Table 1. Patient profiles

Factor	Iron depletion	Control	*P* value
Demographics			
Number of patients	35	40	
Sex (M/F)	17/18	21/19	0.912
Age (years)	61 (34–75)	58 (35–75)	0.523
History of blood transfusion	14 (40%)	10 (25%)	0.255
History of interferon therapy	20 (57%)	23 (58%)	0.975
Observation period (months)	114 (44–144)	106 (31–144)	0.411
Laboratory data			
Albumin (g/dl)	4.2 (3.9–5.0)	4.2 (3.5–4.9)	0.992
Bilirubin (mg/dl)	0.7 (0.4–2.1)	0.7 (0.3–1.2)	0.564
ALT (IU/l)	96 (60–421)	87 (60–278)	0.143
GGTP (IU/l)	40 (16–186)	49 (15–224)	0.912
Platelet count ($\times 10^3/\mu l$)	141 (71–197)	142 (84–192)	0.445
AFP (ng/ml)	5.5 (1.5–139)	5.4 (1.5–220)	0.553
Type IV collagen (ng/ml)	150 (83–362)	144 (42–342)	0.465
HCV serotype			
Group 1	30 (86%)	37 (92%)	0.568
Group 2	5 (14%)	3 (8%)	
HCV concentration			
High	32 (91%)	37 (93%)	0.872
Low	5 (9%)	3 (7%)	
Ferritin (ng/ml)	371 (77–1150)	178 (3–1506)	0.971
Histological grade			
Moderate fibrosis	18 (51%)	22 (55%)	0.942
Severe fibrosis	17 (49%)	18 (45%)	
Iron deposition	4 (0–26)	4.5 (0–24)	0.719

ALT, alanine aminotransferase; GGTP, -glutamyl transpeptidase; AFP, -fetoprotein; HCV, hepatitis C virus

Table 2. Changes in iron-related parameters after iron depletion therapy

	Iron depletion group		Control	
Variable	Baseline	End point	Baseline	End point
Hemoglobin (g/dl)	13.9 (11.8–15.2)	10.7 (9.8–11.7)*	13.7 (11.2–15.4)	13.8 (11.1–15.6)
Serum iron (μg/dl)	152 (57–247)	26 (12–40)*	120 (22–321)	142 (36–441)
Serum ferritin(μg/l)	371 (77–1150)	8 (4–21)*	178 (3–1506)	184 (12–1550)
Serum ALT (IU/l)	96 (60–421)	28 (15–65)*	87 (60–278)	84 (58–312)

Data represent medians (range). *$P < 0.01$ vs. baseline

Risk factors affecting hepatocarcinogenesis

Factors associated with hepatocarcinogenesis were analyzed by Cox regression analysis in all 75 CHC patients to determine the effect of iron depletion therapy on disease progression. The univariate analysis showed that the following four factors significantly affected the crude hepatocarcinogenesis rate in all patients: sex ($P = 0.0058$), age ($P = 0.0328$), iron depletion therapy ($P = 0.021$), and platelet count ($P = 0.0131$) (Table 3). Multivariate regression analysis was then performed with these four statistically significant variables. As a result, three factors were significantly and independently associated with hepatocarcinogenesis. The relative risk for hepatocarcinogenesis was greater in male patients than in female patients [odds ratio (OR) = 2.07], in patients 60 years old compared with those <60 years old (OR = 1.72), and in patients who did not receive iron depletion compared with in control patients (OR = 1.76) (Table 4).

Serum ALT and serum ferritin levels and hepatocarcinogenesis rates

In the iron depletion group, the average serum ALT level during the maintenance period declined to less than 60 IU/l in all patients and became normal (<40 IU/l) in 24 patients (69%). Figures 2 and 3 show the cumulative hepatocarcinogenesis rates based on

Table 3. Univariate analysis of factors associated with hepatocarcinogenesis in patients with chronic hepatitis C

Factors	Category	Odds ratio (95% CI)	*P* value
Demographics			
Sex (M/F)	Female	1	0.00580
	Male	2.21 (1.26–4.60)	
Age (years)	<60	1	0.0328
	60	1.78 (1.06–4.81)	
History of blood transfusion	(+)	1	0.782
	(−)	1.07	
History of interferon therapy	(−)	1	0.735
	(+)	1.08	
Treatment			
Iron depletion	(+)	1	0.0210
	(−)	1.86 (1.11–3.52)	
Laboratory data			
Albumin (g/dl)	4.0	1	0.628
	<4.0	1.05	
Bilirubin (mg/dl)	1.2	1	0.262
	>1.2	1.51	
ALT (IU/l)	<100	1	0.591
	100	1.15	
GGTP (IU/l)	<50	1	0.533
	50	1.16	
Ferritin (ng/ml)	<50	1	0.455
	50	1.07	
Platelet count	10×10^4	1	0.0131
	$<10 \times 10^4$	1.73 (1.04–3.06)	
AFP (ng/ml)	<8.5	1	0.220
	8.5	1.88	
Type IV collagen (ng/ml)	<150	1	0.826
	150	1.21	
HCV serotype	2	1	0.415
	1	1.50	
HCV concentration	Low	1	0.897
	High	1.06	
Histological stage	Moderate fibrosis	1	0.213
	Severe fibrosis	1.35	

CI, confidence interval

Table 4. Multivariate analysis of factors associated with hepatocarcinogenesis in patients with chronic hepatitis C

Factor	Category	Odds ratio (95% CI)	*P* value
Sex (M/F)	Female	1	0.00980
	Male	2.07 (1.18–4.35)	
Age (years)	<60	1	0.0483
	60	1.72 (1.00–3.27)	
Iron depletion	(+)	1	0.0337
	(−)	1.76 (1.05–3.33)	
Platelet count	10×10^4	1	0.1493
	$<10 \times 10^4$	1.73 (0.85–2.56)	

average serum ALT (Fig. 2) and ferritin (Fig. 3) levels, respectively, in patients who underwent iron depletion therapy. The hepatocarcinogenesis rate in patients with serum ALT 40IU/l or serum ferritin 20ng/ml was significantly lower than that in patients with serum ALT > 40IL/l ($P = 0.0377$) or serum ferritin >20ng/ml ($P = 0.0057$). When a cutoff value for serum ferritin of 10ng/ml was used, the hepatocarcinogenesis rate was also significantly ($P = 0.017$) lower than that in patients with serum ferritin >10ng/ml. Furthermore, the multivariate

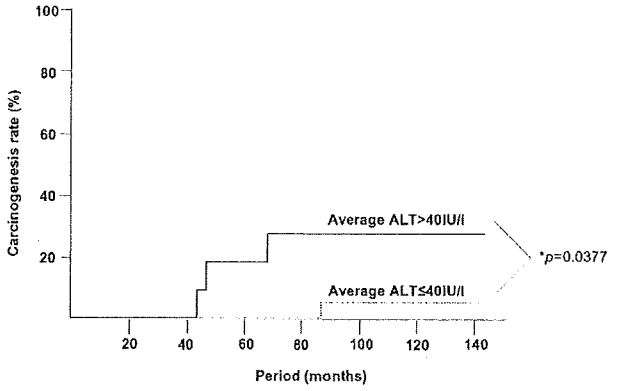

Fig. 2. Hepatocarcinogenesis rate in the iron depletion group. The rate in patients with serum alanine aminotransferase (*ALT*) of 40 IU/l was significantly lower than in those with serum ALT > 40 IU/l (log rank test)

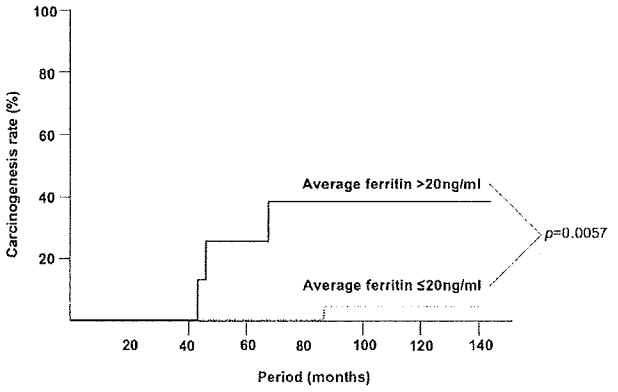

Fig. 3. Hepatocarcinogenesis rate in the iron depletion group. The rate in patients with serum ferritin 20 ng/ml was significantly lower than in those with serum ferritin >20 ng/ml (log rank test)

Cox regression analysis indicated that the average serum ferritin level independently affected the hepatocarcinogenesis rate and the relative risk of HCC (OR = 3.48 for patients with average serum ferritin levels of >20 ng/ml vs. those with levels of 20 ng/ml).

