test was used to test the null hypothesis that estimates were equal across hormone receptor-defined breast cancer subtypes. In addition to matching factors, the following variables were adjusted for as potential confounders: family history of breast cancer (yes, no), history of benign breast disease (yes, no), age at menarche (continuous), menopausal status and age at menopause [premenopausal women, age at menopause for postmenopausal women (<45, 46–49, 50–51, >52) for the three populations combined, (<47, 48–49, 50–51, >52) for Japanese, (<47, 48–49, 50–52, >53) for Japanese Brazilians, and (<43, 44–47, 48–50, >51) for non-Japanese Brazilians], number of births (0, 1, 2, 3, >4), age at first birth (<21, 22–24, 25–26, >27, nulliparous for the three populations combined; <23, 24–25, 26–27, >28, nulliparous for Japanese; <24, 25–26, 27–28, >29, nulliparous for Japanese Brazilians; and <18, 19–21, 22–24, >25, nulliparous for non-Japanese Brazilians), breast feeding (yes, no, nulliparous), body mass index (BMI) (continuous), alcohol drinking (no, occasional, regular drinkers), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week) and vitamin supplement use (yes, no). All reported P values are two-sided, and significance level was set at $P < 0.05$. All statistical analyses were performed with SAS software version 9.1 (SAS Institute, Inc., Cary, NC).

Results

Characteristics of case patients and control subjects have been described elsewhere [18, 19]. For Japanese, the proportion of premenopausal women, current smokers, and vitamin supplement users was higher in cases than in controls; and cases tended to have a family history of breast cancer and history of benign breast disease. Cases were less likely than controls to breast-feed and be physically active. For Japanese Brazilians, cases were less likely than controls to give birth and be physically active. For non-Japanese Brazilians, the proportion of premenopausal women and current smokers was higher in cases than in controls, whereas the proportion of physically active women and vitamin supplement users was lower (data not shown).

Allele frequencies of the SNPs among controls in each population are presented in Table 1. Genotype frequencies of each SNP were consistent with the Hardy–Weinberg equilibrium. The prevalence of the minor allele in the FcgRIIa H131R polymorphism was lower in the Japanese and Japanese Brazilian controls than in the non-Japanese Brazilian controls, while that of the minor allele in the FcgRIIIa F158V polymorphism was similar among the three populations.

ORs for breast cancer by SNP are shown in Table 2. We found no statistically significant association between either of the two SNPs and breast cancer risk regardless of population. Further, no statistically significant association was observed in analyses of the three populations combined: adjusted ORs were 0.93 (95% CI 0.66–1.32) for women with the R/R versus H/H genotype of the FcgRIIa H131R polymorphism and 1.04 (95% CI 0.69–1.57) for the V/V versus F/F genotype of the FcgRIIIa F158V polymorphism.

We next calculated adjusted ORs according to the combination of the two SNPs (Table 3). Overall, we found no significant association. However, compared to women with both the R/R genotype of the FcgRIIa H131R polymorphism and the F/F genotype of the FcgRIIIa F158V polymorphism, the adjusted OR for women with both the H/H and V/V genotype was 0.68 (95% CI 0.37–1.27). In contrast, adjusted ORs were 1.90 (95% CI 0.42–8.69) for women with both the R/R and V/V genotype and 1.63 (95% CI 0.73–3.66) for women with both the H/R and V/V genotype.

We performed further stratified analyses by menopausal status. The association between the two SNPs and risk did not substantially differ between two strata regardless of population (data not shown). Moreover, stratified analyses by parity (nulliparous and parous) to determine whether parity modified the association between the two SNPs and risk showed no remarkable difference for either of the two SNPs (data not shown).

The association between these two SNPs in the FcgR gene and the risk of hormone receptor-defined breast cancer is shown in Table 4. Information on the combined ER and PR status of the breast tumor was available for 730 cases (84%). The following subtypes were used for

Table 1 Minor allele frequencies of single nucleotide polymorphisms among control groups

	Minor allele	Japanese living in Nagano, Japan		Japanese Brazilians living in São Paulo, Brazil		Non-Japanese Brazilians living in São Paulo, Brazil	
		Minor allele frequency	P value[a]	Minor allele frequency	P value[a]	Minor allele frequency	P value[a]
FcgRIIa H131R	R	0.20	0.36	0.19	0.15	0.53	0.52
FcgRIIIa F158V	V	0.25	0.86	0.29	0.51	0.29	0.53

[a] Hardy–Weinberg equilibrium

Table 2 Odds ratios (ORs) and 95% confidence intervals (CIs) for breast cancer according to genetic polymorphism

	Three populations combined						Japanese living in Nagano, Japan				Japanese Brazilians living in São Paulo, Brazil				Non-Japanese Brazilians living in São Paulo, Brazil			
	No.		OR[a]	95% CI	OR[b]	95% CI	No.		OR[c]	95% CI	No.		OR[c]	95% CI	No.		OR[c]	95% CI
	Case	Control					Case	Control			Case	Control			Case	Control		
FcgRIIa H131R																		
H/H	403	399	1.00		1.00		269	261	1.00		57	50	1.00		77	88	1.00	
H/R	335	338	0.98	(0.78–1.22)	1.00	(0.78–1.28)	120	123	0.84	(0.58–1.22)	23	29	0.65	(0.21–2.08)	192	186	1.14	(0.75–1.73)
R/R	131	132	0.98	(0.72–1.33)	0.93	(0.66–1.32)	14	19	0.69	(0.28–1.69)	0	1	–	–	117	112	1.08	(0.69–1.69)
H/R + R/R	466	470	0.98	(0.79–1.21)	0.99	(0.78–1.25)	134	142	0.82	(0.57–1.18)	23	30	0.54	(0.18–1.69)	309	298	1.11	(0.75–1.64)
FcgRIIIa F158V																		
F/F	431	448	1.00		1.00		207	221	1.00		36	37	1.00		188	190	1.00	
F/V	351	337	1.08	(0.89–1.32)	1.16	(0.93–1.45)	162	146	1.29	(0.92–1.82)	34	33	1.61	(0.52–4.97)	155	158	1.06	(0.75–1.51)
V/V	59	56	1.09	(0.75–1.58)	1.04	(0.69–1.57)	21	23	0.86	(0.42–1.76)	5	5	0.60	(0.09–3.77)	33	28	1.21	(0.66–2.20)
F/V + V/V	410	393	1.08	(0.90–1.30)	1.14	(0.92–1.41)	183	169	1.22	(0.88–1.69)	39	38	1.23	(0.48–3.18)	188	186	1.09	(0.78–1.52)

[a] Crude OR

[b] Conditional model adjusting for family history of breast cancer (yes, no), history of benign breast disease (yes, no), age at menarche (continuous), menopausal status and age at menopause [premenopausal women, age at menopause for postmenopausal women (<45, 46–49, 50–51, >52)], number of births (0, 1, 2, 3, >4), age at first birth (<21, 22–24, 25–26, >27, nulliparous), breast feeding (yes, no, nulliparous), body mass index (continuous), alcohol drinking (no, occasional, regular drinkers), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week) and vitamin supplement use (yes, no)

[c] Conditional model adjusting for family history of breast cancer (yes, no), history of benign breast disease (yes, no), age at menarche (continuous), menopausal status and age at menopause [premenopausal women, age at menopause for postmenopausal women (<47, 48–49, 50–51, >52) for Japanese, (<47, 48–49, 50–52, >53) for Japanese Brazilians, and (<43, 44–47, 48–50, >51) for non-Japanese Brazilians], number of births (0, 1, 2, 3, >4), age at first birth (<23, 24–25, 26–27, >28, nulliparous for Japanese; <24, 25–26, 27–28, >29, nulliparous for Japanese Brazilians; and <18, 19–21, 22–24, >25, nulliparous for non-Japanese Brazilians), breast feeding (yes, no, nulliparous), body mass index (continuous), alcohol drinking (no, occasional, regular drinkers), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week) and vitamin supplement use (yes, no)

Table 3 Odds ratio (OR) and 95% confidence interval (CI) for breast cancer according to combination of FcgRIIa H131R and FcgRIIIa F158V polymorphisms among three populations combined

	FcgRIIIa F158V			*P* for interaction
	F/F	F/V	V/V	
FcgRIIa H131R				
R/R				
No. of cases/no. of controls	83/82	37/41	6/3	
OR[a]	1.00	0.77	1.90	
95% CI		(0.41–1.42)	(0.42–8.69)	
H/R				
No. of cases/no. of controls	158/176	145/141	25/12	0.15
OR[a]	0.90	1.10	1.63	
95% CI	(0.59–1.38)	(0.71–1.70)	(0.73–3.66)	
H/H				
No. of cases/no. of controls	190/190	169/155	28/41	
OR[a]	0.95	1.15	0.68	
95% CI	(0.60–1.48)	(0.73–1.82)	(0.37–1.27)	

[a] Conditional model adjusting for family history of breast cancer (yes, no), history of benign breast disease (yes, no), age at menarche (continuous), menopausal status and age at menopause [premenopausal women, age at menopause for postmenopausal women (<45, 46–49, 50–51, >52)], number of births (0, 1, 2, 3, >4), age at first birth (<21, 22–24, 25–26, >27, nulliparous), breast feeding (yes, no, nulliparous), body mass index (continuous), alcohol drinking (no, occasional, regular drinkers), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week) and vitamin supplement use (yes, no)

Table 4 Odds ratios (ORs) and 95% confidence intervals (CIs) of hormone receptor-defined breast cancer according to genetic polymorphism among three populations combined

