genotype of the rs6259 polymorphism in the SHBG gene for postmenopausal Japanese and Japanese Brazilians and to women with at least one A allele for non-Japanese Brazilians. Our findings support the hypothesis that polymorphisms in genes related to the biosynthesis, metabolism, and bioavailability of endogenous hormones may modify the association between isoflavone intake and breast cancer risk.

The rs605059 polymorphism of the 17β-HSD1 gene results in an amino acid change from serine (A allele) to glycine (G allele) at position 312 but does not affect the catalytic or immunological properties of the enzyme (24). Previous studies have found no overall association between the rs605059 polymorphism and risk of breast cancer, which is in general agreement with our findings (24–26). Although interactions between phytoestrogen exposure and polymorphisms in the 17β-HSD1 gene in the risk of breast cancer has not been investigated, Dai et al.'s (33) population-based case-control study in Shanghai reported a significant interaction between isoflavone intake and the rs605059 polymorphism in the risk of endometrial cancer in which an inverse association was only seen among premenopausal women with at least one A allele. In this study, the risk of breast cancer decreased with increasing isoflavone intake only for women with at least one A allele of the rs605059 polymorphism in all 3 populations. Although the interactions were not statistically significant, the overall consistency of findings in the 3 populations suggests that isoflavones may reduce the risk of breast cancer via a mechanism involving the 17β-HSD1 gene.

We found that the risk of breast cancer decreased with increasing isoflavone intake only for women with at least one A allele of the rs605059 polymorphism, even though this polymorphism does not change the catalytic properties of the enzyme. Although the mechanism underlying this observation remains unclear, one possibility comes from Dai et al. (33) who hypothesized from their findings that the amino-acid alteration from serine to glycine may produce a structural change in the 17β-HSD1 binding domain, which in turn results in a substantial loss in enzyme affinity with isoflavones.

The rs6259 polymorphism of the SHBG gene results in an amino acid change substitution of asparagine (G allele) to aspartic acid (A allele) at position 327. The A allele is thought to create SHBG molecules with reduced clearance, which results in higher circulating SHBG levels (34). It has therefore been hypothesized that the A allele is associated with a decreased risk of breast cancer. Overall, however, the few studies that have been reported to date do not support this hypothesis, which is consistent with our findings (27,28). In their cross-sectional study of 1988 healthy postmenopausal women from the European Prospective Investigation of Cancer and Nutrition-Norfolk cohort, moreover, Low et al. (34) reported that plasma SHBG levels were positively associated with urinary isoflavones in women carrying at least one A allele but not in women carrying the GG genotype. This implies that the decrease in breast cancer risk associated with isoflavone exposure might be more prominent among women with at least one A allele. We found that the inverse association between isoflavone intake and breast cancer risk was limited to women with at least one A allele for non-Japanese Brazilians, which supports the hypothesis, but was limited to women with the GG genotype for postmenopausal Japanese and Japanese Brazilians. This inconsistency in findings might reflect the amount of intake on the basis that the results were in fact consistent among populations with low intake. Nevertheless, the reason for the inconsistency remains unclear, and both findings might merely be due to chance. If at least one finding is not due to chance, however, our findings suggest that isoflavones may reduce the risk of breast cancer via a mechanism that involves the SHBG gene.

We failed to replicate previous studies, which have shown an interaction between lignan exposure and the rs743572 polymorphism in the risk of premenopausal breast cancer (15,16). In this study, we found no association between isoflavone intake and breast cancer risk among premenopausal Japanese women. Because of the small number of cases for both Japanese Brazilians and non-Japanese Brazilians, we did not perform stratified analyses according to menopausal status, which might account for the inconsistency. In addition, we found no remarkable difference in the association between isoflavone intake and breast cancer risk by rs10046 polymorphism. Because we evaluated only one SNP in the CYP19 gene, further studies based on a comprehensive evaluation of the gene would clarify the gene-nutrient interaction.

Our study has several methodological advantages over previous studies. First, and unique to this study, we assessed gene-nutrient interactions using 3 populations with different isoflavone intake. For example, isoflavone intake differed considerably among the 3 populations, with median levels (interquartile rage) in the control group (mg/day) of 40.7 (25.8–61.4) among Japanese, 13.4 (7.9–31.1) among Japanese Brazilians, and 0 (0–0) among non-Japanese Brazilians. Second, we analyzed data from 3 populations, meaning that the generalizability of those results that were consistent among them is greater than would be possible for a single population.

Several limitations of the study also warrant mention. First, dietary intake of isoflavone was assessed after the diagnosis of breast cancer and is therefore sensitive to recall bias. Second, although the substantially high participation rates among both eligible cases and controls minimized potential biases related to control selection, the use of controls from medical checkup examinees and cancer-free patients, whose dietary habits may differ from those of the general population due to health consciousness or disease, might have lead to selection bias. Third, because the evaluation of gene-nutrient interactions was performed in a relatively small number of cases, power to evaluate interactions between isoflavone intake and genotype was limited. This may have also limited the interpretability of the results.

Allowing for these methodological issues, our findings support the hypothesis that polymorphisms in the 17β-HSD1 and

SHBG genes may modify the association between isoflavone intake and breast cancer risk. Further, they provide additional evidence that the mechanisms by which isoflavone may reduce the risk of breast cancer might be mediated via alteration of the biosynthesis, metabolism, and bioavailability of endogenous hormones.

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The Breast Cancer Working Group Presentation was Divided into Three Sections: The Epidemiology, Pathology and Treatment of Breast Cancer

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Epidemiology of breast cancer: The incidence and mortality of breast cancer are lower in Asia than in the West, particularly in post-menopausal women, but they are increasing. The age patterns of the incidence of breast cancer in Asia differ from in the West: in most Asian countries the peak incidence of breast cancer is at about age 45–50, whereas in western countries the incidence continues to increase even at older ages. Mortality is decreasing in western countries, whereas it is still increasing in Asian nations. There are many epidemiological factors involved in breast cancer, and important known risk factors include diet, obesity and diabetes. Asian studies found that high intake of isoflavones reduced the risk of breast cancer. Pathology of breast cancer: With regard to the pathology of breast cancer, for the molecular subtype, luminal A and luminal B are being used, while HER2 expression and rapid proliferation are also employed. Study results showed a somewhat higher prevalence of luminal A in Japanese compared with Americans. Ductal carcinoma *in situ* breast cancer is less frequent in Asian breast cancer patients than in Americans. The Working Group resolved to establish an international committee for pathological assessment of breast cancer in Asia. **Treatment of Breast Cancer:** Pharmacokinetics–pharmacodynamics studies are needed between ethnic backgrounds, investigating aromatase inhibitors and tamoxifen (endoxifen), as well as the effects of demographic factors such as diet, medical care, body mass index, etc. Correlations between adverse events and the clinical outcome also need to be studied.

Key words: breast cancer – epidemiology – hormone receptor – aromatase – HER2

EPIDEMIOLOGY

The incidence of breast cancer is increasing rapidly in most Asian countries. There is still a typical pattern for the incidence, which is age-specific and remarkably different from in western women. However, an important issue is how the incidence will change in the next 20 years. In most Asian countries, including Singapore, Japan, India, Korea, China and Thailand, but not in the Philippines, the peak incidence of breast cancer is at about age 45–50 (Fig. 1). In Western countries, the pattern shows a continuous increase in the incidence, even at older ages. There are minor differences among Asian nations, with a bell-shaped pattern in Japan, China, Korea, etc., but a flatter pattern after the peak in the Philippines and Singapore. In Japan, comparison of the incidence of breast