Discussion

We previously demonstrated in a 6-year cohort trial that CHC patients who received iron depletion therapy showed significant improvements in serum ALT levels, histological hepatic fibrosis score, and hepatic 8-OHdG levels.[10] However, it was unclear at the time whether continuation of iron depletion therapy for CHC could decrease the risk of HCC development. Ideally, to tests such a hypothesis, a prospective randomized trial with an observation period of at least 10 years would be required. However, such a prospective trial would be ethically questionable, because during the study period new effective treatments might emerge for HCV patients who do not respond to IFN therapy, and it would be unethical to withhold such treatment from the study participants, particularly those in the control group. In fact, in the last 10 years, Peg-IFN/ribavirin therapy has become a standard treatment for CHC,[5,6] and clinical trials are currently evaluating other new antiviral agents such as VX-950, a novel inhibitor of HCV NS3.4A serine protease, and siRNA for HCV.[19,20]

Therefore, we conducted a cohort study of two groups of CHC patients over a 12-year observation period. The two groups were comparable because no significant differences were observed between them with respect to parameters such as sex, age, serum ALT levels, history of previous IFN treatment, or liver histological stage (Table 1). The study results clearly indicated that the incidence of HCC development in iron-depleted patients (0.9% per year) was significantly lower than that in control patients (3.9% per year). This reduced incidence of HCC in iron-depleted patients is reasonably low even when compared with previously reported rates of HCC development in CHC patients with moderate to severe fibrosis (2.7%–3.8% per year).[4]

A practical difficulty of the present study was the establishment of an effective protocol to maintain low hepatic iron levels over the study period. Although the mechanism underlying hepatic iron accumulation in patients with CHC remains unclear, excess hepatic iron is considered to be derived from daily food intake,[10] and if dietary iron intake is not restricted, phlebotomy can lead to enhanced iron absorption. Thus, the protocol we adopted for iron depletion therapy was phlebotomy combined with a low-iron diet. This protocol was well tolerated during the study. We advised the patients to reduce their daily iron intake to a maximum of 7 mg, and the actual daily iron intake levels were found to be 5.6 mg/day (range, 5.0–6.8 mg/day), which is approximately half the average dietary iron intake in Japan (11–12 mg per day according to a national nutrition investigation by the Ministry of Welfare of Japan conducted in 1996). As a result, mean serum ferritin levels were kept low and we were able to greatly reduce the mean number of phlebotomies during the maintenance phase. However, in some patients, dietary guidance was not successful and their serum ferritin levels were >20 ng/ml. In such patients, serum ALT levels also fluctuated upward. Hence, multivariate analysis demonstrated that both serum ferritin levels 20 ng/ml and serum ALT levels 40 IU/l were independent risk factors for HCC development in the iron depletion group. Accordingly, strict adherence to a low-iron diet is essential for a successful outcome with this treatment modality.

Another practical concern with this modality is its inapplicability to anemic patients (e.g., those with liver cirrhosis) with normal or high serum ferritin levels. In the future, this limitation could conceivably be overcome by using erythropoietin in combination with iron depletion therapy or by using an iron-chelating agent. Nonetheless, iron depletion therapy appears to be a safe and promising means of preventing the progression of CHC to HCC, and is suitable for use until such time as more powerful and less toxic antiviral agents are developed.

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Early- and Late-Onset Breast Cancer Types Among Women in the United States and Japan

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Abstract

Background: **Although differences in breast cancer incidence among Occidental and Asian populations are often attributed to variations in environmental exposures and/or lifestyle, fewer studies have systematically examined the effect of age-related variations.**

Methods: **To further explore age-related geographic breast cancer variations, we compared age-specific incidence patterns among cases of female invasive breast cancer from the Surveillance, Epidemiology, and End Results (SEER) program and the Osaka Cancer Registry (1978-1997).**

Results: **In SEER, there were 236,130 Whites, 21,137 Blacks, and 3,304 Japanese-Americans in Hawaii with invasive breast cancer. In Osaka, there were 25,350 cases. Incidence rates per 100,000 woman-years ranged from 87.6 among Whites to 21.8 in Osaka. Age-specific incidence rates increased rapidly until age 50 years for all race/ethnicity groups, and then continued to increase more slowly for Whites, Blacks, and Japanese-Americans in Hawaii but plateaud for Osaka. Age-specific incidence rates in SEER reflected bimodal (early-onset and late-onset) breast cancer populations, whereas Osaka had only an early-onset age distribution. These age-specific differences in incidence among SEER and Osaka persisted after adjustment for calendar-period and birth-cohort effects using age-period-cohort models.**

Conclusions: **Results confirm striking age-specific differences among Occidental and native Japanese breast cancer populations, probably due to complex age-related biological and/or environmental variations among Occidental and Asian breast cancer populations.** (Cancer Epidemiol Biomarkers Prev 2007;16(7):1437–42)

Introduction

Breast cancer incidence rates are generally higher in Occidental than in Asian populations (1-4), possibly due to a combination of environmental, lifestyle, and/or biological factors. For example, presumptive environmental and/or lifestyle factors shift breast cancer incidence among migrant Asian women from the baseline rate in their native country to the rate in their adopted country (5-8). Biological effects seem to alter the shape of the age-specific incidence rate curve among Occidental and native Asian women (1, 3, 4, 9-15). Among Occidental women, age-specific incidence rates increase rapidly until menopause, and then continue to increase more slowly. Among native Asian women, rates increase rapidly until menopause, and then plateau or decrease. These age-related biological effects have generated interest and debate for decades.

In 1980, Moolgavkar et al. fit a two-stage breast cancer model to six high-risk and low-risk populations, including Connecticut and Osaka (14). The model viewed breast cancer as the end result of two discrete and irreversible events, without distinction for premenopausal (early-onset) and postmenopausal (late-onset) breast cancer types. In this model, among native Asian women, the late-onset drop in incidence was due to a birth-cohort artifact (1, 9) in which the progressive increase in risk from one generation to the next gives the appearance of a decreasing age-specific incidence rate curve. In 1981, Pike and colleagues developed the concept of breast tissue "aging," modified by the timing of certain reproductive risk factors such as the age at menarche, first full-term pregnancy, and menopause (15). Still others have suggested that the different age-specific incidence rate patterns among different breast cancer populations result from the mixing of distinct breast cancer types according to age at onset (16-19). Rates that increase rapidly until age 50 years, and then flatten, reflect mostly early-onset breast cancer populations, whereas rates that increase continuously with aging result from mixed early-onset and late-onset breast cancer types.

To further explore geographic age-related variations among Occidental, migrant Asian, and native Asian breast cancer populations, we examined age-specific incidence patterns (rates and age distributions) using data from the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program and the Osaka Cancer Registry (OCR). To account for calendar-period and/or birth-cohort effects, we used age-period-cohort models to simultaneously adjust for age, calendar-period, and birth-cohort effects.

Materials and Methods

Subjects. Female breast cancer case data for Whites, Blacks, and Japanese-Americans in Hawaii (JAHI) were obtained from the SEER 9-Registry database (November 2004 submission; ref. 20). The SEER 9-Registry database includes data from San Francisco-Oakland, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, and Atlanta, covering ~10% of the U.S. population. Case data for native Japanese women were obtained from the OCR (21). The OCR is a population-based registry in Osaka Prefecture, the second most populous prefecture in Japan, covering ~8 million people or ~7% of

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Japan's population (22). All primary malignant cases recorded in the SEER 9-Registry database and OCR during the period 1978 to 1997 were included in the analysis.

Although case data were available for all race/ethnicity groups, population data for JAHI could not be directly obtained from SEER. The state cancer registry for Hawaii reports only case data to SEER; however, it reports both case and population data to the International Agency for Research on Cancer (IARC; ref. 23). Similarly, OCR reports both case and population data to the IARC. To calculate crude incidence rates for JAHI and Osaka, we obtained corresponding population data from the IARC database, which were either actual census data or population counts estimated from census data. For consistency, we also used population data from the IARC database for Whites and Blacks in SEER.

Demographic and Tumor Characteristics. Female breast cancer cases were stratified by four 5-year calendar-periods of diagnosis (1978-1982, 1983-1987, 1988-1992, and 1993-1997), premenopausal and postmenopausal surrogates (age <50 and 50+ years; refs. 24, 25), stage, grade, and histology. SEER and OCR tumor stage categories were matched to approximate localized, regional, and distant breast cancer (26). Localized disease was confined to the breast tissue and fat, including the nipple and/or areola. Regional disease included breast cancers with regional nodal involvement. Distant disease included systemic metastases.

Tumor grade was dichotomized into low-risk and high-risk groups. Low grade included grade I (well differentiated) and grade II (moderately differentiated) tumors. High grade included grade III (poorly differentiated) and grade IV (undifferentiated or anaplastic) tumors. Histopathologic subtypes were categorized into ductal and lobular groups, using the International Classification of Diseases for Oncology, 3rd edition; and the General Rules for Clinical and Pathological Recording of Breast Cancer by the Japanese Breast Cancer Society (27, 28). All other subtypes were designated as other or unknown. Although there is some variation with respect to histologic typing between the two classification systems, they are comparable with respect to breast cancer overall.

Age-Adjusted Incidence Rates. Breast cancer incidence rates were calculated using case data from SEER and OCR and population data from the IARC. Rates were age-adjusted to the World Standard population (29). Our calculated age-adjusted rates were similar to those recorded in the IARC database. Relative risks were expressed as incidence rate ratios (IRR), in which a given characteristic was compared to a referent characteristic with an assigned IRR of 1.0. Secular trends were plotted on a log-linear scale, as previously described (30).

Age-Specific Incidence Rates. Age-specific incidence rates for the study period 1978 to 1997 were calculated according to 12 5-year age groups (25-29 to 80-84). Slope changes in overall rates at age 50 years for each race/ethnicity group were formally tested using piecewise linear Poisson regression models (PROC GENMOD, SAS, v.8e, SAS Institute Inc.). The statistical model was defined as:

$$\log(\text{incidence rate}) = \beta_0 + \beta_1 \times \text{age} + \beta_2 \times (\text{age} - 50) \times I$$

where I was the indicator variable for age 50 years or older, $\exp(\beta_1)$ was the change in incidence per year of age before 50, and $\exp(\beta_1 + \beta_2)$ was the corresponding change in incidence for age 50 years or older. We allowed for overdispersion in the model by including a deviance variable parameter. A change in slope was considered to be statistically significant when we rejected the null hypothesis β_2 equal to zero ($\alpha = 0.05$).

To determine the age effects after adjustment for calendar-period and birth-cohort effects, we fit age-period-cohort models to incidence data for Whites, Blacks, JAHI, and Osaka using Poisson regression (PROC GENMOD, SAS). We used 12 5-year age groups (25-29 to 80-84), 5 5-year calendar-periods (1973-1977 to 1993-1997), and 16 5-year birth-cohorts, referred to by the mid-year of birth (1893 to 1968) for all populations.