	No. of controls	ER+/PR+			ER+/PR−			ER−/PR−			Unknown			*P* for hetero-geneity[b]
		No. of cases	OR[a]	95% CI	No. of cases	OR[a]	95% CI	No. of cases	OR[a]	95% CI	No. of cases	OR[a]	95% CI	
FcgRIIa H131R														
H/H	399	192	1.00		72	1.00		88	1.00		37	1.00		
H/R	338	146	1.10	(0.82–1.47)	41	0.70	(0.44–1.10)	71	0.92	(0.63–1.35)	65	1.12	(0.69–1.84)	0.71
R/R	132	37	0.91	(0.56–1.46)	18	0.88	(0.46–1.68)	29	0.92	(0.53–1.59)	37	1.20	(0.67–2.15)	
H/R + R/R	470	183	1.06	(0.80–1.41)	59	0.73	(0.48–1.13)	100	0.92	(0.64–1.33)	102	1.15	(0.71–1.84)	0.28
FcgRIIIa F158V														
F/F	448	191	1.00		62	1.00		84	1.00		73	1.00		
F/V	337	153	1.09	(0.83–1.43)	48	1.06	(0.69–1.62)	87	1.43	(1.01–2.02)	50	0.81	(0.54–1.24)	0.42
V/V	56	22	0.82	(0.47–1.45)	13	1.63	(0.82–3.24)	11	0.96	(0.47–1.96)	11	0.96	(0.46–2.02)	
F/V + V/V	393	175	1.05	(0.81–1.37)	61	1.15	(0.77–1.71)	98	1.36	(0.97–1.90)	61	0.84	(0.56–1.24)	0.41

[a] Unconditional model adjusting for age (continuous), study population (Japanese living in Nagano, Japan; Japanese Brazilians living in São Paulo, Brazil; non-Japanese Brazilians living in São Paulo, Brazil), family history of breast cancer (yes, no), history of benign breast disease (yes, no), age at menarche (continuous), menopausal status and age at menopause [premenopausal women, age at menopause for postmenopausal women (<45, 46–49, 50–51, >52)], number of births (0, 1, 2, 3, >4), age at first birth (<21, 22–24, 25–26, >27, nulliparous), breast feeding (yes, no, nulliparous), body mass index (continuous), alcohol drinking (no, occasional, regular drinkers), smoking status (never, past, current smokers), moderate physical activity in the past 5 years (no, less than 3 days/month, 1–4 days/week, more than 5 days/week) and vitamin supplement use (yes, no)

[b] *P* for the null hypothesis that estimates were equal across hormone receptor-defined breast cancer subtypes

modeling in an unconditional polytomous logistic regression model: positive for both receptors (ER+/PR+), ER-positive and PR-negative (ER+/PR−), negative for both receptors (ER−/PR−), and unknown. Overall, we found no remarkable difference in risk by hormone receptor-defined subtype.

Discussion

In these case–control studies, we found no statistically significant association between either of the two SNPs examined and breast cancer risk. Although we expected that women harboring the favorable H/H genotype of the FcgRIIa H131R polymorphism and V/V genotype of the FcgRIIIa F158V polymorphism would show more potent ADCC activity, as mentioned in "Introduction" [11–14], no statistically significant decrease in risk was seen, albeit that the adjusted OR was 0.68. To our knowledge, this is the first study to test the hypothesis that functional SNPs in the FcgR gene are associated with the risk of breast cancer. Our findings do not support this hypothesis and suggest that ADCC might not play a major role in the etiology of breast cancer.

We observed that the prevalence of the minor allele in the FcgRIIa H131R polymorphism was lower in the Japanese and Japanese Brazilian controls than in the non-Japanese Brazilian controls, while that of the minor allele in the FcgRIIIa F158V polymorphism was similar among the three populations, which is in general agreement with previous studies [16, 20, 21]. Although prevalence differed between the populations, no association was found for FcgRIIa H131R polymorphism regardless of population.

Several possible explanations for the observed absence of associations with breast cancer risk can be considered. First, we examined two SNPs, namely the FcgRIIa H131R and FcgRIIIa F158V polymorphisms. Although differences in the level of phagocytic or cytotoxic activities among genotypes of FcgRIIa H131R and FcgRIIIa F158V have been suggested [11–14], the absence of associations indicates that they might not be large enough to contribute to a difference in breast cancer risk among genotypes.

Second, FcgRs are expressed on leukocytes and are composed of three distinct classes: FcgRI, FcgRII, and FcgRIII. The latter two are further divided into FcgRIIa, FcgRIIb, and FcgRIIc, and FcgRIIIa and FcgRIIIb. FcgRI exhibits high affinity for IgG and can bind to monomeric IgG, whereas FcgRII and FcgRIII show weaker affinity for monomeric and hence can only interact effectively with multimeric immune complexes. FcgRIIa and FcgRIIIa activate FcgRs expressed on monocytes/macrophages and on both monocytes/macrophages and NK cells, respectively. Given that FcgRI exhibits high affinity for IgG, and that FcgRIIc on NK cells also induced ADCC [17, 22], the two SNPs in the FcgR gene we examined might not necessarily be the major determinants of inter-individual variation in ADCC or phagocytosis.

Third, although our study included a total of 869 pairs, it may not have had sufficient statistical power to detect a small increase or decrease in the risk of breast cancer. In fact, this study had approximately 80% statistical power, with a two-sided α error level of 5% and a proportion in the 'heterozygous and minor homozygous' group of 35% to detect a true OR of 1.32 or 0.74 for breast cancer among the 'heterozygous and minor homozygous' versus 'major homozygous' groups. While our findings, therefore, suggest that the two SNPs examined are not associated with an approximately 30% or greater increase or decrease in the risk of breast cancer, they cannot deny the possibility of a smaller increase or decrease in risk. In addition, analyses for the combination of FcgRIIa H131R and FcgRIIIa F158V polymorphisms showed a lower risk of breast cancer among women with the two favorable genotypes (H/H and V/V), albeit without statistical significance. This is partly because of the small proportion of women with these two favorable genotypes (5%), mandating a larger sample size.

Fourth, as a methodological issue, analyses for the three population combined might be subject to misclassification due to the difference in study methods between Japan and Brazil, albeit that the two studies were conducted under a similar protocol. For example, the control group in Japan was selected from medical checkup examinees with matching for age (within 3 years) and residential area, whereas that in Brazil was selected from cancer-free patients with matching for age (within 5 years) and ethnicity. If such difference leads to misclassification, this might also explain the observed absence of associations.

Although we found no overall association between these two SNPs in the FcgR gene and breast cancer risk, they might nevertheless be associated with breast cancer risk among specific subgroups. Analyses for the combination of the two SNPs showed a lower risk of breast cancer among women with the two favorable genotypes (H/H and V/V), which might be explained by the difference in ADCC. However, the reason for the higher risk of breast cancer among women with the R allele of the FcgRIIa H131R polymorphism and V/V genotype of the FcgRIIIa F158V polymorphism compared to those with both the R/R and F/F genotype is unclear. The adjusted ORs were not statistically significant, and these findings might merely be due to chance given the small number of subjects in these groups.

Hormonal milieu substantially differs between premenopausal and postmenopausal women, and previous studies have suggested differences in several risk factors between premenopausal and postmenopausal breast cancer [23, 24]. In addition, the age-specific breast cancer incidence rate in Japan shows a unique pattern: while rates in Western countries continue to increase after menopause, those in Japan increase before age 50 years but decrease or flatten after 50 years [25]. In this regard, although we were particularly interested in stratified analysis by menopausal

status in this study, we found no remarkable difference for either of the two SNPs examined regardless of population.

Given that the presence of antibodies against tumor-associated antigens is essential for the induction of ADCC, the association between polymorphisms in the FcgR gene and breast cancer risk might be more prominent among women with antibodies against tumor-associated antigens than in those without these antibodies. Although antibodies against most tumor-associated antigens are found in only 0–3% of healthy individuals, anti-MUC1 antibodies are found in 23.3% for IgG (weighted average of five studies) and 53% for IgM (weighted average of two studies) [7]. It is known that women develop MUC1 and anti-MUC1 antibodies during pregnancy and breast-feeding, presumably due to changes within the breast or uterus that alter MUC1 expression, glycosylation, or shedding [8]. Moreover, serum from multiparous women contained antibodies which selectively mediated ADCC against established mammary carcinoma cell lines [10]. In this regard, however, our stratified analyses showed no association between the two SNPs in the FcgR gene and risk of breast cancer regardless of parity. Further studies using information on the presence of antibodies against tumor-associated antigens will clarify the association between polymorphisms in the FcgR gene and breast cancer risk.

Previous studies have shown that risk factors such as parity and BMI differ among breast cancer subtypes defined by ER or PR status [23, 26]. We, therefore, examined whether the association of the two SNPs in the FcgR gene differed across subtypes, but found no significant difference in risk. On the other hand, given that ADCC is a potential anti-tumor mechanism behind targeted therapy with the humanized monoclonal antibody trastuzumab for HER2-positive breast cancer [15], the two SNPs in the FcgR gene might be more closely associated with the risk of HER2-positive breast cancer. Moreover, gene expression profiling in tumor tissues suggests that breast cancers may be divided into molecular subtypes consisting of two ER+ types (luminal A and B) and three ER− types [HER2-expressing, basal-like, and unclassified (normal-like)], with distinctive clinical outcomes [27, 28]. It is, therefore, of particular interest to test the hypothesis that the association of the two SNPs in the FcgR gene might differ by HER2 status or molecular subtype. However, the present study was not designed to collect tumor tissues or information on HER2 status at the start of recruitment. Further large studies are required to test this hypothesis.

In conclusion, we found no statistically significant association between two SNPs in the FcgR gene and breast cancer risk. Our findings suggest that ADCC might not play a major role in the etiology of breast cancer. Further studies are needed to clarify the role of the immune system in the etiology of breast cancer.

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Conflict of interest All authors declare that we have no conflict of interest in connection with this paper.