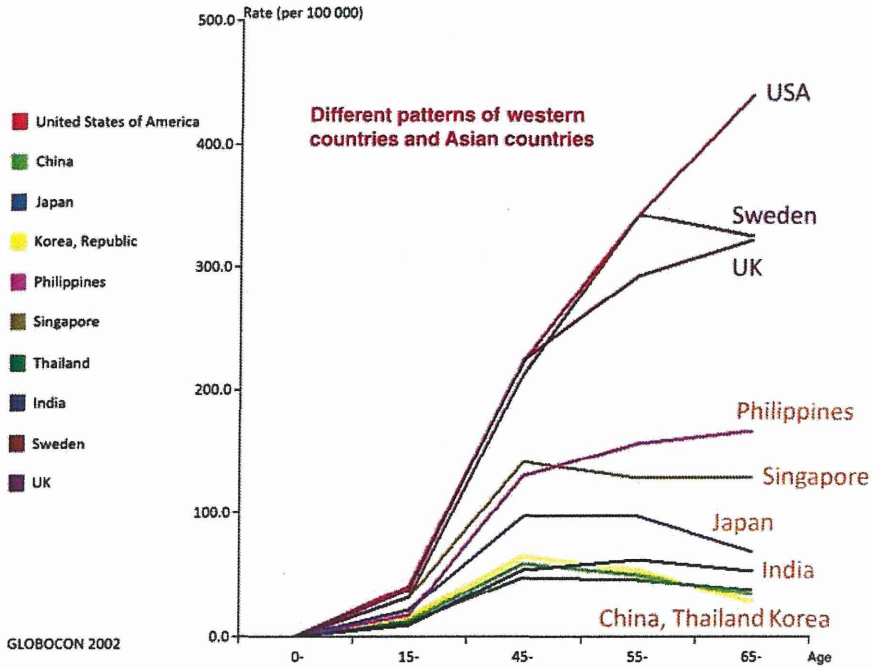

Figure 1. Age-specific incidence rate for breast cancer.

cancer over the last 25 years shows that the peak age remains the same, at ~50 years of age, but the number of cases has increased in each 10-year survey period. In Korea, as well, the same pattern as seen in Japan has prevailed, with the peak age for occurrence of breast cancer at about 50, and the number of patients has continued to increase with time between 1996 and 2006. Data for Malaysia investigated the three ethnic groups of Malay, Indian and Chinese. The results showed that the pattern was similar in the three groups, with decreasing incidence in post-menopausal women (1).

A comparison was made of the age-specific breast cancer incidence rates in Sweden and Singapore at 5-year intervals from 1968 through 1993. The incidence in Sweden continued to increase even after menopause, whereas the incidence in Singapore peaked at ~50 years of age and then plateaued. Also, although Singapore maintained the same age-related pattern over the years, the number of cases increased a bit (2). In Singapore, they also looked at the age-specific incidence of breast cancer by birth cohort, and the results suggested that breast cancer may be associated with some event at a younger age (3). This may be important with regard to the incidence of breast cancer in Asia. There is a need to focus on what factors are important in order to change the incidence of breast cancer in the future.

The mortality of breast cancer is decreasing in many western countries such as the USA and UK, whereas it is still increasing in Japan.

In conclusion, the incidence and mortality of breast cancer are lower in Asia than in the West, particularly in post-menopausal women, but they are increasing. The age patterns of the incidence of breast cancer in Asia differ from those in the West.

There are many epidemiological factors involved in breast cancer, and important known risk factors include diet, obesity and diabetes. Passive smoking may be important in the development of breast cancer, and this issue requires more investigation (4). The body mass index (BMI), as well, is a very important factor in the incidence of breast cancer, and perhaps in mortality. In premenopausal Asian and Pacific women, the relative risk of breast cancer per 5 kg/m^2 was reported to be 1.16, whereas it was 1.31 in post-menopausal women, indicating that the BMI might be a risk factor in Asian women (5).

Soybeans, which contain isoflavones, have been studied both in the West and Asia. An important point is the daily dose of isoflavones that is ingested. The Western studies revealed a high dose of ~0.8 mg/day vs. a low dose of ~0.15 mg/day, whereas the Asian studies revealed doses over the range of 5–20 mg per day, or more than 20 times higher. A clear difference was not shown in the Western studies, but the Asian studies showed that high intake of isoflavones reduced the risk of breast cancer (6). Isoflavones thus inhibit the development of breast cancer (Table 1).

With regard to diabetes and breast cancer, a controversial issue, first reported by a German group, is whether insulin administration promotes the development of breast cancer. Various other groups issued follow-up reports, regarding both insulin and antidiabetic therapy (7). Metformin, a drug that is administered to control diabetes, was reported to result in a higher pathologic complete response rate to neoadjuvant chemotherapy compared with a non-metformin group and a non-diabetic group (8). It is known from pharmacologic studies that metformin modulates the metabolism of the M2 pathway, and that may

Table 1. Soy beans: isoflavone

Description	No. of studies	Odds ratio	95% confidence interval	
Western study				
Highest (~0.8 mg or more isoflavone per day) vs. lowest (~0.15 mg or less isoflavone per day)				
All		11	1.04	(0.97–1.11)
Cohort/nested case–control		4	1.08	(0.95–1.24)
Case–control studies		7	1.02	(0.95–1.11)
Asian study				
Highest (~20 mg or more isoflavone per day) vs. lowest (~5 mg or less isoflavone per day)				
All studies	8		0.71	(0.60–0.85)
All studies in Asia	7		0.73	(0.61–0.89)
Case–control studies	7		0.75	(0.62–0.89)
Premenopausal women	6		0.65	(0.50–0.85)
Post-menopausal women	6		0.63	(0.46–085)
Moderate (~10 mg isoflavone per day) vs. lowest (~5 mg isoflavone or less per day)				
All studies	8		0.88	(0.78–0.98)

Adapted from Wu et al. (6).

result in improving the chance of a complete response to chemotherapy.

The Working Group discussions regarding the epidemiology of breast cancer concluded that (i) the changes in the incidence and mortality of breast cancer in Asia may be due mainly to lifestyle changes; (ii) a prevention (lifestyle modification and screening) strategy should be planned and implemented based on evaluation of its impact and (iii) a database including a cancer registry should be established for identification and evaluation of new risk factors.

The Working Group members also completed a questionnaire regarding various issues relating to breast cancer in each of their Asian homelands. With regard to the anticipated age-specific incidence pattern in the next 20 years, more than half of the members thought that the pattern would be between the current Asian and Western patterns, perhaps similar to that seen in Singapore. About 90% of the members thought that diet is important, with ~45% thinking it very important. There was an opinion that the diet before 20 years of age might be most important. A solid majority of ~60% also thought that passive smoking is probably important to breast cancer development. The importance of exercise was also discussed, and it was concluded that studies are needed to investigate the amount of exercise, timing and energy balance. Other factors that were debated included type II diabetes, which was thought to be important by all, and very important by 50% of the members. Diabetes will be an important issue in the future of breast cancer. Nearly 90% of the members thought that insulin treatment is probably important, but this thus remains a questionable issue. Metformin is a pharmacological issue and also remains controversial, and over 30% of the members felt a need for a clinical trial in the near future to elucidate this issue, whereas the remaining members believe there is a need for more information.

PATHOLOGY

With regard to the pathology of breast cancer in relation to the biological behavior and therapeutic regimen, the intrinsic subtype classification according to the molecular concept had been the topic that attracted attention the most. Luminal type, which indicates hormone receptor positive, is subdivided into luminal A and luminal B. HER2 (erbB-2) type is hormone receptors [both estrogen receptor (ER) and

progestrogen] negative. Triple-negative tumors are all negative for ER, progestrogen receptor (PR) and HER2, and many of them express basal-like features [i.e. CK5 and/or *epidermal growth* factor receptor (EGFR) positive]. All participants agreed to use CK5/6 and/or EGFR for this differential diagnosis. Other markers such as androgen receptor should be investigated in further depth.

The proportion of these intrinsic subtypes among breast cancer cases was assessed by some participants. Data from Malaysia showed that the proportions of ER and/or PR positive cases in Malaysians were quite similar to those among Black Americans, and lower than in White Americans. However, a report from Japan compared the prevalence of the breast cancer subtypes in Japanese, African Americans and non-African Americans (Table 2). These results as well as the subsequent discussion suggested that the proportion of various intrinsic subtypes may be different among the different races or countries. The prevalence of luminal A subtype may be higher, and the triple negative subtype may be lower in Asian women; especially the prevalence of triple-negative may be relatively low among Japanese patients with breast carcinoma, but this may require further investigations to clarify whether the same criteria of determining ER or PR positivity was employed in these cases or not as discussed below. The investigation of the Asian whole will be expected in the future.