In addition to overall age-specific rates, we calculated age-specific incidence rates for 12 5 year age groups (25-29 to 80-84) according to calendar-period (i.e., cross-sectional rates) and birth-cohort (i.e., longitudinal rates). Cross-sectional age-specific rates were determined according to four 5-year calendar-periods (1978-1982 to 1993-1997). Longitudinal age-specific rates were determined according to 15 5-year birth-cohorts, referred to by the mid-year of birth (1898 to 1968).

Age Distributions. We graphed density plots for age at diagnosis by race/ethnicity group and calendar-period (1978-1982 to 1993-1997) using S-PLUS (version 6.2 for Windows, Insightful Corp.). S-PLUS uses kernel density estimation to produce smoothed histograms of the age distributions, and is described in detail elsewhere (31-33). In brief, a Gaussian kernel was used to estimate the underlying probability density function for breast cancer diagnosis conditioned on age. A more detailed description is given in Appendix 1.

Results

Demographic and Tumor Characteristics. Demographic and tumor characteristics for SEER and Osaka for the study period 1978 to 1997 are shown in Table 1. In the nine SEER areas, there were 236,130 White and 21,137 Black female cases of invasive breast cancer. In Hawaii, there were 3,304 Japanese cases. In OCR, there were 25,350 cases. The overall age-adjusted incidence rates per 100,000 woman-years for the study period 1978 to 1997 were highest in Whites (87.6), followed by Blacks (80.0), JAHI (72.4), and Osaka (21.8). Median age-at-diagnosis was oldest in Whites (64 years) and youngest in Osaka (51 years). Similarly, the IRR for cases diagnosed after age 50 years compared with cases diagnosed before age 50 years was highest in Whites (IRR, 10.84) and lowest in Osaka (IRR, 4.73).

All race/ethnicity groups had lower rates of regional stage disease than local stage disease (i.e., IRR for regional compared with local stage disease < 1.0), although the relative differences were greater for Whites and JAHI. For example, IRRs for regional compared with local stage among Whites (IRR, 0.58) and JAHI (IRR, 0.41) were lower than among Blacks (IRR, 0.77) and Osaka (IRR, 0.77). Whites (IRR, 0.86) and JAHI (IRR, 0.60) also had lower rates of high-grade tumors compared with low-grade tumors. In contrast, Blacks were more likely to be diagnosed with high-grade tumors (IRR, 1.46). Grade data for Osaka could not be interpreted given that 77.7% of cases were coded as missing or unknown. All groups had lower rates of lobular carcinoma than ductal carcinoma not otherwise specified. However, due to potential inconsistencies between the coding systems used in the United States and Japan, results should be interpreted with caution. All IRRs in Table 1 were statistically significantly different from 1.00 at the 95% confidence level.

Age-Adjusted Incidence Rates. Breast cancer incidence rates increased among all four groups from the earliest calendar-period 1978-1982 to the latest calendar-period 1993-1997 (Table 1; Fig. 1A). The most rapid increase in rates was observed in JAHI; the age-adjusted rate increased 75%, from 51.1 to 89.2 per 100,000 woman-years (IRR, 1.75). The slowest increase was observed in Whites (IRR, 1.27). Increases were intermediate among Blacks (IRR, 1.37) and Osaka (IRR, 1.51).

Table 1. Breast cancer incidence among Whites, Blacks, and JAHI in the United States (nine SEER areas) and Osaka, Japan during the years 1978 to 1997

	SEER (n = 260,571)						Osaka (n = 25,350)	
	White		Black		JAHI		Osaka	
Number (n)	236,130		21,137		3,304		25,350	
Mean age in years (SE)	63 (0.03)		57.7 (0.10)		61.0 (0.22)		53.5 (0.08)	
Median age in years	64		57		62		51	
Overall rate (SE)	87.6 (0.19)		80.0 (0.56)		72.4 (1.34)		21.8 (0.14)	
	n	Rate SE IRR	n	Rate SE IRR	n	Rate SE IRR	n	Rate SE IRR
Year of diagnosis								
1978-1982	45,152	74.3 0.37 ref	3,481	64.9 1.12 ref	458	51.1 2.47 ref	4,170	16.9 0.26 ref
1983-1987	56,999	87.8 0.39 1.18	4,780	78.0 1.16 1.20	717	65.7 2.59 1.29	5,868	21.1 0.28 1.25
1988-1992	64,519	92.7 0.39 1.25	5,847	84.1 1.14 1.30	961	76.7 2.71 1.50	6,956	22.7 0.28 1.34
1993-1997	69,460	94.0 0.38 1.27	7,029	88.6 1.10 1.37	1,168	89.2 2.91 1.75	8,356	25.5 0.29 1.51
Age at diagnosis								
<50	48,651	29.5 0.02 ref	7,010	31.7 0.12 ref	683	29.0 0.99 ref	11,137	12.5 0.01 ref
50+	187,479	319.9 0.13 0.84	14,127	272.8 1.11 8.61	2,621	245.8 5.03 8.48	14,213	59.1 0.05 4.73
Summary stage								
Local	137,799	50.6 0.15 ref	10,192	38.6 0.39 ref	2,263	48.8 1.10 ref	12,721	11.0 0.10 ref
Regional	75,191	29.1 0.11 0.58	7,841	29.9 0.35 0.77	864	20.0 0.72 0.41	9,771	8.5 0.09 0.77
Distant	13,940	5.1 0.05 0.10	1,996	7.5 0.17 0.19	138	2.9 0.27 0.06	1,453	1.2 0.03 0.11
Other/unknown	9,200	2.7 0.03 0.05	1,108	3.8 0.12 0.10	39	0.6 0.12 0.01	1,405	1.2 0.03 0.11
Grade								
Low (I-II)	61,962	22.7 0.10 ref	4,194	16.0 0.25 ref	1,167	24.4 0.77 ref	5,530	4.8 0.07 ref
High (III-IV)	50,910	19.6 0.09 0.86	6,109	23.3 0.31 1.46	661	14.6 0.61 0.60	119	0.1 0.01 0.02
Other/unknown	123,258	45.2 0.14 1.99	10,834	40.7 0.40 2.54	1,476	33.4 0.92 1.37	19,701	16.9 0.12 3.52
Histology								
Duct NOS	183,553	68.1 0.17 ref	16,439	62.2 0.50 ref	2,839	62.2 1.24 ref	7,821	6.7 0.08 ref
Lobular	17,384	6.3 0.05 0.09	972	3.7 0.12 0.06	102	2.2 0.23 0.04	390	0.3 0.02 0.04
Other/unknown	35,193	13.2 0.08 0.19	3,726	14.0 0.24 0.23	363	8.0 0.45 0.13	17,139	14.8 0.11 2.21

NOTE: Rates per 100,000 woman-years, age-adjusted to the World Standard; ref, referent group; all rate ratios were statistically significantly different from the referent group at the 95% confidence level; duct NOS, ductal carcinoma not otherwise specified (histology codes 8000, 8500, 8010, and 8140); lobular carcinoma (histology code 8520).

Age-Specific Incidence Rates. Overall age-specific rates for the calendar-period 1978-1997 increased rapidly until age 50 years then continued to increase more slowly among Whites, Blacks, and JAHI (Fig. 1B). In contrast, rates increased rapidly until age 50 years then flattened or plateaued among women in Osaka. Poisson regression analyses confirmed significant changes in slope at age 50 years for all race/ethnicity groups ($P < 0.001$).

The differences in age-specific incidence rate patterns persisted after adjusting for calendar-period and birth-cohort effects using age-period cohort-models. However, there were statistically significant ($P < 0.05$) birth-cohort effects in all populations. Among Whites and Blacks, there were significant effects for birth-cohorts (referred to by the mid-year of birth) 1918, 1938, and 1943. Among Blacks, the 1908 birth-cohort effect was also significant. Among JAHI, there were significant effects for birth-cohorts 1903, 1923, and 1943. In Osaka, we observed several more significant birth-cohort contrasts, i.e., for 1918, 1928, 1933, 1943, and 1948.

Among Whites and Blacks, cross-sectional age-specific rates for all calendar-periods increased rapidly until age 50 years then continued to increase more slowly after age 50 years (Fig. 2A and B). Rates among JAHI more closely resembled rates among native Japanese in Osaka for the earliest calendar-period (1978-1982), but were more like rates among Whites and Blacks for subsequent calendar-periods (Fig. 2C). Age-specific rates in Osaka increased rapidly until age 50 years then plateaued for all calendar-periods (Fig. 2D).

Longitudinal age-specific rates were presented for 8 of the 15 birth-cohorts (for clearer graphical depiction; the overall interpretation of the results was the same irrespective of which birth-cohort were plotted; Fig. 3). Similar to cross-sectional age-specific rates (Fig. 2), longitudinal age-specific rates among Whites and Blacks increased rapidly until age 50 years then continued to increase at a slower pace with successive birth-cohort (Fig. 3A and B). Age-specific rates in Osaka increased rapidly until age 50 years then tended to plateau, although rates for individual birth-cohorts were not completely flat (Fig. 3D). Rates for JAHI were intermediate to the patterns for Whites, Blacks, and Osaka (Fig. 3C).