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Review Article

Risk factors for breast cancer: epidemiological evidence from Japanese studies

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Although our understanding of the etiology of breast cancer has improved, many well-known risk factors are not modifiable and present knowledge has proved insufficient to allow the disease to be overcome. Indeed, incidence and mortality among Japanese women have increased over the past three decades. Here, we review epidemiological evidence from our cohort and case–control studies among Japanese women in comparison with other published findings. Our studies confirm the important role of established factors derived primarily from Western populations, such as menstrual and reproductive factors, anthropometric factors, physical activity, and alcohol intake, in the development of breast cancer. In addition, we provide further evidence to better understand the role of traditional Japanese foods in the etiology of breast cancer. Our cohort study found that a higher intake of isoflavone and higher levels of plasma genistein, but not daidzein, were associated with a decreased risk of breast cancer. Our case–control studies reveal a dose–response pattern for these compounds; specifically, decreased risk as women move from "no" to "moderate" intake and leveling off thereafter. In addition, gene–environment interactions have been revealed in the effects of isoflavones. The evidence reviewed suggests that isoflavone has a protective effect against breast cancer in Asian populations. Conversely, our cohort study did not observe an inverse association between breast cancer risk and the intake of green tea and/or the plasma level of tea polyphenols, but we did find an association between increased risk and active and passive smoking. In conclusion, based on current knowledge, primary prevention according to individual lifestyle modification should focus on alcohol intake, weight control, physical activity, and tobacco smoking. (*Cancer Sci* 2011; 102: 1607–1614)

The incidence and mortality rates of breast cancer vary considerably across countries and regions, with a four to five-fold variation in incidence. Rates are highest in Europe and North America and lowest in Asia.[1] Despite Japan's status as a low-risk country, the incidence and mortality of breast cancer among Japanese women have increased over the past three decades (Fig. 1),[2–5] with age-standardized incidence rates (per 100 000 population) of 17.0 in 1975 compared with 44.4 in 2005 according to the Monitoring of Cancer Incidence in Japan (MCIJ) project.[6] Breast cancer is the most common cancer diagnosis and the fourth-leading cause of cancer death among Japanese women. For example, in 2005 the MCIJ estimated that more than 47 583 Japanese women were diagnosed with breast cancer[6] and that 10 721 died of it.[7] In contrast, mortality rates in the UK and US have been in decline since the early 1990s, possibly attributable to improvements in screening practices and treatment effectiveness.[3,8] Moreover, incidence rates in the US and several other developed countries have decreased since 2002, due, in part, to the results of the Women's Health Initiative's randomized trial in July 2002, which saw a rapid fall in the use of hormone-replacement therapy (HRT).[9]

In addition to differences in the incidence and mortality rates of breast cancer between Asian and Western countries, age-specific incidence curves also differ: in Japan, the incidence of breast cancer increases until 50 years of age and decreases or plateaus thereafter, whereas in Western countries the incidence of breast cancer continues to increase after 50 years of age (Fig. 2).[2] This pattern may be explained by differences in the distribution of risk factors for postmenopausal breast cancer, particularly the low prevalence of obesity and HRT use in Japan.[10,11] Of note, the rapid rise in rate with increasing age slows somewhat around 50 years of age, near the time of menopause, which strongly suggests a role for reproductive hormones in the etiology of this disease.

Geographical distribution and secular trends in cancer incidence and mortality, as well as studies of migrants, highlight the relative importance of environmental and lifestyle influences in cancer etiology. Studies in migrants have shown increases in breast cancer incidence and mortality following migration from a lower- to a higher-risk country.[12–14] For example, Japanese immigrants in Los Angeles County had a clearly higher rate of breast cancer than Japanese in Japan.[12] Furthermore, the incidence of breast cancer in first-generation Japanese immigrants in São Paulo from 1968 to 1978 was higher than that among Japanese living in Japan, whereas mortality increased from 1979 to 2001 to a rate intermediate between that of Japanese living in Japan and Brazilians living in the state of São Paulo.[13,14] These findings strongly suggest that breast cancer risk is influenced by factors associated with the lifestyle or environment of the destination country.

Current knowledge of preventive or risk factors

Accumulating evidence obtained mainly from Western countries has established a relatively large number of preventative or risk factors for breast cancer (Table 1).[15–17] Many established risk factors are linked to ovarian hormones, and estrogens in particular, and prospective studies in postmenopausal women have shown a direct association between higher levels of estrogens and their androgen precursors and an increased risk of breast cancer.[18] One possible biological mechanism of the effect of ovarian hormones on risk is that both endogenous and exogenous hormones increase cellular proliferation in the breast, thereby increasing the likelihood of random genetic errors during cell division.[19]

Although our understanding of the etiology of breast cancer has improved, many well-known risk factors, such as menstrual

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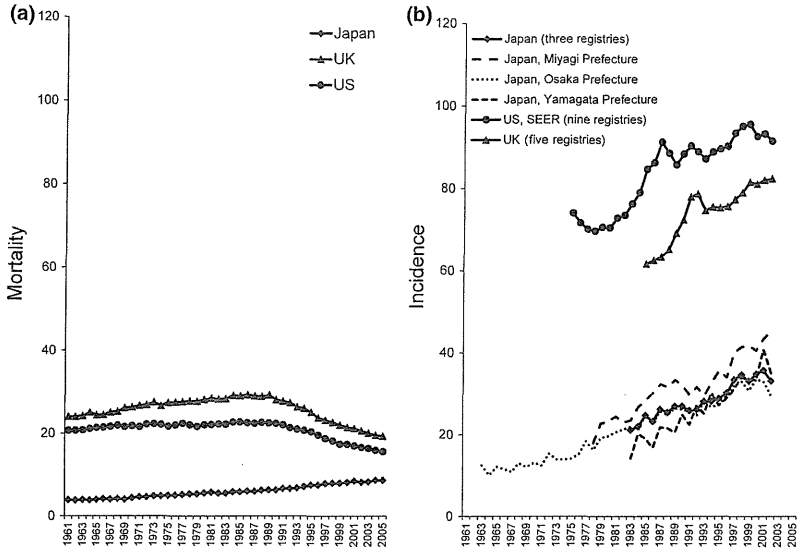

Fig. 1. Annual (a) mortality and (b) incidence rates of breast cancer in Japan, the US, and UK (standardized rate per 100 000 by age to world population). Data for the incidence rate are from Ferlay *et al.*:[2] Japan (three registries: Miyagi, Yamagata, and Osaka) from 1963 to 2002; US (Surveillance Epidemiology and End Results [SEER]; nine registries: Atlanta, Connecticut, Detroit, Hawaii, Iowa, New Mexico, San Francisco-Oakland, Seattle-Puget Sound, and Utah) from 1975 to 2002; and UK (five registries in England: Birmingham and West Midlands Region, Merseyside and Cheshire, North Western, Oxford, and Yorkshire) from 1985 to 2002. Mortality data are from Ferlay:[3] Japan, US, and UK from 1961 to 2005.

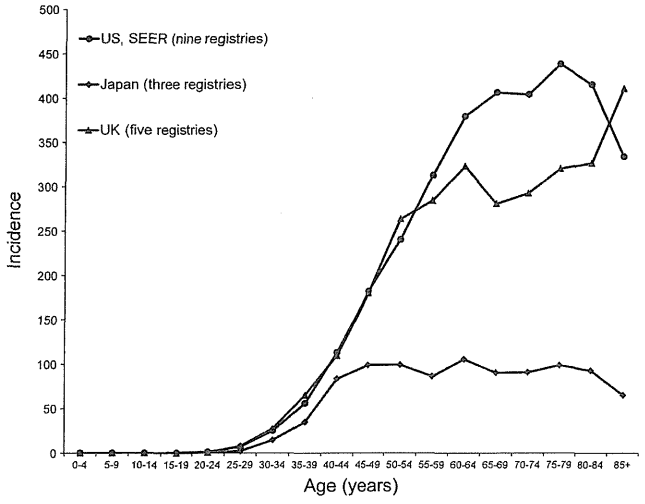

Fig. 2. Age-specific breast cancer incidence rate (per 100 000) in 2002 in Japan (three registries: Miyagi, Yamagata, and Osaka), the US (Surveillance Epidemiology and End Results [SEER]; nine registries: Atlanta, Connecticut, Detroit, Hawaii, Iowa, New Mexico, San Francisco-Oakland, Seattle-Puget Sound, and Utah), and UK (five registries in England: Birmingham and West Midlands Region, Merseyside and Cheshire, North Western, Oxford, and Yorkshire). Data are from Ferlay *et al.*[2]

and reproductive factors, are not modifiable for the purpose of reducing risk. In addition, only a few dietary factors have been causally related to the etiology of breast cancer, even thought diet is an environmental factor that may contribute to the population distribution of breast cancer risk (Table 1). Not surprisingly, present knowledge has proved insufficient to allow the disease to be overcome and the identification of other important etiological factors is thus required.

Rational for epidemiological studies among Japanese

Given that the population distribution of breast cancer risk is determined by variations in exposure, the substantial difference in lifestyle and environment between Japan and Western countries leads to the following general hypothesis: if a factor is characterized by high exposure in Japan (a low-risk country), but low exposure in those Western countries that are considered high-risk countries, it may be associated with a decreased risk of breast cancer. Good examples are traditional foods in Japan, such as soy foods and green tea. Similarly, a factor with low exposure in Japan but high exposure in Western countries may be associated with increased risk. We have used this hypothesis to conduct population-based cohort and hospital-based case–control studies among Japanese women with the goal of identifying risk factors and to further our understanding of the etiology of breast cancer, as detailed below.[20,21]

Briefly, the Japan Public Health Center-based Prospective (JPHC) study, which began in 1990 for Cohort I and in 1993 for Cohort II, enrolled 140 420 subjects (68 722 men and 71 698 women) living in municipalities supervised by 11 public health centers.[20] The study population consisted of registered Japanese inhabitants aged 40–59 years in Cohort I and 40–69 years in Cohort II. Approximately 55 000 women returned a self-administered questionnaire (response rate ~83%) and approximately 25 000 women provided a blood sample (response rate ~45%) in the baseline survey from 1990 to 1995. We conducted 5- and 10-year follow-up surveys to collect information regarding dietary habits, changes in lifestyle, and disease occurrence, as well as information regarding residential status, mortality, and incidence of cancer and cardiovascular diseases.