Although it is considerable that there were some biological variations among different ethnic groups, there are still some problems according to the staining methodology and even technology. For example, even the cut-off line of ER positivity has not been the same among different countries in Asia, even though each participant noticed that the cut-off line of hormone receptor immunostainings had been changed at the St Gallen consensus conference in 2009. The 1% rule had been employed in Philippines on the basis of the guideline; in Malaysia, the line had changed from 10 to 1% recently. However, there are some controversies and each institute deals separately with this issue in Korea and in Japan (Table 2).

It is necessary to clarify clinicopathologic significance of basal-like breast carcinomas. There was no consensus of distinct basal cell markers, but all participants agreed to use CK5/6 and/or EGFR for this differential diagnosis. In Hong Kong, they are routinely stained in some centers, but in other countries they may still be considered a mere academic exercise. Unfortunately, no specified medications have been discovered for CK5/6 or EGFR positive carcinomas.

One of the new methods is to use Ki-67 (MIB-1) immunostains for ER-positive/HER2-negative (namely luminal A type) carcinomas to decide the indication for adjuvant chemotherapy. It is routinely stained for invasive breast carcinomas in Hong Kong, but it is not widely used in each case in other countries. Finally, the incidence of ductal carcinoma *in situ* (DCIS) in Asian women is still rare among Asian women.

Table 2. Pathology

	Japan	Philippines	Malaysia	Singapore	Hong Kong	Korea
ER cut-off	1% or 10%	1% by guideline	10% previously, 1% since 2009		Clinically 1%, 10% for research	Clinically 1%, 10% for research
Basal marker	Research purpose		CK5/6 and EGFR, not in daily practice		Need good evidence, Routine in some centers	Research purpose
Ki67	Depends on institute	Not in daily practice	Not in daily practice		Routinely	Routinely
Micro-metastasis	No unique Asian data available					
Other issues to be discussed	Androgen receptor, molecular markers of DCIS					

EGFR, epidermal growth factor receptor; DCIS, ductal carcinoma *in situ*.

In conclusion, three major issues have been discussed, comprising of the biology, the methodology and the quality of assessment. The Working Group, on the basis of the discussions, decided to establish an international committee for pathological assessment, with registration of the assay methodology for ER, HER2, basal-like markers, etc., performance of quality assurance, preferably externally and collaboration on epidemiological studies.

Yet another issue is mass screening mammography. In Hong Kong, this diagnostic technique was not very popular, and one Working Group member, Dr Chow, conducted a questionnaire survey of the knowledge, perceptions and attitudes of women to determine why. The major reasons cited were a lack of time and the cost. Insufficient knowledge regarding the procedure was another prime reason, with many women being unsure of the benefit. Thus, more comprehensive thinking is needed with regard to mammography, especially concerning the cost, i.e. who will pay, and how to promote this diagnostic procedure. Problems associated with breast screening in Asia include the younger incidence of breast cancer in Asia compared with the West, dense breasts in the younger generation, and a lack of evidence for mammographic screening in the younger generation or a screening program for Asian females. Moreover, a good education program is needed.

TREATMENT

The metabolic pathway of tamoxifen leads to endoxifen, which is more than 100 times more potent than the parent molecule (tamoxifen). The important enzyme in this metabolism is CYP2D6, which carries out the conversion from the relatively inactive molecule (tamoxifen) to the more active molecule (endoxifen) (9). Ethnic differences are seen in the distribution of the alleles of *CYP2D6*, and the inactive *4 allele is relatively common in Caucasians but rare in Asians. The *10 allele, with reduced activity, is more common in Asians, and rare in Caucasians (10). The inhibitor of CYP2D6, paroxetine, suppresses the endoxifen level almost to that with the *4 homozygote (9). It is necessary to take into account the genotype and external factors, and their combinations can make it possible to predict the clinical outcome of breast cancer (Fig. 2).

A questionnaire survey was conducted of the Working Group members from Korea, Hong Kong, Singapore, Malaysia, the Philippines and Japan, who were mostly breast surgeons. Case scenarios were presented regarding pharmacogenetics. The scenario, for hypothetical Country X, consisted of the use of a cytotoxic drug, A, that is inactivated by enzyme B, which can be detected in the population by *single-nucleotide polymorphisms* (SNPs). The population ratio of normal- to poor-metabolizers in Country X was 7–3. The scenario postulated that 100 mg was the recommended dose of drug A for normal metabolizers, whereas 50 mg would be ineffective. For poor metabolizers, the

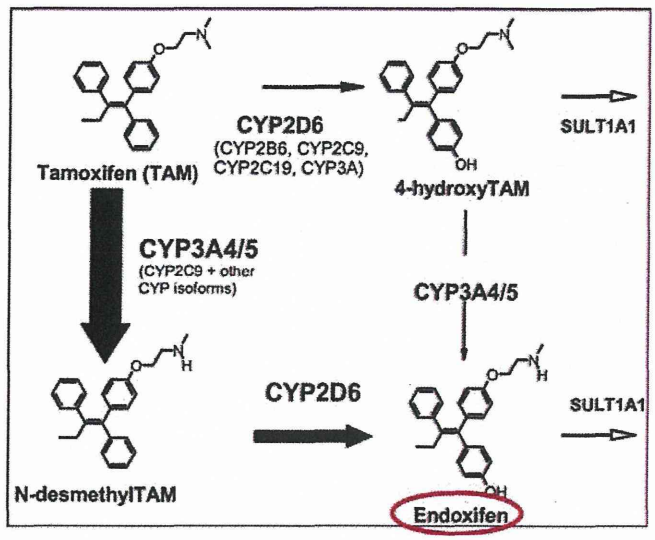

Figure 2. Tamoxifen and its active metabolites. Adapted from Jin et al. (9).

recommended dose was 50 mg, while 100 mg would be too toxic. The surveyed members were asked, 'In this situation, what would you recommend as the drug A dose?' Most of the members recommended PGx-based dosing, and otherwise recommended pharmacokinetics (PK)-based dosing followed by 100 mg in all patients. For hypothetical Country Y, the scenario postulated the reverse population ratio for normal- to poor-metabolizers, i.e. 3–7. Again, the scenario postulated that 100 mg was the recommended dose of the drug for normal metabolizers, while 50 mg was ineffective. For poor metabolizers, the recommended dose was postulated to be 50 mg, while 100 mg would be too toxic. The most common response of the surveyed members again was that the dose should be PGx based, followed by PK-based and then 50 mg with intra-patient dose escaration. As the third scenario, in hypothetical Country Z, the assumptions were similar to those in Country X with regard to the population ratio of normal- to poor-metabolizers (7:3) as well as the recommended dose (50 mg) and toxicity (100 mg) of drug A. However, for Country Z, it was postulated that a herb that is commonly prescribed actually inhibits drug metabolism by enzyme B. To the question 'What dose of drug A would you recommend?' the members' recommendation was 'No allowance for tea intake'. Also, it was felt that the package insert should probably mention about this herb–drug interaction. PK-based dosing was the second most common answer.

Southeast and East Asian countries share similar ethnic backgrounds but have different external factors, including the diet, medical practice, culture, etc. In this reality, 'What is needed to decide the recommended dose?' One-third of the surveyed Study Group members thought that the same recommended dose should be used across Asia, which would mean performing limited number of Phase I clinical trial within Asia. Another third thought that each country should conduct a separate Phase I clinical trial, and another third

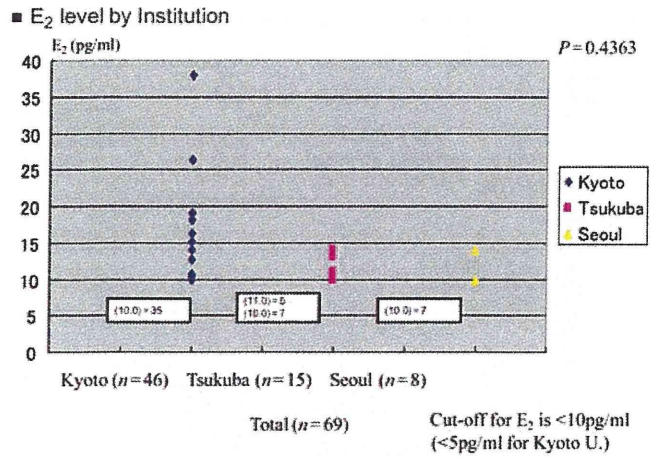

Figure 3. Inter-ethnic variation in the pharmacodynamic parameters of aromatase inhibitors.

thought the recommended dose should be decided by a PK- [or pharmacodynamics- (PD)] based approach.