Age Distributions. The age density plots varied during the study period 1978 to 1997 according to race/ethnicity group

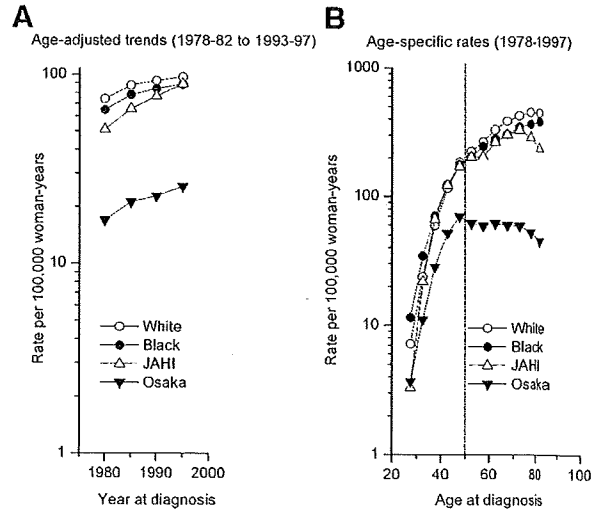

Figure 1. Breast cancer incidence rates among Whites, Blacks, and JAHI in the United States (nine SEER areas) and Osaka, Japan. **A**, trends in age-adjusted rates by calendar-period (1978-1982, 1983-1987, 1988-1992, and 1993-1997). **B**, age-specific rates for the study period 1978 to 1997.

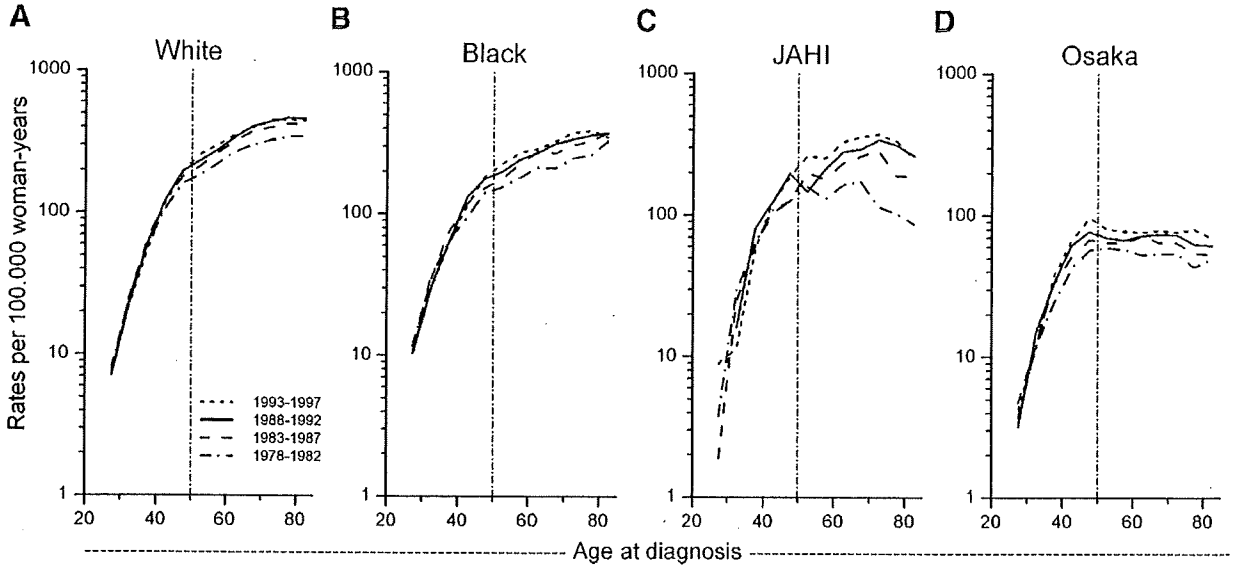

Figure 2. Observed cross-sectional age-specific breast cancer incidence rates by calendar-period (1978-1982, 1983-1987, 1988-1992, and 1993-1997). **A**, Whites in SEER. **B**, Blacks in SEER. **C**, JAHI in SEER. **D**, native Japanese in Osaka, Japan.

(Fig. 4). Although the relative proportions of early-onset and late-onset breast cancer types varied, the peak frequencies (or modes) were generally constant near ages 50 (early-onset) and 70 (late-onset) years. In the earliest calendar-period (1978-1982), the age distributions had single modes among all populations (Fig. 4, *first column*). Osaka had the earliest mode (age 46 years), followed by JAHI (54 years), Blacks (58 years), and Whites (63 years). By the latest calendar-period (1993-1997), a bimodal pattern had emerged among Whites, Blacks, and JAHI (Fig. 4, *fourth column*), although the late-onset peak was not as prominent among Blacks. Among Whites, the modes were at ages 51 and 71 years. Among Blacks, the modes were at ages 48 and 71 years. Among JAHI, the modes were at ages 52 and 68 years. In contrast with the other three groups, Osaka maintained a single early-onset age distribution during the latest calendar-period (Fig. 4, *fourth row*), with a mode at age 48 years.

Discussion

Although age-adjusted breast cancer incidence rates increased in both SEER and Osaka from 1978-1982 to 1993-1997, age-specific patterns (rates and age distributions) suggest that the nature of this increase differed among the various cancer populations (Fig. 4). In SEER, Whites and Blacks had bimodal (early-onset and late-onset) breast cancer populations. In Osaka, there was a consistent early-onset age distribution of the breast cancer population. JAHI were intermediate to Whites and Blacks, and Osaka. The early-onset age distribution in Osaka was observed despite having an older general population. For example, according to the 2000 census data, the median age for women in Osaka Prefecture was older (41.3 years) than for Whites (39.7 years) and Blacks (31.5 years) in the United States (34-36).

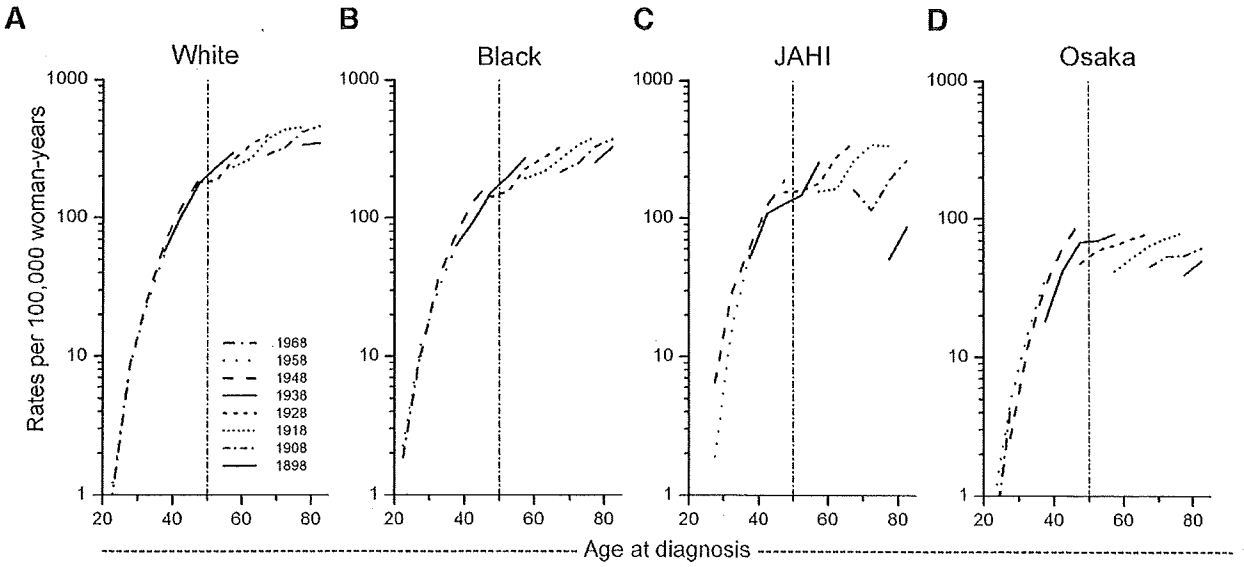

Figure 3. Observed longitudinal age-specific breast cancer incidence rates by the mid-year of birth-cohort (1898, 1908, 1918, 1928, 1938, 1948, 1958, and 1968). **A**, Whites in SEER. **B**, Blacks in SEER. **C**, JAHI in SEER. **D**, native Japanese in Osaka, Japan.