Regarding the multicenter, hospital-based case–control studies, these were conducted from 2001 to 2005 at four hospitals in Nagano Prefecture, Japan, and from 2001 to 2006 at eight hospitals in São Paulo, Brazil.[21] Cases were recruited from a consecutive series of female patients aged 20–74 years who were newly diagnosed with histologically confirmed invasive breast cancer. In the Nagano study, healthy controls were selected from

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Table 1. Established risk factors for breast cancer and corresponding results from the Japan Public Health Center-based Prospective (JPHC) study

Factor	High-risk group	Results from the JPHC study	
		Category	HR (95% CI)
Endogenous and exogenous hormones			
Endogenous estrogen levels	Higher levels	NA	
Oral contraceptive use	Users	NA	
Hormone replacement therapy	Users	NA	
Menstrual and reproductive factors			
Age at menarche	Earlier age	≥16 years *vs* <14 years†	0.73 (0.53–1.00)
Age at menopause	Later age	≥54 years *vs* <48 years‡	1.98 (1.12–3.52)
Parity	Nulliparity	Nulliparous *vs* parous†	1.92 (1.38–2.65)
Age at first birth	Later age	≥30 years *vs* <22 years†	1.63 (1.05–2.52)
History of breast feeding	No history	Have history *vs* no history†	0.86 (0.65–1.15)
Anthropometric factors			
Height	Taller women	≥160 cm *vs* 148 cm‡	2.39 (1.43–3.98)
Body fatness (postmenopausal)	Heavier women	BMI ≥30 kg/m^2 *vs* BMI <19 kg/m^2‡	2.28 (0.94–5.53)
Body fatness (premenopausal)	Leaner women	NA	
Diet and physical activity			
Alcohol intake	Drinkers	Regular drinkers (>150 g ethanol/week) *vs* never drinkers†	1.75 (1.16–2.65)
Physical activity	Inactive women	≥3 days/week *vs* <3 days/month†§	0.73 (0.54–1.00)
Other factors			
History of benign breast disease	Have history	NA	
Mammographically dense breasts	More dense	NA	
Family history in first-degree relatives	Have history	NA	
Ionizing radiation	Exposure	NA	

†All women (both premenopausal and postmenopausal women). ‡Postmenopausal women. §Participation in sports and physical activity in leisure time. Data are from Iwasaki *et al.*[23,26] and Suzuki *et al.*[34,37] BMI, body mass index; CI, confidence interval; HR, hazard ratio; NA, not available.

medical checkup examinees who were confirmed to be cancer free, with one control matched for each case according to age and residential area. In the São Paulo study, controls were preferentially selected from cancer-free patients who visited the same hospital as the index cases with one control matched for age and ethnicity. Eventually, a total of 877 matched pairs participated (405 Japanese in Nagano, along with 83 Japanese Brazilians and 389 non-Japanese Brazilians in São Paulo).

Here, we review our findings in the JPHC study and case–control studies in Nagano and São Paulo in comparison with those from other Japanese and Western studies.

Epidemiological evidence from Japanese studies: established risk factors

Menstrual and reproductive factors. Menstrual and reproductive factors play an important role in the development of breast cancer. A meta-analysis of eight case–control studies in Japan showed that early age at menarche, nulliparity and low parity, and late age at first birth were associated with increased risk.[22] Similar to previous studies from both Western and Asian countries,[15–17] the JPHC study confirmed that early age at menarche, late age at menopause, nulliparity and low parity, and late age at first birth were associated with an increased risk of breast cancer (Table 1).[23] Although a 2007 report of the World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR) concluded that lactation protects against breast cancer,[24] the JPHC study failed to replicate this association.[23] Furthermore, although a recent pooled analysis of 35 568 invasive breast cancer cases showed that nulliparity and late age at first birth were more closely associated with hormone receptor-positive than -negative tumors,[25] the JPHC study observed no significant difference in association by hormone receptor-defined breast cancer.[23]

Anthropometric factors. The 2007 WCRF/AICR report identified adult height as a convincing risk factor for postmenopausal breast cancer and a probable factor for premenopausal breast cancer.[24] The causal factor is unlikely to be tallness itself, but factors that promote linear growth in childhood, including energy intake and exposure levels to growth hormone and insulin-like growth factor.[24] Consistent with the WCRF/AICR report, the JPHC study observed an increased risk associated with greater height, primarily among postmenopausal women (Table 1).[26]

The 2007 WCRF/AICR report documented that the association between body fatness and breast cancer risk depends on menopausal status: although greater body fatness probably protects against premenopausal breast cancer, convincing evidence suggests that it is a cause of postmenopausal breast cancer.[24] In addition, adult weight gain is a probable cause of

postmenopausal breast cancer. The mechanism of this association likely relates to levels of circulating estrogen: specifically, a decrease in levels due to an increased frequency of anovulatory cycles in premenopausal women and an increase in levels due to both an increase in estrogen production by aromatase in adipose tissue and a decrease in circulating level of sex hormone-binding globulin (SHBG) in postmenopausal women.[27]

In the JPHC study, we found a positive association between body mass index (BMI) and breast cancer risk, with the association being stronger in post- than premenopausal women (Table 1).[26] We also found an association between an increase in BMI from age 20 years to recent age with increased risk among postmenopausal women.[28] These findings generally agree with those of studies in Japan and other Asian countries.[29,30] A recent meta-analysis of cohort studies showed that risk was increased by 16% and 31% per 5 kg/m^2 increment of BMI in pre- and postmenopausal Asian women, respectively, but decreased by 9% in premenopausal and increased by 15% in postmenopausal North American women.[30] The lack of an inverse association among premenopausal women may be due to the lower prevalence of overweight women in Asian countries, with few who are sufficiently overweight to likely develop anovulation. Conversely, risk reduction due to greater body fatness in early adulthood appears to continue into the postmenopausal years, which may explain the stronger association among postmenopausal Asian than North American women. In addition, a recent meta-analysis showed a 10% decrease in risk per 5 kg/m^2 increment of BMI among premenopausal women and a 33% increase among postmenopausal women for estrogen and progesterone receptor-positive (ER^+PR^+) tumors, although no association was seen for estrogen receptor-positive and progesterone receptor-negative (ER^+PR^-) or estrogen and progesterone receptor-negative (ER^-PR^-) tumors.[31] In the JPHC study, BMI was more strongly associated with estrogen receptor-positive (ER^+) than -negative (ER^-) tumors in postmenopausal women. These findings may support the involvement of an ER-mediated estrogen-dependent mechanism.

Physical activity. The 2007 WCRF/AICR report concluded that the evidence that any type of physical activity, including occupational, household, transport, and recreational activity, protects against breast cancer is limited-suggestive for premenopausal and probable for postmenopausal breast cancer.[24] A meta-analysis showed a 6% decrease in risk for each additional hour of physical activity per week.[32] The proposed mechanisms behind this association include the beneficial effect of physical activity on body fatness, effects on endogenous sex hormone levels, and possible improvement of immune function.[33]

In the JPHC study, we observed an inverse association between leisure time physical activity and breast cancer risk (Table 1).[34] Compared with women who participated in sports and physical activity on <3 days/month, adjusted hazard ratio (HR) and 95% confidence intervals (CI) for women who participated in sports on >3 days/week was 0.73 (0.54–1.00; P_{trend} = 0.037) for overall breast cancer and 0.43 (0.19–1.00; P_{trend} = 0.022) for ER^+PR^+ tumors. Conversely, we did not observe an inverse association between daily total physical activity and risk of overall breast cancer, but did see an inverse association for ER^+PR^+ tumors. In addition, we also investigated associations between age- and intensity-specific leisure time physical activity and the risk of hormone receptor-defined breast cancer in the case–control study in Nagano.[35] Strenuous, but not moderate, physical activity at age 12 years was inversely associated with breast cancer risk regardless of menopausal status and hormone receptor-defined breast cancer. Among postmenopausal women, moderate physical activity in the previous 5 years was somewhat more closely associated with ER^+PR^+ than ER^+PR^- and ER^-PR^- tumors. Our findings generally agree with those of the WCRF/AICR report and other Japanese studies.[24,36] Moreover, our findings regarding hormone receptor-defined breast cancer may support the involvement of an ER-mediated estrogen mechanism.

Alcohol intake. We found a significant positive association between alcohol intake and the risk of breast cancer in the JPHC study (Table 1).[37] An increase in consumption of 10 g ethanol/day (continuous) was associated with a 6% (95% CI 1–13; P_{trend} = 0.047) increase in the risk of breast cancer. Our findings generally agree with those from the WCRF/AICR report.[24] A meta-analysis of cohort studies reported a 10% increase in risk per 10 g increment of ethanol/day.[24] However, Nagata *et al.* concluded that epidemiological evidence from Japanese populations remains insufficient, given that a systematic review revealed that only three of three cohort and eight case–control studies observed a positive association.[38]

Several biological mechanisms for this association have been proposed, including an increase in circulating hormone levels, a direct carcinogenic effect of alcohol metabolites (e.g. acetaldehyde, a known mutagen), and an antagonistic effect on folate absorption and metabolism.[39] In the JPHC study, we found positive associations for both ER^+PR^+ and ER^+PR^- tumors, but not for ER^-PR^- tumors, although the associations failed to reach statistical significance. A recent meta-analysis showed that the relative risk (RR) and 95% CI per 10 g increment of ethanol/day was 1.12 (1.08–1.15) for all ER^+ tumors, 1.07 (1.00–1.14) for all ER^- tumors, and 1.11 (1.07–1.14) for ER^+PR^+, 1.15 (1.02–1.30) for ER^+PR^-, and 1.04 (0.98–1.09) for ER^-PR^- tumors.[40] These findings suggest that the biological mechanism involves both an ER-mediated estrogen-dependent and hormone-independent mechanism.

Notable epidemiological evidence from Japanese studies

Body weight at age 20 years. A number of epidemiological studies have shown that greater body fatness during childhood and adolescence is associated with a decreased risk of breast cancer.[41–43] The proposed biological mechanism behind this risk reduction is that obese women tend to have an increased frequency of menstrual irregularities and anovulatory cycles, which reduces their lifetime number of ovulations and alters their circulating hormone levels.[27] To date, most studies have been conducted in Western countries, where the prevalence of obesity is high, and little is known about whether greater body fatness during childhood and adolescence is associated with a decreased risk of breast cancer among the lean population.

In the JPHC study, we found a significant inverse association between BMI at age 20 years and the risk of breast cancer. This inverse association was not modified by menopausal status or recent BMI level. Adjusted HR for each 5 unit increment was 0.75 (95% CI 0.61–92).[28] Similarly, the Miyagi Cohort Study also observed a decreased risk associated with higher BMI at age 20 years.[44] These findings from a lean population generally agree with those from Western countries. Interestingly, few women are likely to be sufficiently overweight to cause anovulation in Japan. Moreover, the Nurses' Health Study II reported that the observed inverse association of BMI in early adulthood with risk was not eliminated after adjustment for ovulatory disorders.[41] Therefore, our findings from Japan imply the presence of other biological mechanisms apart from anovulation.

