blood and single-void urine specimens from a random 3% sample of study enrollees. Details of the biospecimen collection, processing and storage procedures have been described previously (15). If the subject refused to donate blood, buccal cell samples were requested and collected if the subject consented. Out of 1059 female cohort participants contacted for biospecimen donation, blood (n = 514) or buccal cells (n = 164) were collected from 678 subjects, representing a participation rate of 64%. The control group for the present study comprised of this subcohort of women who were free of a history of breast cancer as of April, 30, 2003.

Six hundred and sixty-five subjects satisfied this criterion (four had positive history at enrollment and nine developed breast cancer during the follow-up).

We identified incident breast cancer cases through the population-based cancer registry in Singapore (16). As of April 30, 2003, 466 cases of incident breast cancer had developed among female cohort subjects. Histological and staging information on all breast cancer diagnoses were confirmed by manual review of the pathology reports and clinical charts. Blood (n = 233) or buccal cells (n = 64) specimens were available on 297 (63.7%) incident breast cancer cases.

Compared with breast cancer patients who donated a blood or buccal sample, those who did not were less educated (40.7 versus 30.3% had no formal education). Slightly more Cantonese gave biospecimens (54.5%) compared with Hokkiens (45.5%). The two cancer groups were otherwise similar with respect to age at cancer diagnosis (mean, 60 versus 61 years).

Genotyping methods

The method for genotyping the D/I and A-240T gene polymorphisms has been previously described (15). Briefly, the TaqMan assays were performed using a TaqMan PCR Core Reagent kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. The oligonucleotide primers used for the ACE D/I genotyping were GC008f (5′-CCCTCTCCCTACCTCTCAGCCTG-3′), GC008rev1 (5′-GCTCAGAGATTTCAGAGCTGGA-3′) for the D allele and GC008rev2 (5′-GAATCCGGGCTACTGCACTC-3′) for the I allele. In addition, the fluorogenic oligonucleotide probes used to detect each of the alleles were GC008F (5′-TGCTTATACAGTCTTTTATGTGGTTTGCGCC-3′) labeled with FAM to detect the D allele and GC008C (5′-CTTCGCTCTGTGCGCCAGGCTC-3′) labeled with CY3 (Bioresearch Technologies, Novato, CA) to detect the I allele. The oligonucleotide primers for amplification of the region of the ACE gene containing the A-240T polymorphism were GC015 (5′-GATTTGGGCAGAATTTCAGAGCTGGA-3′) and GC015rev (5′-CGGAGAGAGACCTGAGGAGC-3′). The fluorogenic oligonucleotide probes used to detect each of the alleles were GC015F (5′-ACCTTCTTCTTGAAGATGGGAC-3′) labeled with FAM to detect the A allele and GC015C (5′-GCAATGCCTCTCTGTGAAATACGCTGGA-3′) labeled with CY3 to detect the T allele. Following PCR amplification, the fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System (Applied Biosystems) and the results analyzed with Sequence Detection Software (Applied Biosystems). Experimental samples were compared with 12 controls to identify the 3 genotypes at each locus. Any samples outside the parameters defined by the controls were identified as non-informative, and were retested. All samples were processed without the knowledge of their case/control status.

The present study included 297 incident breast cancer cases and 665 control subjects who remained negative for a history of breast cancer as of April 30, 2003. Six cases and nine control subjects with noninformative ACE genotypes were excluded from the relevant data analyses.

Statistical analysis

The distributions of demographic characteristics and risk factors for breast cancer between the case and the control groups were compared and P values for the differences in the distributions were derived from χ²-test. For variables with more than two levels of ordinal values, the Mantel-Haenszel χ²-test with one degree of freedom was used to test the differences in their distributions between the two groups.

Data were analyzed by standard methods for unmatched case–control studies (17). Unconditional logistic regression models were used to examine the associations between tea intake frequency and risk of breast cancer and the possible modifying effect of ACE gene polymorphism on tea-breast cancer association. The associations were measured by odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) and P values. Age at recruitment, year of recruitment and dialect group (Cantonese, Hokkien) were adjusted for in all analyses. Identified independent risk factors for breast cancer among cohort women included level of education (none, primary, secondary school or higher), age when period became regular (<12, 13–14, 15–16, 17+ years or period never became regular) and the number of live births (none, 1–2, 3–4, 5+). (13). These latter variables were adjusted for during analyses. The number of cups of black tea consumed per month was further adjusted for when we examined the effect of green tea on breast cancer risk. Conversely, the number of cups of green tea consumed per month was adjusted for when we examined the effect of black tea on breast cancer risk.