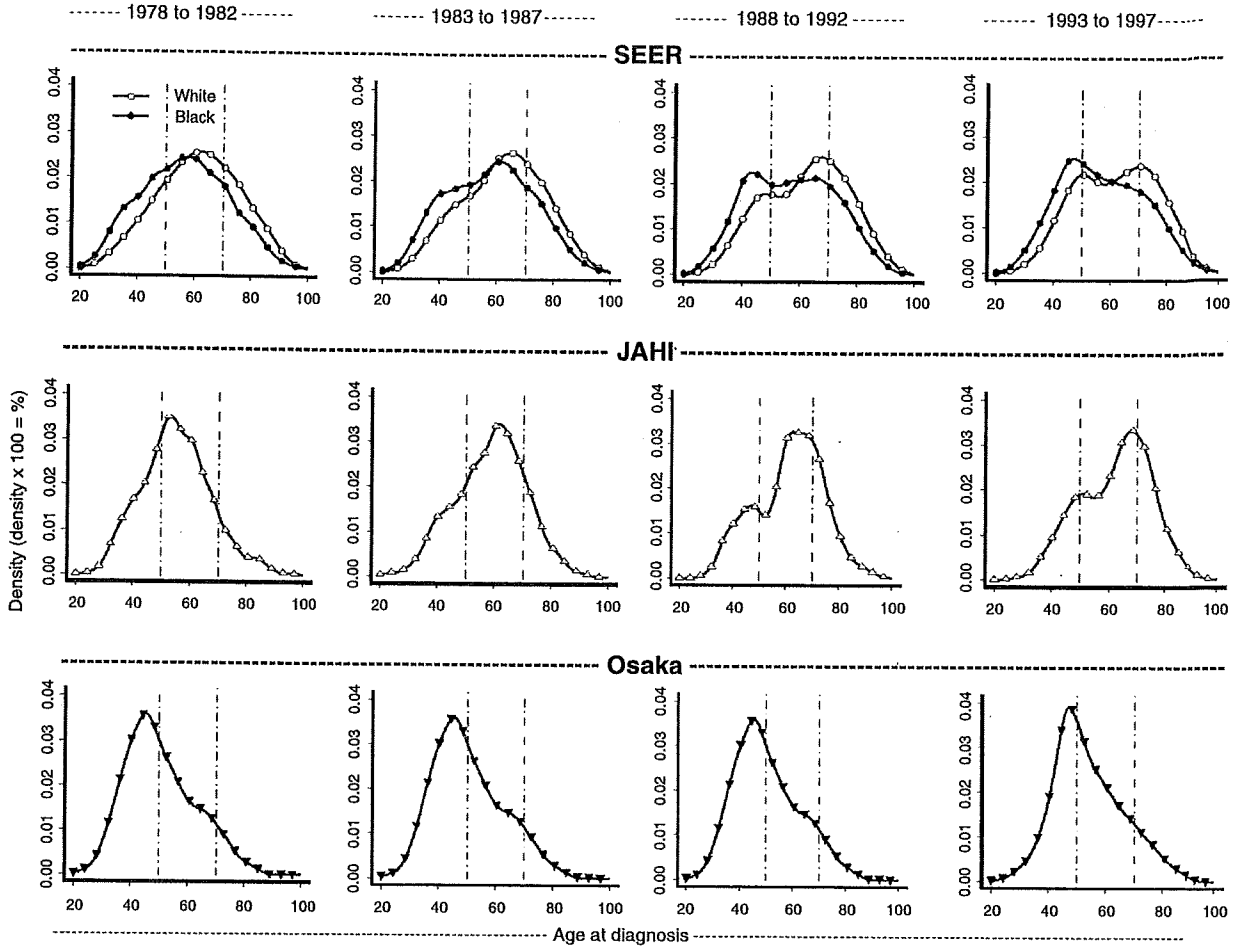

Figure 4. Age distributions among Whites, Blacks, and JAHI in the United States (nine SEER areas) and Osaka, Japan during the calendar-periods 1978 to 1982, 1983 to 1987, 1988 to 1992, and 1993 to 1997. The probability density function is a smoothed estimate of the frequency of women diagnosed at a given age. Reference lines are shown for ages 50 and 70 y.

The changing age distributions among the various breast cancer populations also seemed to affect the shape of the age-specific incidence rate curves. Increasing late-onset breast cancer populations among Whites, Blacks, and JAHI corresponded with successively steeper slopes in the age-specific rates after age 50 years. On the other hand, a constant early-onset age distribution for Osaka corresponded to consistently flattened or plateaued age-specific incidence rates after age 50. Although we observed significant birth-cohort effects for all populations, the age effects were 10-fold higher, and the age-period-cohort–fitted age curves were very similar to the overall (unadjusted) age-specific rate curves (data not shown). This supports that age effects and differences between populations cannot be explained by birth-cohort or calendar-period artifacts.

Although the differences in overall breast cancer incidence rates among Occidental and Asian populations have been often attributed to environmental exposures and/or lifestyle (37), our results suggest important age-specific differences as well. For example, body mass index is inversely associated with premenopausal breast cancer, but is positively associated with postmenopausal breast cancer (38, 39). The much lower prevalence of obesity (defined as body mass index of 30 kg/m^2 or more) in Japan than in the United States (40, 41) may in part explain why the IRRs for postmenopausal relative to premenopausal breast cancer were highest in SEER and lowest in Osaka.

Geographic variations in screening mammography may also explain some of the differences between SEER and Osaka. For example, in the United States, screening mammography became widely implemented during the 1980s, and the coverage rate among women age 40 years and older in 2000 was estimated to be >70% (42). In this study, cross-sectional age-specific incidence rates in SEER increased for all calendar-periods, particularly among women age 50 years and older, i.e., those targeted for screening. The same effect has been shown previously in the United States (43) and in several European countries where screening mammography has been fully established (44, 45). In contrast, screening mammography was not implemented in Japan until the year 2000 (46). In the absence of screening mammography, the age-specific incidence rates in Osaka increased relatively evenly across both younger and older ages for each succeeding calendar-period.

This study is not without limitations. Because we have used registry data, key considerations include the completeness and accuracy of data. Moreover, we obtained data from a number of different sources, so results should be interpreted carefully, as comparability across populations can be hindered by differences in screening practices, disease classification, and data collection. However, both SEER and the OCR meet IARC standards, which ensure a certain degree of data quality and comparability based on a number of factors (47). Indeed, in all groups, >94% of cases were microscopically confirmed in each

5-year calendar-period (1978-1982 to 1993-1997); and the proportion of death certificate only cases was <1% in SEER and 7% in Osaka.

In sum, although breast cancer incidence rates in SEER and Osaka have increased over time, the emergence of a late-onset peak observed in SEER was absent in Osaka. These distinct age-specific patterns may reflect differences in detection, but may also be due to the differential effect of certain age-related exposures. Further hypothesis-driven studies are needed to distinguish the age effects from calendar-period and/or birth-cohort effects on geographic variations in breast cancer patterns. More specifically, studies are needed to further assess whether established risk factors are differentially associated with early- and late-onset breast cancer and the effect this may have on breast cancer patterns worldwide.

Appendix A

The aim of kernel smoothing is to nonparametrically estimate a continuous probability density function f, defined as the derivative of a cumulative probability distribution function. Although a histogram provides such a nonparametric estimate of f, it is not smooth, and is also very dependent on the width and starting points of the intervals or bins. Kernel smoothing avoids both problems. The kernel density estimator for observed data points $x_1, \ldots, x_n$ is of the form

$$\hat{f} = \frac{1}{n}\sum_{i=1}^{N} K(x - x_i; h),$$

where K is a probability density function, known as the kernel function, the variance of which is controlled by h. The variable h is often referred to as the bandwidth or smoothing variable, as it dictates the smoothness of $\hat{f}$, with larger values of h corresponding to smoother curves.

We use a Gaussian kernel, $K(x) = \frac{1}{h\sqrt{2\pi}} exp(-x^2/2h)$ implemented in S-PLUS (31-33).

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ORIGINAL REPORT

Plasma Isoflavone Level and Subsequent Risk of Breast Cancer Among Japanese Women: A Nested Case-Control Study From the Japan Public Health Center-Based Prospective Study Group

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ABSTRACT

Purpose
Because they have large variations in consumption, Asian countries are suitable settings for studies of the effect of relatively high-dose isoflavone intake on breast cancer risk. Nevertheless, no prospective study from Asia has assessed blood or urine levels as biomarkers of isoflavone intake.

Patients and Methods
A total of 24,226 women ages 40 to 69 years in the Japan Public Health Center–based prospective study who responded to the baseline questionnaire and provided blood in 1990 to 1995 were observed to December 2002. During a mean 10.6 years of follow-up, 144 patients newly diagnosed with breast cancer were identified. Two matched controls for each patient were selected from the cohort. Isoflavone levels were assessed by plasma level and food frequency questionnaire, and the odds ratio of breast cancer according to isoflavone level was estimated using a conditional logistic regression model.

Results
We found a statistically significant inverse association between plasma genistein and risk of breast cancer, but no association for plasma daidzein. Adjusted odds ratios for the highest versus lowest quartile of plasma level were 0.34 for genistein (95% CI, 0.16 to 0.74; *P* for trend, .02) and 0.71 for daidzein (95% CI, 0.35 to 1.44; *P* for trend, .54). Median plasma genistein values in the control group were 31.9 ng/mL for the lowest and 353.9 ng/mL for the highest quartile groups. Regarding dietary intake of isoflavones, nonsignificant inverse associations were observed for both genistein and daidzein.

Conclusion
This nested case-control study found an inverse association between plasma genistein and the risk of breast cancer in Japan.

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INTRODUCTION

Soy foods, a traditional staple dish in Asian countries, are a primary source of isoflavones, such as genistein and daidzein, which are classified as phytoestrogens. Because breast cancer risk is substantially lower in Asian than Western countries,[1] the contribution of a high isoflavone intake to low breast cancer risk has been hypothesized.[2] This hypothesis has been supported by in vitro studies at high genistein concentrations and in the majority of animal studies, which together have demonstrated various anticancer effects of isoflavones acting via both estrogen-dependent and -independent mechanisms.[3,4] Estrogen-dependent mechanisms arise through the mediation of estrogen receptor α and β, owing to the similar chemical structure of isoflavones to the human estrogen hormone and their binding affinity to estrogen receptors.[4,5] For this reason, they have been hypothesized to behave like selective estrogen receptor modulators. In contradiction to potential protective effects, however, genistein exhibits estrogenic properties at low concentrations, which could theoretically enhance breast cancer risk.[3,4] In fact, some animal studies have reported that genistein stimulates tumor development and growth.[6,7] Although a recent meta-analysis found that soy intake was associated with a

small reduction in breast cancer risk, the authors concluded that in view of these risk-enhancing effects, recommendations for high-dose isoflavone supplementation to prevent breast cancer or its recurrence were premature.[8] Phytoestrogen supplements, however, are commercially marketed for use by postmenopausal women as natural and safe alternatives to hormone replacement therapy. The effect of relatively high-dose isoflavone on breast cancer risk is now of concern.