Aromatase is a major source of estradiol (E_2) in post-menopausal women. Compared with without treatment, aromatase inhibitor (letrozole or anastrozole) administration suppressed the plasma E_2 level to a non-detectable level (11). In one patient, despite anastrozole being taken only every 3 or 4 days due to hot flashes, the plasma E_2 level was sufficiently suppressed, whereas in another patient who took letrozole daily the plasma E_2 level was not suppressed at all. On the basis of those observations, a study was conducted regarding ethnic variation in the pharmacodynamic parameters of aromatase inhibitors in post-menopausal patients in Kyoto, Tsukuba and Seoul. Even within the two Japanese populations, a regional difference was found for the E_2 level, which was not suppressed in many Kyoto patients, although statistically not significant due to the small sample size (Fig. 3). The reason for this is unclear. There seemed to be also differences in terms of the toxicity (hot flashes, sweating, and joint pain) relative to the E_2 level (20th Asia Pacific Cancer Conference, P-3, 2009 presented by Ishiguro H).

A proposal was made for future prospective studies. A pharmacogenomics study between ethnic backgrounds is needed, looking at differences in SNPs for CYP genes (2D6, 19) and estrogen-metabolizing enzyme genes (UGT, etc.). PK–PD studies are needed between ethnic backgrounds, investigating aromatase inhibitors and tamoxifen (endoxifen), as well as the effects of demographic factors such as diet, concomitant medications, BMI, etc. Correlations with the clinical outcome also need to be studied with regard to adverse events (hot flashes, sweating, and joint pain) and the efficacy endpoints.

Conflict of interest statement

The author, Yasuo Ohashi, received consulting fee/honorarium from AstraZeneca.

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Breast Cancer RESEARCH

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Green tea drinking and subsequent risk of breast cancer in a population to based cohort of Japanese women

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Abstract

Introduction: Although many *in vitro* and animal studies have demonstrated a protective effect of green tea against breast cancer, findings from epidemiological studies have been inconsistent, and whether high green tea intake reduces the risk of breast cancer remains unclear.

Methods: In this Japan Public Health Center-based Prospective Study, 581 cases of breast cancer were newly diagnosed in 53,793 women during 13.6 years' follow-up from the baseline survey in 1990 to 1994. After the five-year follow-up survey in 1995 to 1998, 350 cases were newly diagnosed in 43,639 women during 9.5 years' follow-up. The baseline questionnaire assessed the frequency of total green tea drinking while the five-year follow-up questionnaire assessed that of two types of green tea, *Sencha* and *Bancha/Genmaicha*, separately.

Results: Compared with women who drank less than one cup of green tea per week, the adjusted hazard ratio (HR) for women who drank five or more cups per day was 1.12 (95% confidence interval (CI) 0.81 to 1.56; *P* for trend = 0.60) in the baseline data. Similarly, compared with women who drank less than one cup of *Sencha* or *Bancha/Genmaicha* per week, adjusted HRs for women who drank 10 or more cups per day were 1.02 (95% CI 0.55 to 1.89; *P* for trend = 0.48) for *Sencha* and 0.86 (0.34 to 2.17; *P* for trend = 0.66) for *Bancha/Genmaicha*. No inverse association was found regardless of hormone receptor-defined subtype or menopausal status.

Conclusions: In this population-based prospective cohort study in Japan we found no association between green tea drinking and risk of breast cancer.

Introduction

Green tea is regularly consumed in Japan and China as a traditional habit and cultural characteristic. Although produced from the same plant, *Camellia sinensis*, differences in the manufacturing process mean that green tea has a higher catechin content than black tea [1-3], which might contribute to its beneficial effects on cancer as well as cardiovascular diseases, and other conditions [3,4]. Specifically, (-)-epigallocatechin-3-gallate (EGCG), the most abundant and biologically active catechin in green tea, might play an important role in cancer prevention [1-3,5,6]. Because breast cancer risk is substantially lower in Asian than Western countries [7], a contribution of high green tea intake to low breast cancer risk has been hypothesized. This hypothesis has been supported by *in vitro* and animal studies, which have demonstrated various protective effects of green tea and tea polyphenols acting via strong antioxidant activity, inhibition of cell proliferation and angiogenesis, induction of apoptosis, and antiestrogenic properties [1-3,5,6,8].

In contrast to *in vitro* and animal studies, few epidemiological studies have examined the association between green tea intake and risk of breast cancer, and their findings have been inconsistent [9-16]. An inverse association was found in three case-control studies among Asian-American and Chinese populations [9-11], whereas no association was observed in two cohort

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studies in Japan [12,13] or one nested case-control study in Singapore [14]. This inconsistency might be in part explained by differences in study design and exposure variation. In the three case-control studies which reported an inverse association, the reference groups were non-green tea drinkers, which included approximately 36 to 68% of control subjects [9-11], whereas in the two Japanese cohort studies the reference groups were women who drank less than one cup per day or one or fewer cups per day [12,13]. Inclusion of green tea drinkers in the reference group might attenuate the difference in risk between the reference and the higher drinking groups. Moreover, the highest consumption group in the two Japanese cohort studies were women who drank five or more cups per day, which included a relatively large portion of subjects (approximately 26 to 43%) [12,13]. Several studies have suggested a possible protective effect of very large amounts of green tea intake: one case-control study in Japan showed a decreased risk of gastric cancer among those who drank 10 or more cups per day [17], for example, while a cohort study in Japan observed a decreased risk of cancer in all sites among those who drank 10 or more cups per day [18]. However, a better understanding of the role of green tea in the etiology of breast cancer would be obtained from studies which included non- to heavy green tea drinkers. In addition, given that *in vitro* studies have suggested the inhibition of aromatase by green tea extracts [8] and of estrogen binding with its receptor by EGCG [6], the effect of green tea intake on the risk of breast cancer may differ according to hormone receptor to defined subtype. Nevertheless, no study has evaluated these associations.

To address these issues, we conducted a large-scale population-based prospective cohort study in Japan on the association between green tea intake and the risk of breast cancer.

Materials and methods

Study population

The Japan Public Health Center-based Prospective Study (JPHC Study), which began in 1990 for Cohort I and in 1993 for Cohort II, included 140,420 inhabitants (68,722 men and 71,698 women) in the municipalities supervised by 11 public health centers (PHC). Details of the study design have been described elsewhere [19]. Study participants were informed about the objectives and methods of the study in written form and those who responded to the survey questionnaire were regarded as consenting to participate in the study. In addition, participants were also notified in writing that they could withdraw from the study. Given no relevant ethical guideline and committee at the time of the survey, the institutional review board of the National Cancer Center, Tokyo, Japan considered return of the questionnaire to be classified as informed consent and approved the study protocol.

The study population comprised registered Japanese inhabitants living in each PHC area, aged 40 to 59 years in Cohort I and 40 to 69 years in Cohort II. In the present analysis, one PHC area was excluded because data on cancer incidence were not available. Thus, after exclusion of ineligible women (n = 98), we defined a population-based cohort of 67,422 women.

Baseline and five-year follow-up survey

Surveys of the cohort were conducted twice by self-administered questionnaire, the baseline in 1990 to 1994 and the five-year follow-up in 1995 to 1998. Of 67,422 women, a total of 55,886 women (83%) returned the baseline questionnaire. Of these, 1,510 who reported a history of cancer in the baseline survey were excluded, leaving 54,376 women as the baseline questionnaire respondents. Of the 67,422 women, we identified 62,788 as eligible for the five-year follow-up survey after exclusion of those who had died, moved out of the study area, or been lost to follow-up before the survey, of whom 52,485 (84%) returned the questionnaire. We also excluded 1,860 women who had a history of cancer at the five-year follow-up survey and 5,813 women who did not respond to the baseline questionnaire, leaving 44,812 women as the five-year follow-up questionnaire respondents.