Soy foods and isoflavone. Soy foods, which are rich in isoflavones, are habitually consumed by Asian populations in large amounts. Isoflavones, of which genistein and daidzein are the major examples, are classified as phytoestrogens, which are plant-derived non-steroidal compounds with estrogen-like biological properties. A high intake of isoflavones has been hypothesized to contribute to the lower incidence of breast cancer in Asian compared with Western countries.[45]

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In the JPHC study, we observed an approximate 50% decrease in breast cancer risk associated with higher isoflavone intake, as assessed by a food frequency questionnaire.[46] Moreover, a nested case–control study within the JPHC study revealed a decrease in risk associated with a higher level of plasma genistein, but not plasma daidzein (Fig. 3).[47] Although accumulating evidence suggests that risk is reduced with higher isoflavone intake,[48,49] there is little available evidence for a dose–response relationship. In the case–control studies in Nagano and São Paulo, we evaluated the dose–response relationship using the three populations combined, because the respective amount of and variation in isoflavone intake is high and large for Japanese, intermediate and relatively large for Japanese Brazilians, and low and small for non-Japanese Brazilians.[21] We found that breast cancer risk decreased linearly from "no" to "moderate" isoflavone intake (20–30 mg/day) and thereafter leveled off (Fig. 4), suggesting that isoflavones have a risk-reducing rather than risk-enhancing effect on breast cancer within the range achievable from dietary intake alone.

Several biological mechanisms have been proposed to explain how isoflavones may reduce the risk of breast cancer. Isoflavones and human estrogen share similar chemical structures; given the consequent binding affinity of isoflavones to estrogen receptors, they may act as estrogen agonists and antagonists that compete for estradiol at the receptor complex.[50,51] Isoflavones may also influence risk by altering the biosynthesis, metabolism, and bioavailability of endogenous hormones.[52,53] In this regard, isoflavones have been shown to inhibit aromatase[52] and 17β-hydroxysteroid dehydrogenase Type I (17β-HSD1),[52] as well as to increase the synthesis of SHBG.[53] Considering these mechanisms, we tested the hypothesis that polymorphisms in estrogen receptor genes and genes related to the biosynthesis, metabolism, and bioavailability of endogenous hormones may modify the association between isoflavone intake and breast cancer risk in the case–control studies in Nagano and São Paulo.[54,55] The results showed several suggestive interactions between isoflavone intake and polymorphisms of *estrogen receptor beta* (*ESR2*), *17β-HSD1*, and *SHBG*: an inverse association between intake and risk in women with the GG genotype of the rs4986938 polymorphism in *ESR2* among postmenopausal Japanese, Japanese Brazilians, and non-Japanese Brazilians (Fig. 5);[54] an inverse association in women with at least one A allele of the rs605059 polymorphism in *17β-HSD1* among the three populations;[55] and an inverse association in women with the GG allele of the rs6259 polymorphism in *SHBG* among Japanese populations and women with at least one A allele among non-Japanese Brazilians.[55] Our findings support the idea that isoflavones may reduce the risk of breast cancer via mechanisms that involve estrogen receptors or the biosynthesis, metabolism, and bioavailability of endogenous hormones.

A recent meta-analysis observed risk reduction with higher isoflavone intake among Asian, but not Western, populations.[49] Overall, our studies suggest that isoflavone intake has a protective effect against breast cancer. Because we found a decreased risk not only in Japanese, but also Japanese Brazilians and non-Japanese Brazilians, our findings are somewhat inconsistent with those of the meta-analysis. This heterogeneity of findings across populations and studies warrants careful consideration. In this regard, Nagata noted that the association between soy isoflavone intake and the risk of breast cancer may be variously modified by the amount of soy isoflavones consumed, the form and food source of the isoflavones, the timing of isoflavone exposure, the estrogen receptor status of tumors, the equol-producer status, and the hormonal profile of individuals.[56]

Green tea. Although rarely consumed in Europe and North America, where black tea is the common tea beverage, green tea is one of the most popular beverages in Japan and China. Green tea has a higher catechin content than black tea, which may contribute to its protective effects against cancer via the strong antioxidant activity of catechin, its inhibition of cell proliferation and angiogenesis, induction of apoptosis, and antiestrogenic properties.[57,58]

In the JPHC study, we found no significant inverse association between green intake and the risk of breast cancer.[59] Compared with women who drank less than one cup of *Sencha* or *Bancha/Genmaicha* per week, the adjusted HR for those who

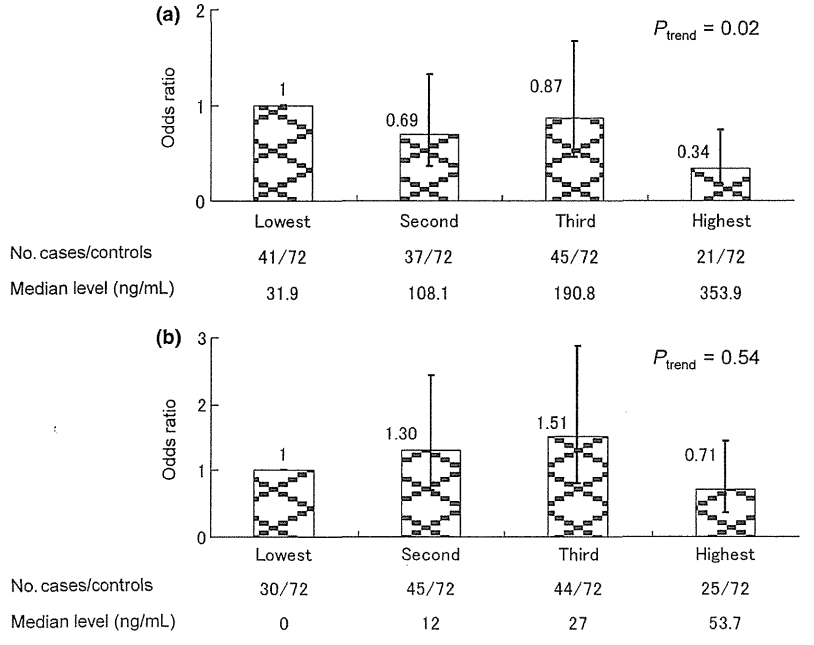

Fig. 3. Plasma isoflavones and the risk of breast cancer: the Japan Public Health Center-Based Prospective (JPHC) study. (a) Genistein; (b) daidzein. Odds ratios were adjusted for the numbers of births and the age at first birth. Data are from Iwasaki *et al.*[47]

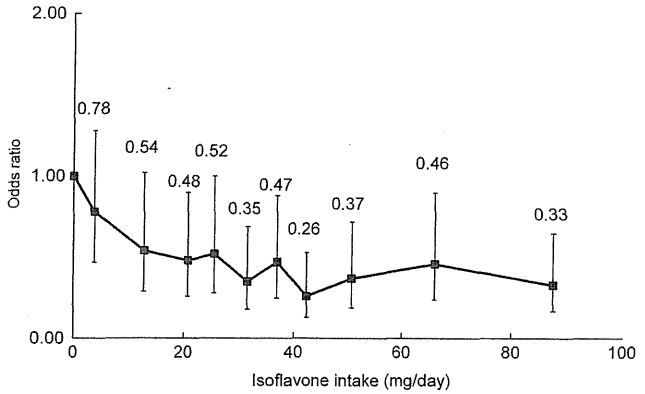

Fig. 4. Isoflavone intake and the risk of breast cancer in hospital-based case–control studies among Japanese, Japanese Brazilians, and non-Japanese Brazilians. Subjects were categorized into 11 groups: non-consumers and deciles of isoflavone consumers based on the control distribution. Odds ratios were estimated using matching pairs with adjustment for menopausal status, number of births, family history of breast cancer, smoking status, moderate physical activity in the past 5 years, and vitamin supplement use. Data are from Iwasaki *et al.*[21]

drank 10 or more cups per day was 1.02 (95% CI 0.55–1.89; P_{trend} = 0.48) for *Sencha* and 0.86 (0.34–2.17; P_{trend} = 0.66) for *Bancha/Genmaicha*. One noteworthy strength of this study over previous studies is its remarkably wide variation in green tea intake, from women who drank less than one cup per week to those who drank 10 or more cups per day.

Tea polyphenol content in green tea varies according to preparation, the type and amount of green tea leaves, the frequency of renewing the tea batch in the pot, water temperature, and brewing time, among others. To reduce misclassification due to these factors, we conducted a nested case–control study within the JPHC study and measured plasma levels of (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (−)-epigallocatechin-3-gallate (EGCG), and (−)-epicatechin-3-gallate (ECG).[60] We found no significant association between plasma tea polyphenol levels and breast cancer risk. Adjusted odds ratios (OR) for the highest versus lowest group were 0.90 (95% CI 0.42–1.96; P_{trend} = 0.98) for EGC, 0.95 (95% CI 0.43–2.08; P_{trend} = 0.86) for EC, 1.21 (95% CI 0.52–2.80; P_{trend} = 0.53) for EGCG, and 1.75 (95% CI 0.81–3.78; P_{trend} = 0.15) for ECG.

To our knowledge, four cohort and three case–control studies have been published on the association between green tea intake and breast cancer, but findings have been inconsistent.[61–67] Our findings generally agree with those of three of the cohort studies, including two Japanese cohorts, which found no association between green tea intake and risk,[64–66] but contradict those of the three case–control studies, which showed an inverse association between green tea intake and risk.[61–63] Possible explanations for these apparent discrepancies in results include the influence of recall and selection bias stemming from the case–control design; differences in the type of tea and drinking methods; and possible effect modification by dietary and genetic factors.[59,63,66] Moreover, among studies investigating the association between circulating tea polyphenol levels and breast cancer risk using prediagnostic biological specimens, the Shanghai Women's Health Study found no dose–response relationship between urinary levels of tea polyphenols and their metabolites and the risk of breast cancer,[68] which is similar to the results of our JPHC study.

Smoking and passive smoking. The JPHC study found that both active and passive smoking were associated with an increased risk of breast cancer among premenopausal women.[69] When the reference group was defined as never-active smokers without passive smoking, adjusted HR (95% CI) for ever-smokers were 3.9 (1.5–9.9) and 1.1 (0.5–2.5) in pre- and postmenopausal women, respectively. In never-active smokers, the adjusted HR (95% CI) for passive smoking was 2.6 (1.3–5.2) in premenopausal women and 0.6 (0.4–1.0) in postmenopausal women. Subsequently, Nagata *et al.*[70] concluded that tobacco smoking possibly increases the risk of breast cancer in the Japanese population, considering that a systematic review of evidence showed a positive association in five of three cohort and eight case–control studies in Japan.