Because they have large variations in consumption among individuals, Asian countries serve as suitable venues for studies of the effect of relatively high-dose isoflavone intake on breast cancer risk. Despite this advantage, only a few epidemiological studies on soy or isoflavone intake and breast cancer risk from Asia have been reported.[9] In particular, no prospective study on isoflavone levels in blood or urine samples has been reported, notwithstanding that, because they are partly determined by individual differences in absorption and metabolism, blood or urine levels might better reflect interperson differences than dietary assessment. The three nested case-control studies which have investigated this association in Western populations have been inconsistent, with one reporting an inverse association with plasma genistein in the Netherlands,[10] the second showing no association with urinary genistein in the Netherlands,[11] and the third finding a positive association with urine and serum phytoestrogens in the United Kingdom.[12] This inconsistency might be in part explained by the apparently small variation in isoflavone levels in Western countries. For example, studies in the Netherlands, which has a high incidence of breast cancer (age-standardized rate per 100,000 world population, 86.7 in 2002),[13] reported a median genistein intake of 0.14 mg/d in women ages 49 to 70 years,[14] and a median plasma genistein level of 4.89 ng/mL in the control group of a nested-case control study.[10] In contrast, a study in Japan, where the incidence of breast cancer is low (age-standardized rate per 100,000 world population, 32.7 in 2002),[13] reported a median genistein intake of 22.3 mg/d and median serum level of 90.2 ng/mL.[15] This substantial variation in isoflavone levels suggests that the Japanese population represents an ideal setting for determining whether an association exists at relatively high levels achievable from dietary intake only.

Herein, to clarify the effect of relatively high-dose isoflavone exposure on breast cancer risk, we conducted a nested case-control study within a large-scale population-based prospective study in Japan.

PATIENTS AND METHODS

Study Population

The Japan Public Health Center–based prospective study, which began in 1990 for cohort I and in 1993 for cohort II, included 140,420 subjects (68,722 men and 71,698 women) living in the municipalities supervised by 11 public health centers (PHC). Details of the study design have been described elsewhere.[16] The study protocol was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

The study population comprised registered Japanese inhabitants living in each PHC area, ages 40 to 59 years in cohort I and 40 to 69 years in cohort II. In this analysis, one PHC area was excluded since data on cancer incidence were not available. We thus defined a population-based cohort of 67,426 women (27,389 in cohort I and 40,037 in cohort II) after the exclusion of ineligible subjects (n = 95).

Questionnaire Survey

A baseline survey was conducted from 1990 to 1994. A total of 55,891 women (83%) returned the questionnaire, which contained questions concerning demographic characteristics, medical history, menstrual and reproductive history, anthropometric factors, physical activity, smoking and drinking habits, and diet.

Blood Collection

Subjects voluntarily provided 10 mL of blood during health check-ups from 1990 to 1995. Blood samples were divided into plasma and buffy layers and stored at −80°C until analysis. Among respondents to the baseline questionnaire, a total of 24,996 women (45%) donated blood.

Follow-Up

All registered subjects were observed from the start of the study period to December 31, 2002. Data on residential relocation were obtained from residential registries. Among study subjects (n = 24,996), 1,289 subjects (5.2%) moved out of the study area and 5 (0.02%) were lost to follow-up within the study at-risk period.

Selection of Patients and Controls

Incidence data on breast cancer were collected for the Japan Public Health Center cancer registry through two data sources—major local hospitals and population-based cancer 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 (Appendix A1, online only) using the International Classification of Diseases for Oncology, third edition, code C500-509. Up to the end of the study period, 144 new breast cancer cases (97 in cohort I and 47 in cohort II) were identified among the 24,226 women (9,689 in cohort I and 14,537 in cohort II) who had returned the baseline questionnaire, reported no history of breast cancer or ovarian cystoma, and provided blood samples. Diagnosis was microscopically verified in 98% of patients, and based on death certificates only in 0.7%. The mortality/incidence ratio was 0.14.

For each patient, two controls were selected using incidence density sampling from subjects who were not diagnosed with breast cancer during the follow-up period when the patient was diagnosed. Control selection was done without reference to incidence of other cancer sites. Controls were matched with each patient for age (within 3 years), PHC area, area (city or town and village), date of blood collection (within 90 days), time of day of blood collection (within 3 hours), fasting time at blood collection (within 3 hours), and baseline menopausal status.

Assessment of Dietary Intake

Dietary intakes of genistein and daidzein were assessed by a food frequency questionnaire of 44 items for cohort I and 52 for cohort II. Isoflavone intake was defined for this study as the sum of genistein and daidzein intake. We documented the questionnaire assessment of isoflavone intake to be reasonably valid (details in Appendix A1).[15,17]

Laboratory Assay

Plasma levels of isoflavone were analyzed using high-performance liquid chromatography with a coulometric array detector in accordance with the modified methods of Gamache and Acworth.[18] Concentrations of genistein and daidzein were determined by linear regression of the peak height for each standard, and adjusted according to the recovery rate of the internal plasma standard. The regression coefficient of peak height and concentration calculated for isoflavones revealed a linearity range of 0 to 0.75 μg/mL, with correlation coefficient values higher than 0.938. Voltametric response for the standard solution displayed coefficients of variation of 8% for intra- and 11% for interday variation. Recovery rates of isoflavones in plasma samples ranged between approximately 73% and 98%. Detection limits were 2.2 ng/mL for genistein and 2.7 ng/mL for daidzein. Laboratory personnel were blinded to case-control status when performing the analyses.

Statistical Analysis

Comparison of baseline characteristics, as well as plasma levels and dietary intake of isoflavones, between cases and controls was evaluated by the Mantel-Haenszel test using matched-set strata. Spearman's correlation coefficients were calculated among plasma levels and dietary intakes of isoflavone

among control subjects. Using a conditional logistic regression model, we calculated odds ratios (ORs) and 95% CIs of breast cancer for plasma levels and dietary intake of isoflavone divided into quartiles based on control distribution. The ORs were adjusted for number of births and age at first birth as potential confounders. The adjusted ORs were calculated based on a total of 405 subjects with complete information for covariates. Linear trends for ORs were tested in the conditional logistic regression model using the exposure categories as ordinal variables. All P values reported are two sided, and significance level was set at $P < .05$. All statistical analyses were performed with SAS software, version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Case subjects and controls had significantly different distribution for number of births (Table 1). Other characteristics, such as age at menarche, age at first birth, body mass index (BMI), alcohol consumption, or dietary intake did not substantially differ between the two groups.

Plasma genistein was significantly lower among cases than controls whereas plasma daidzein values were similar (Table 2). No significant differences between the groups were seen for dietary genistein, daidzein, or isoflavone intake. Median isoflavone intake in the control group was 34.8 mg/d (36.1 in cohort I and 29.9 mg/d in cohort II). Genistein and daidzein were highly correlated for both plasma level ($r = 0.72$) and dietary intake ($r = 0.99$). Correlation coefficients between plasma and dietary levels were relatively low for both genistein ($r = 0.23$) and daidzein ($r = 0.31$).

We found a statistically significant inverse association between plasma genistein and the risk of breast cancer (P for trend, .02), but no statistically significant association for plasma daidzein (P for trend, .54; Table 3). Adjusted ORs for the highest versus lowest quartile of plasma level were 0.34 for genistein (95% CI, 0.16 to 0.74; $P \leq .01$) and 0.71 for daidzein (95% CI, 0.35 to 1.44; $P = .34$). Moreover, the results did not change substantially after adjustment for dietary intake of isoflavone or other potential confounders such as age at menarche, menopausal status at baseline, age at menopause, height, BMI, and alcohol consumption. Further, exclusion of cases diagnosed before the first 3 years of follow-up did not substantially change the results, nor did the exclusion of subjects who used vitamin supplements or who provided a nonfasting blood sample (ie, within 6 hours after a meal). Regarding dietary intake, we observed inverse associations for both genistein and daidzein but neither was statistically significant (Table 3). In addition, adjusted ORs by isoflavone intake were closely similar to those by genistein intake (data not shown).

A stratified analysis according to baseline menopausal status showed no remarkable difference between two strata for either genistein and daidzein, regardless of whether the values were assessed by plasma or questionnaire, although the inverse association between plasma genistein and risk of breast cancer tended to be more stable in postmenopausal than premenopausal women (Table 4).

Table 1. Characteristics of Patients and Matched Control Subjects at Baseline

Characteristic	Patients (n = 144) No.	%	Controls (n = 288) No.	%	P*
Mean age, years	51.7		51.8		
Standard deviation	7.1		7.1		—
Family history of breast cancer	2	1.4	2	0.7	.48
Premenopausal women	59	42	118	42	—
Postmenopausal women					
Natural menopause	70	50	140	50	—
Surgical menopause	10	7.2	20	7.2	—
Mean age at menopause, years	50.0		49.8		.76
SE†	0.38		0.27		
Mean age at menarche, years	14.6		14.8		.33
SE†	0.15		0.10		
Mean No. of births	2.3		2.8		.01
SE†	0.12		0.09		
Mean age at first birth, years	25.7		25.0		.22
SE†	0.30		0.21		
Use of exogenous female hormones (current use)	4	3.0	2	0.8	.10
Mean height, cm	151.7		151.4		.70
SE†	0.46		0.33		
Mean body mass index, kg/m^2	23.4		23.5		.49
SE†	0.25		0.18		
Smoking (current smoker)	5	3.5	17	5.9	.23
Alcohol drinking (regular drinker)	18	13	26	9.1	.28
Leisure-time physical activity (≥ once per week)	30	21	57	20	.42
Vitamin supplement user	33	24	61	23	.65
Green tea intake (≥ five cups per day)	36	25	71	25	.42
Mean total energy intake, kcal/d	1,269.4		1,271.0		.41
SE‡	26.5		19.2		
Mean fish and shellfish intake, g/d	45.4		45.7		.75
SE‡	2.5		1.8		
Mean meat intake, g/d	30.5		28.5		.15
SE‡	1.7		1.2		
Mean vegetable intake, g/d	121.2		115.9		.20
SE‡	5.7		4.1		
Mean fruit intake, g/d	104.8		99.4		.79
SE‡	5.9		4.3		

*P for Mantel-Haenszel test with matched-set strata.
†Adjusted for age.
‡Adjusted for age and cohort.