Exposure measurements

Information on beverages, including green tea, oolong tea, black tea, and coffee, was obtained in the baseline questionnaire in terms of frequency and amount using six precoded categories: less than one cup per week, one to two cups per week, three to four cups per week, and almost daily (further divided into one to two cups per day, three to four cups per day, and five or more cups per day). In the five-year follow-up questionnaire, the consumption of beverages, including two items of green tea, namely *Sencha* (first or second flush of green tea; that is, the first seasonal picking) and *Bancha* (third or fourth flush of green tea; that is, the late seasonal picking)/*Genmaicha* (blend of *bancha* and roasted brown rice), oolong tea, black tea, coffee and canned coffee was assessed in terms of frequency and amount using nine precoded categories: less than one cup per week, one to two cups per week, three to four cups per week, five to six cups per week, one cup per day, two to three cups per day, four to six cups per day, seven to nine cups per day, and ten or more cups per day. *Sencha* and *Bancha* are the two main types of green tea consumed in Japan, and are usually prepared by steeping the tea leaves in hot water. The amounts of *Sencha* or *Bancha*/

Genmaicha consumed (ml per day) were computed by multiplying the frequency by the portion size for each beverage (120 ml per cup). Total green tea intake was defined as the sum of *Sencha* and *Bancha/Genmaicha* intake. The validity of green tea intake reported by the cohort was assessed using dietary records for 28 days (seven-day dietary records in four seasons) or 14 days. Spearman's correlation coefficients for green tea intake between the dietary record data and baseline questionnaire were 0.63 for Cohort I [20] and 0.43 for Cohort II (unpublished data).

Follow-up

All registered women were followed from the start of the study period to 31 December 2006. Data on residential relocation were obtained from residential registries. Among the baseline respondents (n = 54,376), 2,962 women (5.4%) moved out of the study area and 201 (0.4%) were lost to follow-up. The occurrence of cancer was identified by active patient notification from major local hospitals in the study area and data linkage with population-based cancer registries, with permission from the local governments responsible for the registries. Death certificates were used to supplement information on cancer incidence. Site of origin and histologic type were coded by members of our Study Group using the International Classification of Diseases for Oncology, Third Edition (ICD-O-3), code C500-509. Information on estrogen receptor (ER) and progesterone receptor (PR) status was collected from medical records or pathology reports. Up to the end of the study period, 586 new breast cancer cases were identified among the baseline questionnaire respondents (n = 54,376) and 362 cases among the five-year follow-up questionnaire respondents (n = 44,812). Diagnosis was microscopically verified in 95% of 586 cases, and based on death certificates only in 1.0%. Information on ER and PR status was available for 278 (47%) and 262 (45%) of 586 cases, respectively.

Statistical analysis

Since the assessment of green tea intake differed between the baseline and five-year follow-up questionnaires, these were analyzed separately. We excluded 583 women with incomplete information for green tea from 54,376 women, leaving a total of 53,793 women, including 581 breast cancer cases, for inclusion in the baseline data analyses. We also excluded 1,173 women with incomplete information for *Sencha* or *Bancha/Genmaicha* from 44,812 women, leaving a total of 43,639 women, including 350 breast cancer cases, for inclusion in the five-year follow-up data analyses.

In the baseline data analyses, person-years of follow-up were calculated from the baseline survey (1990 to 1994) until the date of diagnosis of breast cancer, date of relocation from the study area, date of death, or end of the study period (31 December 2006), whichever occurred first. Similarly, person-years of follow-up were calculated from the five-year follow-up survey (1995 to 1998) until the date of diagnosis of breast cancer, date of relocation from the study area, date of death, or end of the study period (31 December 2006), whichever occurred first for the five-year follow-up data analyses.

The Cox proportional hazards model was used to estimate hazard ratio (HR) and 95% confidence intervals (CI) of breast cancer by green tea intake using the SAS program (PROC PHREG) (SAS Institute Inc., Cary, NC, USA). The following variables were used for adjustment as potential confounders: age, study area, age at menarche, menopausal status at baseline and age at menopause, number of births, age at first birth, height, body mass index, alcohol intake, smoking status, physical activity, exogenous hormone use, family history of breast cancer, oolong tea intake, black tea intake, coffee intake and canned coffee intake. Linear trends for HRs were tested in the Cox proportional hazards models using the exposure categories as ordinal variables. All P-values reported are two-sided, and significance level was set at $P < 0.05$.

Results

During 733,667 person-years of follow-up (average follow-up, 13.6 years) for 53,793 women between 1990 to 1994 and 2006, a total of 581 cases of breast cancer were newly diagnosed and included in the baseline data analyses; while during 412,801 person-years of follow-up (average follow-up, 9.5 years) for 43,639 women between 1995 to 1998 and 2006, a total of 350 cases of breast cancer were newly diagnosed and included in the five-year follow-up data analyses.

Baseline characteristics of the study participants according to green tea intake are shown in Table 1. Approximately 12% of women drank green tea less than one cup per week while 27% drank five or more cups per day in the baseline data; and 22% and 30% of women did not drink *Sencha* and *Bancha/Genmaicha*, while 5.2% and 2.5% drank 10 or more cups per day, respectively, in the five-year follow-up data. Women who drank five or more cups per day in the baseline data tended to be older, live in towns or villages, be taller, have an earlier onset of first menstruation and fewer births, have a lower use of exogenous female hormones, drink less oolong tea and coffee, and consume more fruits and isoflavones. Similar characteristics was observed for women who drank 10 or more cups of *Sencha* per day in the five-year follow-up data, while women who drank 10 or more cups of *Bancha/Genmaicha* per day tended to be older, have later onset of first

Table 1 Baseline characteristics according to green tea intake*

	Green tea assessed by baseline questionnaire			Green tea assessed by five-year follow-up questionnaire, *Sencha*			Green tea assessed by five-year follow-up questionnaire, *Bancha/ Genmaicha*		
	Less than one cup per week	One to two cups per day	Five or more cups per day	Less than one cup per week	Two to three cups per day	Ten or more cups per day	Less than one cup per week	Two to three cups per day	Ten or more cups per day
No. of subjects	6,202	11,322	14,308	9,638	9,052	2,281	13,053	8,148	1,099
Age (year), mean	50.4	50.8	53.9	58.2	57.0	58.9	57.5	57.8	59.7
Residential area (town or village), %	40.9	57.3	68.5	58.7	66.4	73.2	66.9	64.0	60.2
Family history of breast cancer, %	0.7	1.2	1.1	0.9	1.5	0.8	1.3	1.1	0.9
Premenopausal women, %	46	47	31	21	24	15	23	22	14
Age at menopause (year), mean†	49.2	49.3	49.4	-	-	-	-	-	-
Age at menopause (50 to 54 years old), %	-	-	-	46	50	47	47	49	46
Age at menarche (year), mean†	15.0	14.6	14.7	15.0	14.6	14.7	14.7	14.7	14.9
Number of births, mean†	2.9	2.7	2.7	3.0	2.6	2.7	2.7	2.7	2.9
Age at first birth (year), mean†	24.8	25.0	24.9	24.6	25.0	25.0	25.0	24.8	24.6
Use of exogenous female hormones (current use), %	1.4	1.0	1.0	2.9	2.4	2.1	2.4	2.3	2.3
Height (cm), mean†	150.9	152.0	152.2	151.1	152.0	152.3	151.8	151.7	151.9
Body mass index (kg/m2), mean†	23.6	23.3	23.4	23.7	23.3	23.4	23.4	23.4	23.6
Smoking (current smoker), %	7.9	6.5	7.0	5.6	4.4	8.0	6.1	4.1	8.4
Alcohol drinking (regular drinker), %	11	14	11	9.7	13	13	13	11	12
Leisure-time physical activity (≥ once per week), %	15	18	18	-	-	-	-	-	-
Physical activity (metabolic equivalent to hours per day), mean†	-	-	-	32.0	31.9	32.2	31.8	32.1	32.4
Vitamin supplement user, %	20	19	20	12	16	15	15	15	13
Bancha/Genmaicha intake (≥ four cups per day), %	-	-	-	30	6.2	25	-	-	-
Sencha intake (≥ four cups per day), %	-	-	-	-	-	-	43	14	44
Oolong tea intake (≥ one cup per day), %	20	17	10	11	12	8.8	11	13	11
Black tea intake (≥ one cup per day), %	2.8	5.0	3.2	2.6	5.2	3.8	3.3	5.0	4.4
Coffee intake (≥ one cup per day), %	41	49	28	36	40	22	34	40	27
Canned coffee intake (≥ one cup per day), %	-	-	-	4.3	2.7	2.2	2.8	3.2	2.8
Total energy intake (kcal/ day), mean†‡	1,770.0	1,764.0	1,805.2	1,770.1	1,923.5	2,194.0	1,833.9	1,934.6	2,185.8
Fish and shellfish intake (g/day), mean†‡	105.5	102.8	105.4	79.6	97.5	114.3	88.2	97.0	105.3
Meats intake (g/day), mean†‡	67.2	65.2	66.1	59.7	60.4	67.7	56.6	61.6	71.6