In 2004, the International Agency for Research on Cancer (IARC) endorsed the "lack of carcinogenicity of tobacco smoking in humans for cancers of the female breast".[71] However, large cohort studies published since 2002 have observed an increased risk associated with a long duration and/or high number of pack-years of smoking.[72] Moreover, a meta-analysis found a significant interaction between smoking, *N*-acetyltransferase 2 (*NAT2*) genotype, and risk of breast cancer: higher pack-years were associated with an increased risk among women with the *NAT2* slow genotype, but not among rapid acetylators.[73] Recent reappraisals have therefore suggested an increased risk of breast cancer and the IARC concluded that there is limited evidence that tobacco smoking causes breast cancer.[74] With regard to passive smoking, a meta-analysis published in 2007 showed that this was associated with a 60–70% increase in breast cancer risk among younger, primarily premenopausal women who had never smoked.[75] However, a more recent meta-analysis found an increased risk associated with passive smoking based on case–control, but not cohort, studies.[76]

Conclusions

Evidence establishing menstrual and reproductive factors, anthropometric factors, physical activity, and alcohol intake

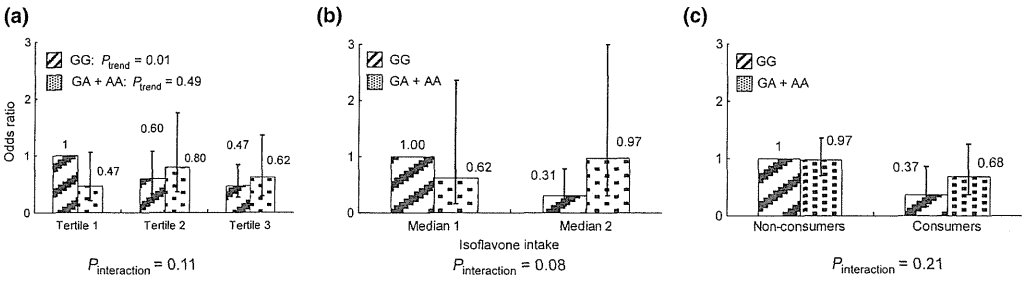

Fig. 5. Isoflavone, polymorphisms in the estrogen receptor beta gene (rs4986938) and breast cancer risk in hospital-based case–control studies among (a) Japanese (postmenopausal), (b) Japanese Brazilians (all), and (c) non-Japanese Brazilians (all). Odds ratios were estimated using matching pairs with adjustment for menopausal status, the number of births, family history of breast cancer, smoking status, moderate physical activity in the past 5 years, and vitamin supplement use. Data are from Iwasaki *et al.*[54]

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as risk factors for breast cancer was derived primarily from Western countries, but only a few dietary factors have been causally related to this disease.[15–17,24] Our studies among Japanese women have confirmed that these previously established factors play an important role in the development of breast cancer.[23,26,34,37] In addition, we have provided further evidence of the role of traditional Japanese foods in the etiology of breast cancer.[21,46,47,54,55,59,60] In particular, our studies of isoflavones and breast cancer have clarified a dose–response relationship and gene–environment interactions.[21,54,55] Given the evidence reviewed above, we suggest that isoflavones exert a protective effect against breast cancer in Asian populations. Finally, current knowledge of protective and risk factors for breast cancer suggest that primary prevention by lifestyle modification in individuals should focus on alcohol intake, weight control, physical activity, and tobacco smoking.

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Disclosure Statement

The authors have no conflicts of interest.

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Cancer Epidemiology, Biomarkers & Prevention

Research Article

Plasma Isoflavones and the Risk of Lung Cancer in Women: A Nested Case–Control Study in Japan

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Abstract

Background: Although several epidemiologic studies have found that isoflavone intake assessed by questionnaire is associated with a decreased risk of lung cancer, no prospective study has investigated this association using blood concentrations of isoflavones.

Methods: We conducted a nested case–control study within a population-based prospective cohort study. A total of 24,127 women aged 40 to 69 years who returned the baseline questionnaire and provided blood samples were observed from 1990 through 2006. During a median follow-up period of 13.5 years, 126 newly diagnosed lung cancer cases were identified. For each case, we selected two controls matched for age, area, smoking status, and condition of blood draw. A conditional logistic regression model was used to estimate the odds ratios (ORs) and 95% CIs of lung cancer in relation to plasma concentrations of genistein, daidzein, glycitein, equol, and total isoflavones.

Results: After exclusion of 20 lung cancer cases diagnosed in the first 3 years after blood collection, an inverse association was found between plasma genistein concentration and lung cancer risk. The multivariate-adjusted OR (95% CI) of lung cancer in the highest quintile of plasma genistein concentration as compared with that in the lowest quintile was 0.31 (0.12, 0.86; P for trend = 0.085). Other isoflavones and total isoflavones were not associated with a significant decrease in the risk of lung cancer.

Conclusion: Plasma genistein concentration was inversely associated with lung cancer risk in Japanese women.

Impact: Our data support the previously observed association between isoflavone intake and lung cancer risk. *Cancer Epidemiol Biomarkers Prev; 20(3); 419–27. ©2011 AACR.*

Introduction

Isoflavones, including genistein, daidzein, and glycitein, are found mainly in soy and soy products in Asian diets. They are similar in structure to the human female hormone 17-beta estradiol. They are also similar in function, as they have a high affinity for the beta-estrogen receptor (1) and act as estrogen agonists and antagonists (2). Therefore, it has been hypothesized that isoflavones protect against the development of cancers related to sex hormones. Indeed, epidemiologic studies have shown an inverse association between isoflavones and the risks of breast (3–5) and prostate cancers (6–8).

In addition to these cancers, it has been suggested that estrogen has a role in lung carcinogenesis (9). Estrogen receptors are expressed in healthy lung tissue and in lung tumors (10), and estrogen induces proliferation of non–small-cell lung cancer (NSCLC) cells (11). Furthermore, randomized controlled trials have indicated that hormone replacement therapy which includes estrogens may increase lung cancer risk in women (12, 13). Thus, isoflavones may be related to the risk of lung cancer, in addition to other hormone-related cancers.

Although several *in vitro* and *in vivo* studies have shown a protective effect of genistein on lung carcinogenesis (14–16), epidemiologic studies have produced conflicting results regarding the association between lung cancer risk and isoflavone intake assessed by food frequency questionnaire (FFQ; ref. 17–20). Notably, 2 recent prospective studies in Asia observed an inverse association in never smokers (19, 20). Epidemiologic

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Note: Study group members are listed in the Appendix.

Authors' Contributions: T. Shimazu, M. Inoue, and S. Tsugane designed research; T. Shimazu, M. Inoue, S. Sasazuki, M. Iwasaki, N. Sawada, T. Yamaji, and S. Tsugane conducted research; T. Shimazu analyzed data and wrote the paper; T. Shimazu had primary responsibility for final content. All authors read and approved the final manuscript.

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studies using blood concentrations of isoflavones might clarify the association with lung cancer risk, because the concentration of isoflavone in blood reflects individual differences in absorption and metabolism, in which intestinal microflora have an important role (21). In particular, due most likely to differences in intestinal bacteria, only 30% to 50% of adults have the capacity to metabolize daidzein into equol, which is known to have stronger estrogenic activity than daidzein (22).

Here, in a nested case–control study within a large-scale, population-based, prospective study, we investigated the association between plasma isoflavone concentration and lung cancer risk among a population of Japanese women that varied substantially in isoflavone intake (3) and had a high prevalence of never smokers (23).

Materials and Methods

Study population

The Japan Public Health Center–based Prospective Study was launched in 1990–1994. The study population was defined as all Japanese inhabitants who had registered their address in administrative districts (city, town, or village) supervised by the 11 public health center (PHC) and were aged 40 to 69 years at the start of the baseline survey (24). Study participants were informed of the objectives and methods of the study in writing, and those who responded to the survey questionnaire and donated blood were regarded as having given informed consent to participate in the study. In addition, participants were notified that they could withdraw from the study at any time. Our study protocol follows the current ethical guidelines for epidemiological research in Japan (25). The study protocol was approved by the Institutional Review Board of the National Cancer Center, Japan.

For the present analysis we excluded one PHC area because data on cancer incidence were not available. After exclusion of ineligible participants (n = 144), we identified 67,522 women as the cohort.

Questionnaire survey

We distributed a baseline self-administered questionnaire survey on various health habits, including personal medical history, menstrual and reproductive history, anthropometric factors, smoking history, and other lifestyle factors in 1990 for Cohort I and in 1993 to 1994 for Cohort II. Women who reported first-degree relatives with lung cancer were considered to have a family history of lung cancer. Never smoking status was determined by answers to the question "Have you ever smoked cigarettes?" in Cohort I and by answers to the question "Are you currently smoking cigarettes?" and information on past history of smoking in Cohort II. Questions regarding age at initiation of smoking and average number of cigarettes smoked per day were also included. Information on passive smoking at the workplace was collected using a question with 4 frequency categories: almost never, 1–3 days/month, 1–4 days/week, and almost daily. We defined women who drank alcoholic beverages less than 1 day/month as nondrinkers. Women who reported past or current use of female hormone drugs were classified as past or current exogenous female hormone users. We had no information on the type, duration, or dosage of such use.

The questionnaire survey also included validated FFQs that asked about average intake during the previous month of 44 food items (for Cohort I) or 52 food items (for Cohort II). The questionnaires had 6 frequency categories for beverages, ranging from 'rarely' to '5 glasses per day', and 4 (Cohort I) or 5 (Cohort II) categories for other items, ranging from 'never' or 'rarely' to 'almost daily'. The intakes of total energy, vegetables, fruit, and fish were calculated from these responses (26, 27), and portion sizes were estimated using data from a validation study (28).