Statistical analysis was carried out using the SAS software Version 9.1 (SAS Institute, Cary, NC). All reported P values are two-sided and P < 0.05 was considered statistically significant.

Results

The mean age of breast cancer patients at the time of diagnosis was 59.7 (standard deviation 7.8) years (range, 46–82 years). The mean time interval between baseline interview and cancer diagnosis was 3.9 years (range, 1 month–9.5 years). Table I shows the distributions of selected demographic characteristics and risk factors for breast cancer in case and control subjects. The case and control groups had similar distributions by dialect group, body mass index, cigarette smoking, alcohol consumption, use of replacement hormone and familial history of breast cancer (first degree relatives). Similar to earlier results (13), cases were more educated, menstrual periods became regular at an earlier age, had menopause at a later age, first birth at a later age and fewer number of live births than controls (Table I).

Table I shows the associations between intake frequencies of green tea and black tea separately and risk of breast cancer. Among controls, 40 and 29% drank green tea at least monthly and at least weekly, respectively. The corresponding figures among cases were 41 and 28%. Overall, green tea intake was not significantly associated with risk of breast cancer. Further adjustment for black tea intake and other potential confounders did not materially alter the null association between green tea intake and risk of breast cancer. Similarly, there was no statistically significant association between black tea intake and risk of breast cancer in the study population (Table II).

Similar to earlier results (13), women with the ‘low-activity’ ACE genotype [i.e. the (AT or AA) and (ID or II) genotypes for the A-240T and D/I gene polymorphisms, respectively] had about half the risk of breast cancer compared with their counterparts with the ‘high-activity’ ACE genotypes (i.e. the TT and DD genotypes for the A-240T and D/I gene polymorphisms, respectively). This ACE genotype–breast cancer association remained unchanged after adjustment for green tea or black tea intake and other potential confounders (Table II).

Table III shows the associations between intake frequencies of green tea and black tea separately and risk of breast cancer stratified by the ACE genotype. Among women with the high-activity ACE genotypes, multivariate-adjusted OR for breast cancer was 0.33 (95% CI = 0.13–0.82) for women drinking...
green tea at least monthly compared with non-drinkers. Among women who drank green tea at least once a week, the adjusted OR for breast cancer was further reduced to 0.29 (95% CI = 0.10-0.79). The inverse association between number of cups of green tea consumed per month and risk of breast cancer was statistically significant (P for trend = 0.039).

On the other hand, green tea intake was not associated with risk of breast cancer among women with the low-activity ACE genotype (Table III). The interaction effect between green tea intake and ACE genotype on risk of breast cancer was statistically significant (P = 0.01). Black tea intake was unrelated to breast cancer risk irrespective of the ACE genotype (Table III).

Soy intake has been found to be inversely related to breast cancer risk in Asian women (18-21) We repeated the analyses with further adjustment for intake of total soy food or total soy isoflavones. This adjustment did not materially alter the observed association between intake of green tea or black tea and breast cancer risk. Among women with the high-activity ACE genotype, the ORs for breast cancer with adjustment for soy intake and other potential confounders were 0.34 (95% CI = 0.13-0.84) for women drinking green tea at least monthly and 0.29 (95% CI = 0.11-0.81) for those drinking green tea at least weekly compared with non-drinkers.

Since preclinical disease might alter a woman’s tea drinking habit, we repeated all analyses, restricted to patients whose diagnosis of breast cancer occurred at least 2 years after enrollment. In this reduced dataset (217 cases), we again observed a statistically significant inverse association between green tea intake and breast cancer risk in women with the high-activity ACE genotype (P for trend = 0.008); the multivariate adjusted ORs for breast cancer were 0.25 (95% CI = 0.08-0.74) for women drinking green tea at least monthly and 0.17 (95% CI = 0.05-0.63) for women drinking green tea at least weekly compared with non-drinkers. There was no association between intake of green tea or black tea and breast cancer risk among all women or women with low-activity ACE genotype in this reduced dataset (data not shown).