Table 1 Baseline characteristics according to green tea intake* *(Continued)*

Vegetable intake (g/day), mean†‡	293.8	293.7	295.7	217.0	247.7	319.9	234.9	245.8	327.0
Fruit intake (g/day), mean†‡	175.9	177.3	180.8	208.5	269.9	359.3	243.1	270.1	323.7
Isoflavone intake (mg/day), mean†‡	31.6	32.2	33.5	37.5	43.3	54.7	41.5	42.0	54.8

* Three categories were chosen from six or nine precoded categories, respectively: less than one cup per week, one to two cups per week, three to four cups per week, one to two cups per day, three to four cups per day, and five or more cups per day for baseline questionnaire, and less than one cup per week, one to two cups per week, three to four cups per week, five to six cups per week, one cup per day, two to three cups per day, four to six cups per day, seven to nine cups per day, and ten or more cups per day for five to year follow-up questionnaire.

†Adjusted for age.

‡ Intake for each subject was estimated from the food frequency questionnaires based on a regression function derived from the validation study data (baseline questionnaire only).

menstruation, earlier age at first birth and a larger number of births.

We found no inverse association between green tea intake and the risk of breast cancer regardless of green tea intake as assessed by the baseline or five-year follow-up questionnaire (Table 2). Compared with women who drank less than one cup of green tea per week, the adjusted HR for women who drank five or more cups per day was 1.12 (95% CI 0.81 to 1.56; *P* for trend = 0.60) in the baseline data analyses. Similarly, compared with women who drank less than one cup of *Sencha* or *Bancha/Genmaicha* per week, adjusted HRs for women who drank 10 or more cups per day in the five-year data analyses were 1.02 (95% CI 0.55 to 1.89; *P* for trend = 0.48) for *Sencha* and 0.86 (0.34 to 2.17; *P* for trend = 0.66) for *Bancha/Genmaicha*. Moreover, compared with women who drank neither *Sencha* nor *Bancha/Genmaicha*, the adjusted HR for women who drank more than 1,320 ml per day was 1.29 (0.60 to 2.79; *P* for trend = 0.70). No substantial change was seen after further adjustment for other potential confounders such as residential area or dietary intake of meat, fish, vegetables, fruit, energy and isoflavones (data not shown). Further, no substantial change was seen in either baseline or five-year follow-up data after the exclusion of women within the first five years of follow-up to minimize the influence of existing preclinical conditions (data not shown), or after the exclusion of women who drank more than one cup of oolong tea or black tea per week to prevent the inclusion of other tea drinkers into the green tea intake reference category (data not shown).

To assess the potential influence of changes in green tea intake during the follow-up period on our findings, we first categorized subjects into three consumption groups for each survey: non-drinkers (corresponding to 'less than one cup per week'), 1 to 719 ml per day, and more than 720 ml per day (corresponding to 'more than five cup per day' in the baseline questionnaire). We next categorized subjects into three groups based on the combination of green tea intake calculated from the both the baseline and five-year follow-up questionnaires: non-drinkers for both questionnaires, more than 720 ml per day for both questionnaires, and other combinations. Compared with women who did not drink green tea for both questionnaires, adjusted HR was 1.12 (95% CI 0.45 to 2.83) for women who drank more than 720 ml per day for both questionnaires.

For analysis of cases by hormone receptor status and among women grouped by menopausal status, we re-categorized women into four groups for the baseline data and three for the five-year follow-up data. In the baseline data analyses, we found no inverse association between green tea intake and the risk of breast cancer regardless of hormone receptor-defined subtype (Table 3). Stratified analyses according to baseline menopausal status showed no remarkable difference between strata. Similar results were obtained when we analyzed the five-year follow-up data (data not shown). Additional stratified analyses according to dietary intake observed no remarkable difference between subgroups defined by dietary isoflavone and folate intake for either the baseline or five-year follow-up data (data not shown).

In additional analyses to investigate the associations of oolong tea, black tea, and coffee intake with breast cancer risk, we found no inverse associations with the baseline (Table 4) or five-year follow-up data (data not shown).

Discussion

In this population-based prospective cohort study, we found no overall association between green tea intake and the risk of breast cancer among Japanese women regardless of menopausal status. Our findings are in general agreement with those of three prospective studies, including two Japanese cohort studies, which found no association between green tea intake and breast cancer risk [12-14]. One noteworthy strength of the present over previous studies is the our remarkably wide variation in green tea intake, from women who drank green

Table 2 Hazard ratio and 95% confidence interval of breast cancer according to green tea intake

	Green tea intake assessed by baseline questionnaire						
	Less than one cup per week	**One to two cups per week**	**Three to four cups per week**	**One to two cup per day**	**Three to four cups per day**	**Five or more cups per day**	***P* for trend**
No. of cases	68	50	36	117	160	150	
Person-years	87,841	56,200	40,912	152,896	201,005	194,813	
Age- and area-adjusted HR (95% CI)	1.00	1.18 (0.82 to 1.71)	1.17 (0.78 to 1.75)	1.11 (0.82 to 1.50)	1.18 (0.88 to 1.59)	1.12 (0.83 to 1.51)	0.58
Multivariate HR (95% CI)*	1.00	1.19 (0.80 to 1.76)	1.13 (0.72 to 1.75)	1.13 (0.81 to 1.58)	1.17 (0.85 to 1.62)	1.12 (0.81 to 1.56)	0.60
	Green tea intake assessed by five-year follow-up questionnaire						
	Less than one cup per week	**One to six cups per week**	**One cup per day**	**Two to three cups per day**	**Four to six cups per day**	**Seven to nine cups per day**	**Ten or more cups per day**	P for trend
Sencha								
No. of cases	82	56	35	63	78	18	18	
Person-years	91,228	75,237	38,328	85,491	73,160	27,641	21,715	
Age- and area-adjusted HR (95% CI)	1.00	0.79 (0.56 to 1.12)	1.02 (0.68 to 1.52)	0.83 (0.59 to 1.16)	1.22 (0.88 to 1.69)	0.74 (0.44 to 1.26)	0.92 (0.55 to 1.55)	0.77
Multivariate HR (95% CI)†	1.00	0.71 (0.46 to 1.08)	0.97 (0.59 to 1.58)	0.85 (0.57 to 1.27)	1.25 (0.84 to 1.86)	0.68 (0.36 to 1.31)	1.02 (0.55 to 1.89)	0.48
Bancha/Genmaicha								
No. of cases	107	90	38	69	30	7	9	
Person-years	123,189	99,201	42,254	76,532	46,563	14,733	10,329	
Age- and area-adjusted HR (95% CI)	1.00	1.02 (0.77 to 1.36)	1.05 (0.72 to 1.53)	1.09 (0.80 to 1.49)	0.80 (0.53 to 1.21)	0.58 (0.27 to 1.26)	1.02 (0.52 to 2.02)	0.41
Multivariate HR (95% CI)†	1.00	1.12 (0.80 to 1.58)	1.23 (0.79 to 1.92)	1.23 (0.85 to 1.78)	0.83 (0.51 to 1.35)	0.70 (0.28 to 1.76)	0.86 (0.34 to 2.17)	0.66
	Total green tea intake assessed by five-year follow-up questionnaire‡						
	0	**1 to 119 ml per day**	**120 to 299 ml per day**	**300 to 599 ml per day**	**600 to 959 ml per day**	**960 to 1319 ml per day**	**1320 ml + per day**	***P* for trend**
No. of cases	13	44	48	85	105	28	27	
Person-years	18,312	50,715	58,499	91,109	114,255	44,745	35,166	
Age- and area-adjusted HR (95% CI)	1.00	1.25 (0.67 to 2.32)	1.25 (0.67 to 2.32)	1.47 (0.81 to 2.67)	1.48 (0.82 to 2.67)	1.02 (0.52 to 2.00)	1.20 (0.61 to 2.36)	0.88
Multivariate HR (95% CI)§	1.00	1.08 (0.52 to 2.23)	1.26 (0.62 to 2.56)	1.59 (0.81 to 3.14)	1.43 (0.73 to 2.83)	0.89 (0.40 to 1.97)	1.29 (0.60 to 2.79)	0.70