Each questionnaire included 3 food items that contained genistein. In Cohort I, the percentages of women reporting 'almost daily' consumption of (i) miso soup, (ii) soybeans, tofu, deep-fried tofu, and natto (fermented soybeans), and (iii) vegetables other than yellow and green vegetables (e.g., Chinese cabbage, radish, tomato, and cucumber) were 79.4%, 52.2%, and 46.7%, respectively. In Cohort II, miso soup, tofu, and natto were consumed almost daily by 63.2%, 32.5%, and 9.5% of women, respectively.

A total of 55,842 women responded to the questionnaire, yielding a response rate of 83%. We then excluded 585 participants with incomplete information on smoking status and 1,525 participants who had received a diagnosis of cancer before the baseline questionnaire survey. Ultimately, a total of 53,732 women were eligible.

Blood collection

Participants voluntarily provided 10 mL of blood during health checkups in 1990–1995. Blood samples were divided into plasma and buffy layers and preserved at −80°C until analysis. Among the eligible participants, a total of 24,127 women (96.7% of participants in health checkups) donated blood.

Follow-up

We followed study participants until December 31, 2006. Participants who died or moved to other municipalities were identified annually through residential registers in the respective PHC areas. Cause of death was confirmed using mortality data from the Ministry of Health, Labour and Welfare. Among the study participants (n = 24,127), 1,160 (4.8%) died, 1,559 (6.5%) moved away, and 51 (0.2%) were lost to follow-up during the study period.

Selection of cases and controls

We determined lung cancer incidence by using voluntary reports from local major hospitals in the study areas

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and data linkage with population-based cancer registries, after obtaining permission. We used death certificate information as a supplementary information source. In our cancer registry system, the proportion of cases for which information was obtained only from death certificates was 5.1% during the study period. During the time from blood collection to the end of the study period, we identified 126 newly diagnosed lung cancer cases.

The site of origin and histologic type were coded using the International Classification of Diseases for Oncology, Third Edition (C34.0-C34.9; ref. 29). Diagnosis of lung cancer was confirmed by histologic or cytologic examination in 89% of cases ($n = 112$), and was based on clinical findings or unspecified evidence in the remaining 11%. Histologic type was classified as adenocarcinoma ($n = 94$; 75%), squamous cell carcinoma ($n = 6$), large cell carcinoma ($n = 4$), small cell carcinoma ($n = 3$), or other histologic types ($n = 5$), according to the World Health Organization histological classification of lung tumors (30).

For each case, 2 controls were selected at random from participants with no history of lung cancer when the case was diagnosed. Controls were matched for each case by age (within 3 years), PHC area, area (city, or town and village), date on which blood was collected (within 60 days), time of day of blood collection (within 3 hours), duration of fasting at blood collection (within 3 hours), and smoking status (never, past, and current).

Laboratory assays

Plasma concentrations of isoflavones (i.e., genistein, daidzein, glycitein, and equol) were analyzed using triple-quadrupole tandem liquid chromatography–mass spectrometry (31). Beta-glucuronidase/sulfatase was added to 0.1 mL of plasma. The aglycones of the isoflavones and their metabolites were recovered by diethyl ether extraction. The diethyl ether extract of the sample was dried under nitrogen flow and redissolved in acetonitrile. The ionizing method was electrospray using negative ions; multiple reaction monitoring was used for mass analysis.

To assure quality control (QC), the precision of laboratory measurement was assessed before and after each assay using a pooled blood sample from healthy volunteers. Based on 20 replicated measurements of the QC sample at a mean concentration of 122.1 ng/mL for genistein, 88.0 ng/mL for daidzein, 10.6 ng/mL for glycitein, and 39.6 ng/mL for equol, the coefficients of variation were 3.0% or less for intraday variation and 3.9% or less for interday variation. Cases and matched controls were assayed in the same batch. Detection limits were less than 1.0 ng/mL for all isoflavones. All samples were analyzed at a single laboratory (SRL, Tokyo, Japan) while blinded to case–control status.

Statistical analysis

Baseline characteristics between cases and controls were evaluated by the Mantel–Haenszel procedure with matched-set strata (32). For genistein and daidzein, study participants were classified into quintiles according to plasma concentration. For glycitein and equol, the lowest category comprised study participants with amounts below the detection limit (<1.0 ng/mL), and those with detectable concentrations were divided into quartiles. Total isoflavones was defined as the sum of genistein, daidzein, glycitein, and equol concentrations and was classified by quintile of plasma concentration. Glycitein and equol concentrations below the detection limit were regarded as zero in the calculation of total isoflavones. Cutoff points for plasma isoflavone concentration were based on the control distribution.

We used a conditional logistic regression model to estimate odds ratios (OR) and 95% CIs of lung cancer risk by category of plasma isoflavones and to adjust for potentially confounding variables. Dummy variables were created for the categories of plasma isoflavone concentration, and the lowest category was used as the reference category. We calculated P values for the analysis of linear trends by assigning ordinal values for categories of plasma isoflavone concentration and entering the number as a continuous term in the regression model. All reported P values are 2-tailed. All statistical analyses were performed using SAS statistical software, version 9.1 (SAS Institute Inc; ref. 33).

Multivariate-adjusted ORs were adjusted for family history of lung cancer (yes or no), pack-years of smoking among current smokers (1–19 or ≥20 pack-years, as defined by multiplying the years of smoking by the average number of cigarettes per day and dividing by 20), passive smoke exposure at work (≤1–3 days/month, 1–4 days/week, or almost daily), past or current use of exogenous female hormones (yes or no), and fruit and vegetable intake (continuous variable). All analyses were repeated after excluding participants who received a diagnosis of lung cancer within 3 years of blood collection ($n = 20$).

Results

The characteristics of cases and controls are shown in Table 1. The prevalence of never smokers among both cases and control was 92.9% ($n = 117$ and $n = 234$, respectively). We found no significant differences in the characteristics of cases and controls. Table 2 shows plasma isoflavone concentrations in cases and controls. The median plasma concentrations of genistein, daidzein, glycitein, equol, and total isoflavones in cases were all slightly lower than those in controls; however, the differences were not statistically significant.

Table 3 shows the associations between plasma isoflavone concentrations and risk of lung cancer. After adjustment for potential confounders, there was a U-shaped association between plasma isoflavone concentrations and lung cancer risk. However, after exclusion of the 20 lung cancer cases diagnosed in the first

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Table 1. Baseline characteristics of cases and controls

Characteristic	Cases (n = 126)	Controls (n = 252)	P[a]
Age, mean (SD), y	57.3 (7.4)	57.0 (7.3)	-
Family history of lung cancer, n (%)	4 (3.2)	3 (12)	0.18
Never smokers, n (%)	117 (92.9)	234 (92.9)	-
Current smokers, n (%)	8 (0.1)	16 (0.1)	-
1–19 pack years, n (%)[b]	5 (31.3)	5 (62.5)	0.15
Passive smoke exposure, almost daily, n (%)	24 (19.5)	46 (18.5)	0.41
Nondrinkers, n (%)	98 (78.4)	209 (82.9)	0.33
Postmenopausal status, n (%)	101 (82.8)	200 (82.0)	0.39
Age at menarche, mean (SD), y[c]	15.8 (2.0)	15.9 (2.1)	0.34
Age at menopause, mean (SD), y[c]	48.7 (4.5)	49.3 (4.0)	0.56
Past or current use of exogenous female hormones, n (%)	19 (17.1)	26 (11.8)	0.21
Dietary intake[d]			
Total energy, mean (SE), kcal/d	1278 (37.5)	1249 (26.5)	0.49
Vegetables, mean (SE), g/d	118 (6.6)	119 (4.6)	0.98
Fruit, mean (SE), g/d	102 (8.7)	106 (6.2)	0.70
Fish, mean (SE), g/d	44 (2.6)	49 (18)	0.14

[a]P value on Mantel-Haenszel test with matched-set strata.
[b]Among current smoking women.
[c]Among postmenopausal women.
[d]Adjusted for cohort.

3 years after blood collection, we found an inverse association between plasma genistein concentration and lung cancer risk. After adjustment for family history of lung cancer, pack-years of smoking among current smokers, passive smoke exposure at work, past or current use of exogenous female hormones, and fruit and vegetable intake, the multivariate-adjusted ORs (95% CIs) of lung cancer across increasing quintiles of plasma genistein, with the lowest quintile as reference, were 1.00, 0.27 (0.10, 0.75), 0.21 (0.08, 0.59), 0.24 (0.08, 0.71), and 0.31 (0.12, 0.86) (P for trend = 0.085). For daidzein, glycitein, and total isoflavones, the ORs of lung cancer were also below unity, but were not statistically significant. We found no association between plasma equol concentration and lung cancer risk, even after lung cancer cases in the first 3 years after blood collection were excluded.

For the purpose of sensitivity analysis, we included additional variables in the model, namely, menopausal status (premenopausal or postmenopausal), ages at menarche (<16 or ≥16 years) and menopause (≤50 or >50 years) among postmenopausal women, and

Table 2. Plasma isoflavone concentrations in cases and controls

Isoflavone	Cases (n = 126)		Controls (n = 252)		P[a]
	Median, ng/mL	IQR	Median, ng/mL	IQR	
Genistein	72.0	(25.4–163.1)	72.4	(29.8–127.0)	0.84
Daidzein	29.3	(9.9–66.3)	31.8	(11.6–61.4)	0.82
Glycitein[b]	1.8	(0–4.1)	2.1	(0–4.1)	0.34
Equol[b]	2.8	(0–20.2)	3.5	(0–15.4)	0.78
Total isoflavones[c]	124.7	(42.2–267.1)	126.4	(51.8–214.6)	0.82

Abbreviation: IQR, interquartile range.
[a]P value on Mantel-Haenszel test with matched-set strata.
[b]Values below the detection limit (<1 ng/ml) were regarded as zero.
[c]Total isoflavones is the sum of genistein, daidzein, glycitein, and equol concentrations.