### Discussion

The present study demonstrated a protective effect of regular green tea intake on the risk of developing breast cancer that is confined to women possessing the high-activity genotype of the ACE gene. This is the first report that shows an interaction effect between green tea intake and ACE genotypes on the risk of developing breast cancer. The findings of the present study directly support a protective role of green tea in breast carcinogenesis and suggest that green tea polyphenols may exert their chemopreventive effect through an angiotensin II-driven pathway.

The current study has several strengths. Singapore is a small city-state where there is good access to specialized medical care. The nation-wide cancer registry has been in place since 1968 and has been shown to be comprehensive in its recording of cancer cases (22). Thus, the ascertainment of incident breast cancer cases can be assumed to be complete. Tea consumption and other known environmental risk factors for breast cancer were assessed prior to cancer diagnosis and hence can be presumed to be free of recall bias. The limitation of the study is its relatively small sample size of breast cancer cases and hence we view our results to be more of a hypothesis-generating than hypothesis-testing nature. Our findings require confirmation in other large cohorts with high exposures to green tea. We intend to continue monitoring this cohort and will revisit this hypothesis in a few years when the numbers of breast cancer cases in the informative tea/genotype categories are considerably larger. Our reason for publishing these results now instead of later, when the study sample size can yield...
10 days showed increasing EGCG levels in blood from days in circulating plasma at peak values ~2 h after tea intake (24).

Green tea intake, black tea intake and ACE genotypes in relation to risk of breast cancer (The Singapore Chinese Health Study 1993–2003)

<table>
<thead>
<tr>
<th>Green tea intake</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-drinkers</td>
<td>174 (58.6)</td>
<td>397 (59.7)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Monthly or more frequent drinkers</td>
<td>123 (41.4)</td>
<td>268 (40.3)</td>
<td>1.03 (0.78–1.36)</td>
<td>1.00 (0.75–1.32)</td>
</tr>
<tr>
<td>Weekly or more frequent drinkers</td>
<td>83 (27.9)</td>
<td>194 (29.2)</td>
<td>0.95 (0.69–1.30)</td>
<td>0.91 (0.66–1.26)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Black tea intake</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-drinkers</td>
<td>200 (67.3)</td>
<td>474 (71.3)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Monthly or more frequent drinkers</td>
<td>97 (32.7)</td>
<td>191 (28.7)</td>
<td>1.21 (0.90–1.63)</td>
<td>1.18 (0.87–1.61)</td>
</tr>
<tr>
<td>Weekly or more frequent drinkers</td>
<td>70 (23.6)</td>
<td>134 (20.2)</td>
<td>1.25 (0.89–1.75)</td>
<td>1.21 (0.86–1.71)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ACE genotypes</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT and/or DD</td>
<td>53 (18.2)</td>
<td>72 (11.0)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>AA or AA and (ID or II)</td>
<td>238 (81.8)</td>
<td>584 (89.0)</td>
<td>0.56 (0.38–0.82)</td>
<td>0.54 (0.36–0.86)</td>
</tr>
</tbody>
</table>

*Adjusted for age at recruitment, year of recruitment and dialect group; OR, odds ratio; CI, confidence interval.

**Further adjusted for level of education, age when period became regular, number of live births and whatever applicable, i.e. the number of cups of green tea consumed per month or the number of black tea consumed per month.

Six cases and nine controls with non-informative ACE genotype were excluded from this analysis.

High-activity ACE genotype.

Green tea intake, black tea intake and ACE genotypes on risk of breast cancer (The Singapore Chinese Health Study 1993–2003)

<table>
<thead>
<tr>
<th>Green tea intake</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-drinkers</td>
<td>42</td>
<td>44</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Monthly or more frequent drinkers</td>
<td>11</td>
<td>28</td>
<td>0.33 (0.13–0.82)</td>
<td></td>
</tr>
<tr>
<td>Weekly or more frequent drinkers</td>
<td>8</td>
<td>24</td>
<td>0.29 (0.10–0.79)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Black tea intake</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-drinkers</td>
<td>41</td>
<td>54</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Monthly or more frequent drinkers</td>
<td>12</td>
<td>18</td>
<td>1.12 (0.43–2.91)</td>
<td></td>
</tr>
<tr>
<td>Weekly or more frequent drinkers</td>
<td>9</td>
<td>12</td>
<td>1.20 (0.40–3.59)</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age at recruitment, year of recruitment, dialect group, level of education, age when period became regular and number of live births; OR, odds ratio; CI, confidence interval.