,†,§ Adjusted for age (continuous), area (10 public health centers), age at menarche (continuous), menopausal status at baseline (premenopausal women, age at menopause for postmenopausal women (- 47, 48 to 50, 51 to 53, 54+; †,§ - 44, 45 to 49, 50 to 54, 55+)), number of births (0, 1, 2, 3, 4, 5+), age at first birth (-21, 22 to 25, 26 to 29, 30+, nulliparous), height (continuous), BMI (continuous), alcohol intake (non-drinkers, occasional drinkers, -150 (g per week) and 150+ (g per week) among regular drinkers (ethanol)), smoking status (never, past, current), *leisure time physical activity (no, one to three days per month, more than one day per week), †,§ daily physical activity (metabolic equivalent-hours per day) (continuous), exogenous hormone use (never, past, current), family history of breast cancer (yes, no), oolong tea intake (*less than one cup per week, one to four cups per week, one or more cups per day; †,§ less than 1 cup per week, one to six cups per week, one or more cups per day), black tea intake (*less than one cup per week, one to four cups per week, one or more cups per day; †,§ less than one cup per week, one to six cups per week, one or more cups per day), coffee intake (*less than one cup per week, one to four cups per week, one to two cups per day, three or more cups per day; †,§ less than one cup per week, one to six cups per week, one or more cups per day), †,§ canned coffee intake (less than one cup per week, one to six cups per week, one or more cups per day), and †Sencha and Bancha/Genmaicha intake.

Models for Sencha or Bancha/Genmaicha intake did not include these variables, respectively.

‡ Total green tea intake was defined as the sum of Sencha and Bancha/Genmaicha intake (ml per day).

Table 3 Hazard ratio and 95% confidence interval of breast cancer according to subgroup analyses

	Green tea intake assessed by baseline questionnaire								
	Less than one cup per day	One to two cups per day		Three to four cups per day		Five or more cups per day		*P* for trend
All subjects								
No. of cases	154	117		160		150		
Age- and area-adjusted HR (95% CI)	1.00	1.01	(0.79 to 1.29)	1.08	(0.86 to 1.36)	1.02	(0.81 to 1.30)	0.75
Multivariate HR (95% CI)*	1.00	1.04	(0.80 to 1.35)	1.08	(0.84 to 1.39)	1.03	(0.80 to 1.34)	0.76
ER+PR+ breast cancer								
No. of cases	29	25		32		32		
Age- and area-adjusted HR (95% CI)	1.00	1.25	(0.73 to 2.17)	1.18	(0.69 to 2.00)	1.05	(0.62 to 1.79)	0.93
Multivariate HR (95% CI)*	1.00	1.33	(0.75 to 2.38)	1.35	(0.77 to 2.36)	1.02	(0.57 to 1.83)	0.92
ER- PR- breast cancer								
No. of cases	23	16		20		16		
Age- and area-adjusted HR (95% CI)	1.00	1.10	(0.58 to 2.11)	1.09	(0.58 to 2.05)	0.82	(0.42 to 1.60)	0.61
Multivariate HR (95% CI)*	1.00	1.13	(0.55 to 2.32)	1.23	(0.63 to 2.41)	0.81	(0.39 to 1.69)	0.69
Premenopausal women								
No. of cases	81	59		71		51		
Age- and area-adjusted HR (95% CI)	1.00	1.01	(0.72 to 1.43)	1.13	(0.81 to 1.58)	0.95	(0.66 to 1.37)	0.99
Multivariate HR (95% CI)*	1.00	1.05	(0.73 to 1.49)	1.12	(0.79 to 1.58)	0.97	(0.66 to 1.41)	0.99
Postmenopausal women								
No. of cases	70	56		86		96		
Age- and area-adjusted HR (95% CI)	1.00	1.01	(0.71 to 1.45)	1.04	(0.75 to 1.45)	1.05	(0.76 to 1.45)	0.76
Multivariate HR (95% CI)*	1.00	1.01	(0.67 to 1.50)	1.02	(0.71 to 1.48)	1.08	(0.75 to 1.55)	0.67

* Adjusted for age (continuous), area (10 public health centers), age at menarche (continuous), menopausal status at baseline (premenopausal women, age at menopause for postmenopausal women (-47, 48 to 50, 51 to 53, 54+)), number of births (0, 1, 2, 3, 4, 5+), age at first birth (-21, 22 to 25, 26 to 29, 30+, nulliparous), height (continuous), BMI (continuous), alcohol intake (non-drinkers, occasional drinkers, -150 (g/week) and 150+ (g/week) among regular drinkers (ethanol)), smoking status (never, past, current), leisure time physical activity (no, one to three days per month, more than one day per week), exogenous hormone use (never, past, current), family history of breast cancer (yes, no), oolong tea intake (less than one cup per week, one to four cups per week, one or more cups per day), black tea intake (less than one cup per week, one to four cups per week, one or more cups per day) and coffee intake (less than one cup per week, one to four cups per week, one to two cups per day, three or more cups per day).

tea less than one cup per week to those who drank 10 or more cups per day. This strength argues against the possibility that the observed absence of associations with breast cancer risk is attributable to insufficient variation in green tea intake. Our findings therefore suggest that green tea intake within a usual drinking habit is unlikely to reduce the risk of breast cancer.

The other major strength of the present study was its prospective design, in which information was collected before the subsequent diagnosis of breast cancer, thereby avoiding the exposure recall bias inherent to case-control studies. Subjects were selected from the general population, the sample was large, the response rate to the questionnaire (more than 80%) was acceptable for study settings such as this, and the loss to follow-up (0.4%) was negligible. Furthermore, the cancer registry in the study population was of sufficient quality to reduce the possibility of misclassification of the outcome.

Several limitations of this study warrant mention. First, since green tea intake was assessed by self-administered questionnaire, misclassification may have been unavoidable, albeit that our validation study showed relatively high validity [20]. Changes in green tea intake during the follow-up period may also have caused misclassification. To assess the potential influence of this misclassification, we calculated HRs for women who drank more than 720 ml per day in both the baseline and five-year follow-up questionnaires versus those who did not drink green tea in either questionnaire. Although we found no association, we cannot deny the possibility that changes in green tea intake during the follow-up period influenced the findings, particularly considering that the questionnaires used in the baseline and five-year follow-up surveys were different. These misclassifications due to inaccurate measurement would in turn have attenuated the true association, which might be one reason for our results. However, we previously found an inverse association between green tea intake and the risk of distal gastric cancer among women [21], and between green tea intake and the risk of advance prostate cancer among men in the JPHC study using the same analytic approach, namely that exposure status was not updated during follow-up [22]. These findings would also argue against the possibility that the observed absence of associations with breast cancer risk was attributable to inaccurate measurement of green tea intake.