DISCUSSION

In this study, we found a statistically significant inverse association between plasma genistein and the risk of breast cancer, but no association for plasma daidzein. This finding suggests that genistein may

Table 2. Plasma Levels and Dietary Intake of Isoflavone in Patients and Matched Controls

Parameter	Patients (n = 144) Median	Interquartile Range	Controls (n = 288) Median	Interquartile Range	P*
Plasma level					
Genistein, ng/mL	131.8	67.9-202.6	144.5	78.8-255.6	.046
Daidzein, ng/mL	16.7	7.0-34.0	17.9	5.5-40.8	.45
Dietary intake					
Genistein, mg/d	19.9	16.6-24.0	21.7	16.8-26.1	.37
Daidzein, mg/d	12.5	10.1-14.8	13.3	10.3-16.3	.36
Isoflavone, mg/d†	32.5	26.8-38.7	34.8	27.0-42.4	.36

*P for Mantel-Haenszel test with matched-set strata.
†Isoflavone intake = sum of genistein and daidzein intake.

Table 3. ORs and 95% CIs of Breast Cancer According to Plasma Level and Dietary Intake of Isoflavone

Parameter	Quartile 1	2	3	4	*P* for trend
Plasma level					
Median genistein, ng/mL	31.9	108.1	190.8	353.9	
No. of patients	41	37	45	21	
No. of controls	72	72	72	72	
OR	1.00	0.84	1.04	0.46	.07
95% CI	Reference	0.47 to 1.51	0.57 to 1.91	0.23 to 0.91	
Adjusted OR*	1.00	0.69	0.87	0.34	.02
95% CI	Reference	0.36 to 1.32	0.45 to 1.67	0.16 to 0.74	
Median daidzein, ng/mL	0	12.0	27.0	53.7	
No. of patients	30	45	44	25	
No. of controls	72	72	72	72	
OR	1.00	1.50	1.44	0.79	.59
95% CI	Reference	0.85 to 2.64	0.80 to 2.61	0.41 to 1.54	
Adjusted OR*	1.00	1.30	1.51	0.71	.54
95% CI	Reference	0.70 to 2.42	0.80 to 2.86	0.35 to 1.44	
Dietary intake					
Median genistein, mg/d	15.7	18.5	22.9	27.3	
No. of patients	42	36	37	29	
No. of controls	69	75	71	73	
OR	1.00	0.78	0.83	0.58	.15
95% CI	Reference	0.46 to 1.35	0.47 to 1.48	0.30 to 1.12	
Adjusted OR*	1.00	0.81	0.92	0.58	.21
95% CI	Reference	0.46 to 1.45	0.50 to 1.70	0.29 to 1.18	
Median daidzein, mg/d	9.4	11.4	14.1	17.1	
No. of patients	40	39	35	30	
No. of controls	70	74	72	72	
OR	1.00	0.91	0.82	0.65	.21
95% CI	Reference	0.52 to 1.58	0.46 to 1.47	0.33 to 1.27	
Adjusted OR*	1.00	0.96	0.94	0.67	.34
95% CI	Reference	0.54 to 1.74	0.50 to 1.74	0.33 to 1.39	

Abbreviation: OR, odds ratio.
*Adjusted for number of births (0, 1, 2, 3, 4, 5+) and age at first birth (−21, 22-25, 26-29, 30+, nulliparous). Adjusted ORs were calculated based on a total of 405 subjects with complete information of covariates.

play a more important role in the etiology of breast cancer than daidzein. Our findings are in general agreement with those of a recent nested case-control study in the Netherlands,[10] albeit that our inverse association occurred at substantially higher plasma concentrations. For example, median plasma genistein values in the control group of the Netherlands study were 3.75 ng/mL for premenopausal and 4.89 ng/mL for postmenopausal women.[10] In contrast, the median value in our control group was 144.5 ng/mL, and only 3.2% of control subjects was under 5 ng/mL. This apparently high level is not surprising considering that the median value of 353.9 ng/mL in our highest plasma genistein quartile group, which had a significantly lower risk of breast cancer than the lowest group, corresponded to a median dietary intake of 28.5 mg/d for genistein and 46.5 mg/d for isoflavone, as estimated by the validation study data. Although some in vivo and in vitro studies have shown risk-enhancing effects of genistein, our study suggests that relatively high-dose isoflavones exposure achievable from dietary intake alone is associated with a decreased rather than increased risk.

We observed an approximately 65% reduction in breast cancer risk in the highest plasma genistein quartile group but no decrease in the other quartiles, indicating that only the highest group benefited from risk reduction. The apparent lack of a dose-response relationship might imply the presence of a threshold level of effect. Interestingly, this idea contradicts findings in Western populations, in whom inverse associations are seen despite materially low levels of isoflavones. Given the differences in hormonal milieu between the two populations, the potential protective effect of isoflavones in breast cancer might act differently between Western and Asian populations: sex hormone levels are higher in Western than Asian women,[19] for example, as is the prevalence of obesity.[20,21] In this regard, a case-control study in Shanghai found that the inverse association between urinary isoflavone level and breast cancer risk was stronger among women in the high BMI, waist-hip ratio, and estradiol level groups and in the low sex hormone-binding globulin level group than in the respectively converse low and high groups.[22] Alternatively, the apparent lack of a dose-response relationship might merely reflect uncontrolled confounding by other dietary characteristics or risk-lowering behaviors.

The reason for a role for genistein but not daidzein in the etiology of breast cancer is unclear, but several possibilities can be speculated. Genistein possesses stronger binding affinity for estrogen receptor than daidzein.[5] Further, a pharmacokinetic study showed higher plasma levels and a 1.5-fold longer half-life for genistein than daidzein

Table 4. ORs and 95% CIs of Breast Cancer According to Plasma Level and Dietary Intake of Isoflavone By Baseline Menopausal Status

Parameter	Quartile 1	2	3	4	*P* for trend
Premenopausal women					
Plasma genistein, ng/mL					
No. of patients	24	14	19	2	
No. of controls	41	28	25	24	
Adjusted OR*	1.00	0.76	1.75	0.14	.20
95% CI	Reference	0.31 to 1.86	0.68 to 4.50	0.03 to 0.69	
Plasma daidzein, ng/mL					
No. of patients	17	21	15	6	
No. of controls	27	45	23	23	
Adjusted OR*	1.00	0.80	1.27	0.49	.48
95% CI	Reference	0.34 to 1.88	0.48 to 3.38	0.15 to 1.57	
Dietary genistein intake, mg/d					
No. of patients	21	16	14	8	
No. of controls	35	31	32	20	
Adjusted OR*	1.00	0.92	0.86	0.62	.43
95% CI	Reference	0.41 to 2.05	0.34 to 2.18	0.21 to 1.84	
Dietary daidzein intake, mg/d					
No. of patients	20	17	14	8	
No. of controls	36	30	32	20	
Adjusted OR*	1.00	1.07	0.93	0.67	.53
95% CI	Reference	0.46 to 2.51	0.37 to 2.34	0.22 to 2.03	
Postmenopausal women					
Plasma genistein, ng/mL					
No. of patients	17	23	25	15	
No. of controls	28	41	46	45	
Adjusted OR*	1.00	0.54	0.57	0.36	.10
95% CI	Reference	0.18 to 1.62	0.20 to 1.65	0.12 to 1.12	
Plasma daidzein, ng/mL					
No. of patients	13	23	27	17	
No. of controls	40	27	47	46	
Adjusted OR*	1.00	2.86	2.06	1.16	.95
95% CI	Reference	1.03 to 7.98	0.82 to 5.17	0.43 to 3.15	
Dietary genistein intake, mg/d					
No. of patients	20	20	22	18	
No. of controls	33	42	35	50	
Adjusted OR*	1.00	0.73	0.93	0.52	.31
95% CI	Reference	0.30 to 1.77	0.38 to 2.27	0.19 to 1.42	
Dietary daidzein intake, mg/d					
No. of patients	19	22	20	19	
No. of controls	33	42	36	49	
Adjusted OR*	1.00	0.89	0.93	0.64	.43
95% CI	Reference	0.38 to 2.10	0.38 to 2.29	0.23 to 1.72	

Abbreviation: OR, odds ratio.
*Adjusted for number of births (0, 1, 2, 3, 4, 5+) and age at first birth (−21, 22-25, 26-29, 30+, nulliparous).

after ingestion of baked soybean powder containing closely similar amounts of the two.[23] Moreover, the absence of an association for plasma daidzein might be attributable to misclassification arising from the metabolization of this compound. Daidzein can be metabolized by intestinal bacteria to equol and O-desmethylangolites; because approximately only 30% to 50% of individuals are capable of equol production, probably due to differences in gut microflora, daidzein-to-equol metabolizers may have lower plasma daidzein levels than nonmetabolizers.[24] Equol has been suggested to have greater biologic activity than daidzein,[24] and an inverse association between equol level and breast cancer risk has been reported.[25] Here, the lowest plasma daidzein quartile group might conversely have had a lower breast cancer risk than the higher groups due to its inclusion of equol metabolizers, and such misclassification, if present, would lead to a null result.