**Further adjusted for the number of cups of black tea consumed per month.

3-sided P for interaction between the number of cups of green tea consumed per month and ACE genotypes on risk of breast cancer = 0.01.

**Further adjusted for the number of cups of green tea consumed per month.

a relatively stable risk estimate for the gene and tea interaction effect of interest, is because our novel observation is supported by a credible biological model and carries important scientific as well as public health implications.

The bioavailability of green tea catechins after ingestion of tea has been extensively studied (23). In humans, orally administered EGCG is rapidly absorbed from the gut and is detected in circulating plasma at peak values ~2 h after tea intake (24). The EGCG concentration in the plasma correlates well with the dosing level. Repeated dosing at 800 mg EGCG per day for 10 days showed increasing EGCG levels in blood from days 1–10 and the maximum concentration of EGCG on day 10 was 2.5-fold more than that of day 1. The elimination half-lives for EGCG also extended from 3.4 h on day 1 to 5.2 h on day 10 (25). In female mice, oral feeding of radio-labeled EGCG resulted in detection of radioactivity in the mammary glands after 1 h and the radioactivity level at 24 h after dosing was 8-fold more than that at 1 h after dosing. A repeated dose at 6 h following the first dosing led to a 2.5-fold increase in radioactivity level in the mammary glands (26), suggesting that frequent consumption of green tea may result in high levels of polyphenols in breast tissues.

There is experimental evidence that ROS play an etiologic role in the initiation and progression of breast cancer (27,28). The major catechin in green tea, EGCG, is an antioxidant capable of inhibiting free radical formation and lipid peroxidation (9,10). Hence, green tea may protect against breast cancer by means of the direct antioxidant property of its catechins. However, since the catechin concentration in blood after intake of green tea is lower than the normal range for other antioxidants such as vitamins C and E (29), other mechanistic pathways may be responsible for the protective effect of green tea on breast cancer.

Angiotensin II is a potent angiogenic factor and may participate in tumorigenesis by promoting angiogenesis in cancer cells (30–32). Angiotensin II can upregulate NADPH oxidase in endothelial cells in a dose-dependent manner (33,34) and the latter is an important source in the generation of ROS. ROS derived from endothelial NADPH oxidase participates in vascular endothelial growth factor (VEGF) signaling and plays a significant role in VEGF-induced angiogenesis in vitro and in vivo (35). VEGF in turn has been identified to play a major role in promoting neovascularization in human breast cancer (36). Lowering of angiotensin II levels by ACE inhibitors has been shown to suppress VEGF-induced angiogenesis and inhibit tumor growth in vivo (37,38). Green tea polyphenols have been shown to decrease the production of ROS generated via the NADPH oxidase-dependent pathway (11). Green tea...
extracts and EGCG also have been shown to reduce the level of VEGF secreted by human breast cancer cells in a dose-dependent manner (2). Therefore, it is biologically plausible that green tea reduces breast cancer risk by inhibiting the angiotensin II/NADPH oxidase-induced ROS/VEGF pathway in breast cancer.

In this study, black tea intake was not associated with a reduced risk of breast cancer. Our findings are consistent with other epidemiological studies that investigated mainly black tea consumption in Western populations and generally found no association between black tea intake and breast cancer risk (39–41). Black tea differs from green tea in the process of tea production. In the processing of green tea, fresh tea leaves are steamed or heated immediately to destroy oxidases after harvest, resulting in minimal oxidation of the naturally occurring tea catechins and gallocatechins in the tea leaves. In the processing of black tea, tea leaves are dried and crushed upon harvesting to encourage oxidation, which converts the indigenous tea catechins and gallocatechins to other polyphenols (mainly theaflavins and thearubigens). Most of the experimental data have attributed the beneficial effects of green tea to its catechins and gallocatechins, whose levels are 3–10 times higher than those in black tea (42, 43).

In summary, the present study provides the first epidemiological evidence of an interaction effect between green tea intake and ACE gene polymorphisms on risk of female breast cancer. Our findings highlight the importance of genetically determined factors in evaluating the role of green tea in the development of breast cancer.

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Conflict of Interest Statement: None declared.

References


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