Table 4 Hazard ratio and 95% confidence interval of breast cancer according to oolong tea, black tea and coffee intake as assessed by baseline survey

	Oolong tea intake						
	Less than one cup per week	One to four cups per week		One or more cups per day			*P* for trend
No. of cases	336	153		76			
Person-years	454,964	148,821		93,630			
Age- and area-adjusted HR (95% CI)	1.00	1.37	(1.13 to 1.67)	1.09	(0.85 to 1.41)		0.08
Multivariate HR (95% CI)*	1.00	1.34	(1.09 to 1.66)	0.98	(0.74 to 1.30)		0.40
	Black tea intake						
	Less than one cup per week	One to four cups per week		One or more cups per day			*P* for trend
No. of cases	441	111		24			
Person-years	557,038	140,312		24,776			
Age- and area-adjusted HR (95% CI)	1.00	1.00	(0.81 to 1.24)	1.29	(0.85 to 1.96)		0.45
Multivariate HR (95% CI)*	1.00	0.84	(0.67 to 1.07)	1.30	(0.84 to 2.02)		0.80
	Coffee intake						
	Less than one cup per week	One to four cups per week		One to two cups per day		Three or more cups per day	*P* for trend
No. of cases	161	180		173		63	
Person-years	233,697	213,979		210,969		69,940	
Age- and area-adjusted HR (95% CI)	1.00	1.20	(0.97 to 1.49)	1.18	(0.94 to 1.48)	1.30 (0.95 to 1.77)	0.09
Multivariate HR (95% CI)*	1.00	1.15	(0.91 to 1.46)	1.12	(0.87 to 1.43)	1.22 (0.87 to 1.71)	0.26

* Adjusted for age (continuous), area (10 public health centers), age at menarche (continuous), menopausal status at baseline (premenopausal women, age at menopause for postmenopausal women (-47, 48 to 50, 51 to 53, 54+)), number of births (0, 1, 2, 3, 4, 5+), age at first birth (-21, 22 to 25, 26 to 29, 30+, nulliparous), height (continuous), BMI (continuous), alcohol intake (non-drinkers, occasional drinkers, -150 (g/week) and 150+ (g/week) among regular drinkers (ethanol)), smoking status (never, past, current), leisure time physical activity (no, one to three days per month, more than one day per week), exogenous hormone use (never, past, current), family history of breast cancer (yes, no), green tea intake (less than one cup per day, one to two cups per day, three to four cups per day, five or more cups per day), oolong tea intake (less than one cup per week, one to four cups per week, one or more cups per day), black tea intake (less than one cup per week, one to four cups per week, one or more cups per day) and coffee intake (less than one cup per week, one to four cups per week, one to two cups per day, three or more cups per day). Models for oolong tea, black tea, or coffee intake did not include these variables, respectively.

Second, in spite of a reasonably large cohort population (53,793 women) and long follow-up period (average 13.6 year), the number of breast cancer cases was relatively low (n = 581) in the baseline data analyses, reflecting the low incidence rate in Japan (age-standardized rate per 100,000 world population in 2002, 32.7 in Japan and 101.1 in United States for comparison) [23]. Although we found no association between green tea intake and breast cancer risk (HR for five or more cups per day versus less than one cup per week = 1.12 in the baseline data analyses), the 95% CI was 0.81 to 1.56, which was a relatively wide. Our relatively small sample size therefore cannot deny the possibility of a smaller increase or decrease in risk.

Third, although we measured and adjusted for several potential confounders in the statistical model as far as possible, the effects of confounding by unmeasured variables and residual confounding cannot be totally discarded.

Fourth, information on hormone receptor status was available for 45 to 47% cases in the present study. The major reason for the unknown cases is that collection of this information began in 2002, while data for 1990 to 2002 were obtained by retrospective review of medical records or pathology reports. The relatively small number of cases weakened the statistical power to detect the association. In addition, despite the generally high agreement between the enzyme-linked immunoassay and immunohistochemical techniques, differences in classification as well as interlaboratory variation may result in misclassification, which, if present, may have also attenuated the true association. These methodological limitations might partly explain our findings, which showed no inverse association between green tea intake and the risk of breast cancer regardless of hormone receptor-defined subtype. Thus, further research to clarify the differential associations by hormone receptor-defined subtype is warranted.

Our findings contradict those of three case-control studies, which showed an inverse association [9-11]. Given that all three previous prospective studies showed no association [12-14], these findings might have been influenced by recall and selection bias stemming from the case-control design. Meanwhile, for the three

case-control studies conducted outside Japan [9-11], inconsistent findings might be in part explained by differences in the type of green tea and drinking method. In general, green tea contains catechins, minerals, and vitamins, and their contents vary among green tea types: *Sencha*, for example, one of most popular green teas in Japan, contains higher levels of tannin, vitamin C and folate than *Bancha/Genmaicha* [24]. The present study, however, found no overall association between green tea intake and the risk of breast cancer regardless of type. Moreover, levels of tea polyphenol and other nutrients in green tea varies according to preparation, amount of green tea leaves, frequency of renewing a tea batch in the pot, water temperature, brewing time, and so on. Although two Chinese case-control studies took account of several of these conditions, including green tea leaf amount, brew strength, and tea batch renewal frequency [9,10], no study has directly measured prediagnostic biomarkers of tea polyphenols. In this regard, we conducted a nested cased-control study within the JPHC study and found no overall association between plasma tea polyphenols and the risk of breast cancer [25]. Differences in tea polyphenols level due to green tea type and drinking method are therefore unlikely to explain these inconsistent findings, in any major way at least.

Alternatively, the inconsistencies reported in previous studies might be explained by possible effect modification by dietary factors and genetic polymorphisms [11,14,26]. In a case-control study in Asian-Americans, a risk-reducing effect of green tea was primarily observed among subjects whose soy intake was low [11], while a nested case-control study in Singapore showed a protective effect of green tea against breast cancer among women with high-activity genotypes of the *methylenetetrahydrofolate reductase* (*MTHFR*) and *thymidylate synthase* (*TYMS*) genes [14]. This association was stronger among those whose dietary folate intake was low. Subsequent studies, including our present study, however, have failed to replicate effect modification by soy and folate intake [9,13]. On the other hand, given a lack of information on genetic polymorphisms, the possibility cannot be excluded that green tea drinking is protective among subgroups of women.

Moreover, a recent cohort study in Shanghai showed a time-dependent interaction between green tea consumption and age of breast cancer onset: HRs for women who started green tea drinking at 25 years of age or younger versus non-green tea drinkers were 0.69 for premenopausal breast cancer and 1.61 for postmenopausal breast cancer [16]. This complex observation might also provide an explanation for the inconsistencies reported in previous studies. However, the present study was not able to examine time-dependent interactions because of a lack of information on green tea drinking habits, such as age at the beginning of green tea drinking and years of drinking.

Although oolong tea, black tea, and coffee are less popular than green tea in Japan, they are also rich in phenolic compounds and have been hypothesized to have a protective effect against the development of breast cancer: oolong tea and black tea contain higher levels of theaflavins and thearubigins than catechins, and coffee contains a high level of chlorogenic acid [1-3]. We also found no association of oolong tea, black tea, coffee and canned coffee intake with breast cancer risk. In addition to the relatively small number of women who drank oolong tea, black tea, and coffee, the potential influence of green tea intake on our findings was not fully excluded, albeit that we adjusted for green tea intake. Our findings for black tea, however, are consistent with those of a previous meta-analysis [27]. In contrast, our findings for coffee intake contradict a recent meta-analysis, which suggested a small risk-reducing effect of coffee intake on breast cancer: for example, relative risk (95% CI) per increment of two cups per day was 0.98 (0.96 to 1.00) based on 18 studies [28]. In addition to this small risk reduction, the small variation in coffee intake in our participants might have contributed in part to our findings.

Conclusions

In this population-based prospective cohort study, we found no overall association between green tea intake and the risk of breast cancer among Japanese women. Our findings suggest that drinking green tea as a beverage is unlikely to reduce the risk of breast cancer regardless of green tea type and number of cups within a usual drinking habit.

Abbreviations

CI: confidence interval; EGCG: (-)-epigallocatechin-3-gallate; ER: estrogen receptor; HR: hazard ratio; JPHC Study: Japan Public Health Center-based Prospective Study; PHC: public health centers; PR: progesterone receptor.

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Competing interests
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