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Table 3. ORs and 95% CIs of lung cancer, by plasma isoflavone concentration[a]

	Quintile of Plasma Isoflavone Concentration[b]					
Plasma concentration	**Q1 (lowest)**	**Q2**	**Q3**	**Q4**	**Q5 (highest)**	***P* for trend**
Genistein, ng/mL	<24.8	24.8–52.3	52.4–88.7	88.8–151.2	>151.2	
No. of cases	34	21	19	17	35	
No. of controls	50	51	50	51	50	
OR1 (95% CI)[c]	1.00 (Reference)	0.51 (0.25, 1.07)	0.47 (0.23, 0.99)	0.43 (0.20, 0.93)	0.88 (0.45, 1.74)	0.915
OR2 (95% CI)[d]	1.00 (Reference)	0.40 (0.17, 0.94)	0.36 (0.15, 0.86)	0.36 (0.14, 0.93)	0.68 (0.30, 1.53)	0.700
OR3 (95% CI)[e]	1.00 (Reference)	0.27 (0.10, 0.75)	0.21 (0.08, 0.59)	0.24 (0.08, 0.71)	0.31 (0.12, 0.86)	0.085
Daidzein, ng/mL	<8.3	8.3–21.7	21.8–40.7	40.8–72.2	>72.2	
No. of cases	31	24	22	19	30	
No. of controls	50	51	50	51	50	
OR1 (95% CI)[c]	1.00 (Reference)	0.71 (0.35, 1.40)	0.68 (0.34, 1.34)	0.57 (0.27, 1.18)	0.94 (0.47, 1.86)	0.709
OR2 (95% CI)[d]	1.00 (Reference)	0.81 (0.37, 1.76)	0.84 (0.39, 1.82)	0.56 (0.23, 1.36)	1.03 (0.46, 2.29)	0.874
OR3 (95% CI)[e]	1.00 (Reference)	0.79 (0.34, 1.86)	0.56 (0.23, 1.36)	0.35 (0.13, 0.97)	0.73 (0.29, 1.82)	0.258
Glycitein, ng/mL	<1.0	1.0–1.9	2.0–3.0	3.1–5.4	>5.4	
No. of cases	48	15	22	22	19	
No. of controls	82	42	41	45	42	
OR1 (95% CI)[c]	1.00 (Reference)	0.59 (0.29, 1.20)	0.90 (0.48, 1.67)	0.80 (0.42, 1.51)	0.74 (0.36, 1.49)	0.513
OR2 (95% CI)[d]	1.00 (Reference)	0.42 (0.19, 0.95)	0.94 (0.47, 1.88)	0.79 (0.35, 1.79)	0.72 (0.32, 1.64)	0.601
OR3 (95% CI)[e]	1.00 (Reference)	0.42 (0.18, 1.03)	0.77 (0.36, 1.63)	0.44 (0.17, 1.19)	0.52 (0.21, 1.31)	0.147
Equol, ng/mL	<1.0	1.0–4.3	4.4–12.1	12.2–26.7	>26.8	
No. of cases	53	15	14	23	21	
No. of controls	99	38	39	38	38	
OR1 (95% CI)[c]	1.00 (Reference)	0.73 (0.36, 1.48)	0.67 (0.33, 1.37)	1.13 (0.61, 2.11)	1.03 (0.53, 2.00)	0.796
OR2 (95% CI)[d]	1.00 (Reference)	0.73 (0.32, 1.66)	0.82 (0.35, 1.94)	0.94 (0.44, 2.01)	1.08 (0.51, 2.31)	0.845
OR3 (95% CI)[e]	1.00 (Reference)	0.86 (0.35, 2.12)	0.78 (0.32, 1.92)	0.97 (0.43, 2.20)	1.07 (0.47, 2.44)	0.889
Total isoflavones, ng/mL	<42.1	42.1–86.0	86.1–148.1	148.2–257.1	>257.1	
No. of cases	30	20	21	20	35	
No. of controls	50	51	50	51	38	
OR1 (95% CI)[c]	1.00 (Reference)	0.57 (0.26, 1.25)	0.63 (0.31, 1.30)	0.56 (0.25, 1.25)	1.06 (0.52, 2.17)	0.590
OR2 (95% CI)[d]	1.00 (Reference)	0.59 (0.24, 1.49)	0.61 (0.26, 1.41)	0.58 (0.22, 1.57)	0.95 (0.41, 2.20)	0.729
OR3 (95% CI)[e]	1.00 (Reference)	0.46 (0.16, 1.34)	0.43 (0.16, 1.12)	0.41 (0.13, 1.29)	0.55 (0.20, 1.49)	0.442

[a]A conditional logistic regression model was used to estimate ORs and 95% CIs.

[b]For genistein and daidzein, study participants were classified into quintiles according to plasma concentration. For glycitein and equol, the lowest category (Q1) comprised study participants with concentrations below the detection limit (<1.0 ng/mL); those with detectable concentrations were divided into quartiles.

[c]Matched variables were age, public health center area, geographic area (city, or town and village), date on which blood was collected, time of day of blood collection, duration of fasting at blood collection, and smoking status.

[d]OR2 was adjusted for family history of lung cancer (yes or no), pack-years of smoking among current smokers (1–19 or >20 pack-years, defined by multiplying the years of smoking by the average number of cigarettes per day and dividing by 20), passive smoke exposure at work (<1–3 days/month, 1–4 days/week, or almost daily), past or current use of exogenous female hormones (yes or no), and fruit and vegetable intake (continuous variable).

[e]OR3 was adjusted for the same variables as OR2, after exclusion of lung cancer cases diagnosed in the first 3 years after blood collection.

fish intake. The results were similar (data not shown). The findings were also similar when the analysis was limited to never smokers: after exclusion of lung cancer cases in the first 3 years, the multivariate-adjusted OR (95% CI) for the highest quintile of genistein concentration versus the lowest quintile was 0.36 (0.13–0.98; *P* for trend = 0.151), when the analysis was restricted to women who provided a fasting

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blood sample (i.e., 6 or more hours after a meal), and when only participants with lung adenocarcinoma or NSCLC (adenocarcinoma, squamous cell carcinoma, or large cell carcinoma) were defined as cases (data not shown).

Discussion

In this nested case–control study within a large-scale, population-based, prospective study of Japanese women, we found that plasma concentrations of genistein, but not daidzein, glycitein, equol, or total isoflavones, were associated with a significant decrease in lung cancer risk after exclusion of lung cancer cases diagnosed within 3 years of blood collection. At the time of blood collection, participants who later developed early lung cancer might have had preclinical lung cancer, which could have changed their dietary behavior. Also, if participants with preclinical lung cancer were more likely due to ill health to have heath checkups than the apparently healthy population at the baseline, they would be more likely to be cases. If indeed this occurred, any association would be distorted. We consider that the results obtained after excluding these early lung cancer cases suggest a preventive effect of genistein on lung cancer incidence. To our knowledge, this is the first study to investigate the association between plasma isoflavone concentrations and lung cancer risk.

We did not find a dose–response relationship between plasma genistein concentration and lung cancer risk, as lung cancer risk remained constant across the second through the fifth quintiles of plasma genistein concentration. Although we cannot characterize the shape of the exposure–disease relation because of the limited number of cases, the results suggest that a low genistein concentration is important in lung carcinogenesis. However, further study of a larger number of lung cancer cases is needed to confirm this hypothesis.

We observed an inverse association only for genistein. If isoflavones have an effect via estrogen-dependent mechanisms, this inverse association with genistein is plausible, as it has been reported to have greater estrogenic activity (34–36) than daidzein. Reports have shown that equol has even higher estrogenic activity than genistein (34, 36); however, the median plasma concentration of genistein in controls was 2.3 to 34.5 times that of other isoflavones, including equol (Table 2), which may explain why we failed to detect an association with isoflavones other than genistein.

In addition to the estrogen receptor-mediated mechanism, we speculate that a mechanism mediated by the epidermal growth factor receptor (EGFR) may be involved. The EGFR mediates signals related to increased cell proliferation and inhibition of apoptosis (37). While mutations in the *EGFR* gene activate the EGFR pathway (38), NSCLC with mutated *EGFR* is highly responsive to gefinitib, an EGFR protein-tyrosine kinase (PTK) inhibitor (39). Interestingly, genistein is reported to be a PTK inhibitor, based on the fact that it inhibited EGFR PTK activity *in vitro* (40). Genistein inhibited growth of NSCLC cell lines, particularly one with mutated *EGFR* (16). Furthermore, a case–control study in Japan found that soy food intake was inversely associated with *EGFR*-mutated NSCLC only (41). Although information was not available on the *EGFR* status of lung cancer in our study, the present participants had characteristics similar to those associated with the *EGFR* mutation, that is, never-smoking status, East Asian ethnicity, and female sex (42). Genistein might exert its preventive effect on lung cancer through the EGFR-mediated mechanism.

Only 2 prospective studies have examined the association between isoflavone intake and lung cancer risk in Asian countries, where isoflavone intake is higher than in Western countries. We previously reported an association between isoflavone intake and lung cancer risk, using data from a 5-year follow-up questionnaire in our cohort (20). In that study, we found a nonsignificant inverse association between isoflavone intake (determined by using genistein intake) and lung cancer risk in women (hazard ratio for the highest vs. lowest quartile of intake: 0.83; 95% CI: 0.54, 1.29; P for trend = 0.409). In the Singapore Chinese Health Study, Seow and colleagues reported an inverse association between isoflavone intake and overall risk of lung cancer in nonsmoking women: the multivariate-adjusted hazard ratio for lung cancer incidence in the highest versus the lowest quartile of isoflavone intake was 0.59 (95% CI: 0.38, 0.91) (19). These findings conform to those of the current study.

The limitations of this study warrant mention. First, we used a single measurement of plasma isoflavone concentration, which may be subject to day-to-day and diurnal variation. However, in a validation study using a subsample of the cohort, high reproducibility of genistein intake was observed: the correlation coefficients for FFQ estimates separated by 1 year and 5 years were 0.72 (43) and 0.61 (28), respectively. Furthermore, our validation study yielded satisfactorily high correlation coefficients for genistein estimates from dietary records (DR) measured repeatedly for a year, a fasting serum sample, and a single FFQ (DR vs. serum: 0.33; DR vs. FFQ: 0.59; ref. 43). As isoflavone intake was likely to have been stable for a long period in this population, we consider it unlikely that day-to-day variation in plasma isoflavone concentrations substantially distorted the association between plasma isoflavone concentration and lung cancer risk. Because of the half-life of genistein and daidzein in blood (7.7 to 9.5 hours; ref. 44), plasma concentrations of isoflavones vary with regard to fasting time. To minimize attenuation in risk estimation due to diurnal variation, fasting time was matched in cases and controls. A second