Our study has several methodological advantages over previous studies of isoflavones and the risk of breast cancer. First, the direct measurement of plasma isoflavone levels provides not only an index of intake but also of the absorption and metabolism of isoflavone, an understanding of which is important to elucidating the mechanisms by which isoflavones might influence breast cancer development. Indirect measurement by dietary intake of genistein is likely a major reason for the present smaller and nonsignificant risk reduction of breast cancer than by plasma genistein. Exposure assessment using

blood samples is therefore likely a more sophisticated means of detecting an association. Second, two case-control studies in Australia and China showed an inverse association between urinary isoflavones and breast cancer risk.[25,26] In view of the retrospective design of these studies, however, blood or urine levels of isoflavones in breast cancer cases might have been influenced by metabolic changes after the breast cancer was detected or by altered eating habits among case subjects. In our nested case-control study within a prospective cohort, in contrast, blood samples were collected before cancer diagnosis, obviating any potential bias due to the presence of cancer. Third, cases and controls were selected from the same cohort, thereby avoiding the selection bias inherent to case-control studies.

Several limitations of this study warrant mention. First, we measured plasma isoflavones only once for each individual. The consumption of soy foods is a personal dietary preference, and intake levels of most individuals are assumed to be relatively stable over time in Japan, as suggested by our validation study, which showed high reproducibility of repeated measurements of genistein intake by food frequency questionnaire (correlation coefficient = 0.72 for 1-year interval and 0.61 for 5-year interval).[15,17] By comparison, plasma isoflavone levels may reflect short-term rather than long-term intake: isoflavones have short half-lives in blood (eg, 6 to 8 hours),[23,27] and plasma levels are particularly affected by time elapsed since the last meal. To minimize the attenuation of risk estimates derived from random measurement errors, we matched fasting time between cases and controls. Second, despite a reasonably large cohort population (24,226 women) and long follow-up period (average, 10.6 years), the number of breast cancer cases was relatively small, reflecting the low incidence rate in Japan (age-standardized rate per 100,000 world population, 32.7 in 2002).[13] The interpretability of our results might therefore be limited, particularly in stratified analyses. Third, although our cohort subjects were selected from the general population, subjects were restricted to the 24,226 women respondents (43%) to the baseline questionnaire who provided blood samples. Although health check-up examinees in our previous report had a different socioeconomic status than nonexaminees and a more favorable lifestyle profile,[28] no apparent difference in isoflavone intake and breast cancer risk factors was found between subjects in the subcohort for this study and the original cohort; median isoflavone intake, for example, was 32.5 and 32.1 mg/d, respectively, and the average number of births was 2.8 and 2.7, respectively.[29] Nevertheless, any extrapolation of the results to the general population should be done cautiously, particularly in view of a previous report showing the difficulty of extrapolating relative risk estimates for a subcohort to an entire cohort. This difficulty might in fact be inherent to prospective studies in general.[30]

Allowing for these methodological issues, we found an inverse association between plasma genistein and the risk of breast cancer in a nested case-control study in Japan. This finding suggests a risk-reducing rather than a risk-enhancing effect of isoflavones on breast cancer, even at relatively high concentrations within the range achievable from dietary intake alone.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

Isoflavone, polymorphisms in estrogen receptor genes and breast cancer risk in case-control studies in Japanese, Japanese Brazilians and non-Japanese Brazilians

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Epidemiologic studies have shown an inverse association between isoflavones and breast cancer risk. Because isoflavones bind estrogen receptors, we hypothesized that polymorphisms in the estrogen receptor genes might modify the association between isoflavone intake and breast cancer risk. We conducted hospital-based case-control studies of patients aged 20–74 years with primary, incident, histologically confirmed invasive breast cancer, and matched controls from among medical checkup examinees in Nagano, Japan, and from cancer-free patients in São Paulo, Brazil. A total of 846 pairs (388 Japanese, 79 Japanese Brazilians and 379 non-Japanese Brazilians) completed validated food frequency questionnaires, and provided blood samples. Five single nucleotide polymorphisms in the estrogen receptor alpha (rs9340799, rs1913474, and rs2234693) and beta (rs4986938 and rs1256049) genes were genotyped. We found no consistent association between the five single nucleotide polymorphisms and breast cancer risk among the three populations. In analyses of combinations of isoflavone intake and single nucleotide polymorphisms, an inverse association between intake and risk was limited to women with the GG genotype of the rs4986938 polymorphism for postmenopausal Japanese (odds ratio for highest *versus* lowest tertile = 0.47; *P* for trend = 0.01), Japanese Brazilians (odds ratio for highest *versus* lowest median = 0.31) and non-Japanese Brazilians (odds ratio for consumers *versus* non-consumers = 0.37) (*P* for interaction = 0.11, 0.08, and 0.21, respectively). We found no remarkable difference for the other four polymorphisms. Our findings suggest that polymorphisms in the estrogen receptor beta gene may modify the association between isoflavone intake and breast cancer risk. (*Cancer Sci* 2009; 100: 927–933)

Soy foods are a traditional staple dish in Asian countries. They are a primary source of isoflavones such as genistein and daidzein, which are classified as phytoestrogens. Because breast cancer risk is substantially lower in Asian than Western countries,[1] the contribution of a high isoflavone intake to low breast cancer risk has been hypothesized.[2] A meta-analysis supported this hypothesis and found a small decrease in breast cancer risk with higher soy intake.[3] On the other hand, a more recent meta-analysis indicated that risk reduction was limited to Asian populations.[4] This discrepancy might reflect differences in exposure levels and genetic factors between Asian and Western populations.

Several mechanisms by which isoflavones may reduce the risk of breast cancer have been proposed.[5,6] The most prominent and thoroughly investigated are those mediated via estrogen receptors, which arise due to the similarity in chemical structures between isoflavones and human estrogen hormone, and the consequent binding affinity of isoflavones for estrogen receptors.[6,7] Isoflavones can therefore act as estrogen agonists and antagonists competing for estradiol at the receptor complex,[5] suggesting in turn that isoflavones might interact with estrogen receptor genes in the development of breast cancer. However, the possible joint effect of isoflavone intake and polymorphisms in the estrogen receptor genes on the risk of breast cancer has not been investigated.

Here, we conducted hospital-based case-control studies in Nagano, Japan and São Paulo, Brazil, targeting three populations with a substantially different intake of isoflavones and distribution of polymorphisms in the estrogen receptor genes: Japanese living in Japan, Japanese Brazilians living in São Paulo, and non-Japanese Brazilians living in São Paulo. In a previous report, we found a non-significant inverse association between isoflavone intake and the risk of breast cancer in postmenopausal Japanese women but a statistically significant inverse association in Japanese Brazilians and non-Japanese Brazilians.[8] Based on this finding, the present study tested the hypothesis that polymorphisms in estrogen receptor genes may modify the association between isoflavone intake and breast cancer risk.

Materials and Methods

Study subjects. These multicenter, hospital-based case-control studies of breast cancer were designed to determine lifestyle factors and genetic susceptibility to the risk of breast cancer, and to compare potential risk factors among Japanese living in Nagano, Japan, and Japanese Brazilians and non-Japanese Brazilians living in São Paulo, Brazil. Eligible cases were a consecutive series of female patients aged 20–74 years with newly diagnosed and histologically confirmed invasive breast cancer. Patients with cancer were recruited between 2001 and 2005 at four hospitals in Nagano, and between 2001 and 2006 at eight hospitals in São Paulo, totaling 405 patients (98%) in Nagano, and 83 Japanese Brazilians (91%) and 389 non-

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Abbreviations: CI, confidence interval; CYP17, cytochrome P450c17α; CYP19, aromatase; CYP2E1, cytochrome P450 2E1; ESR1, estrogen receptor alpha; ESR2, estrogen receptor beta; FFQ, food-frequency questionnaire; NAT2, N-acetyltransferase 2; OR, odds ratio; SNP, single-nucleotide polymorphism.

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Table 1. Single-nucleotide polymorphisms in estrogen receptor genes and their allele frequency

Gene	SNP rs number	Synonym	Region	Major/minor allele	Minor allele frequency among control groups		
					Japanese living in Nagano, Japan	Japanese Brazilians living in São Paulo, Brazil	Non-Japanese Brazilians living in São Paulo, Brazil
Estrogen receptor alpha gene	rs9340799	XbaI	intron 1	A/G	0.19	0.20	0.31
	rs1913474		intron 3	C/T	0.48	0.48	0.21
	rs2234693	PvuII	intron 1	T/C	0.45	0.45	0.42
Estrogen receptor beta gene	rs4986938	AuII	3'-UTR	G/A	0.14	0.13	0.33
	rs1256049	RsaI	exon 6	G/A	0.30	0.20	0.05

SNP, single-nucleotide polymorphism.

Japanese Brazilians (99%) in São Paulo. In the Nagano study, eligible controls were selected from medical checkup examinees in two of the four hospitals and confirmed not to have cancer. One control was matched for each case by age (within 3 years) and residential area. Among potential controls, one examinee refused to participate and two refused to provide blood samples. Eventually, we obtained written informed consent from 405 matched pairs. In the study in São Paulo, eligible controls were preferentially selected from cancer-free patients who visited the same hospital as the index cases. One control was matched for each patient with cancer by age (within 5 years) and ethnicity. Among potential controls, 22 patients refused to participate (participation rate = 96%). Eventually, we obtained written informed consent from 472 matched pairs (83 for Japanese Brazilians and 389 for non-Japanese Brazilians). The study protocol was approved by Comissão Nacional de Ética em Pesquisa (CONEP), Brasília, Brazil and by the institutional review board of the National Cancer Center, Tokyo, Japan.

Questionnaire. Participants in Nagano were asked to complete a self-administered questionnaire, while those in São Paulo were interviewed by trained interviewers using a structured questionnaire. The two questionnaires contained similar questions concerning demographic characteristics, medical history, family history of cancer, menstrual and reproductive history, anthropometric factors, physical activity and smoking habits. For dietary habits, we used a semiquantitative food frequency questionnaire (FFQ) (136 items for the Japanese version and 118 items for the Brazilian version), which was developed and validated in each population.[9–11] In the FFQ, participants were questioned on how often they consumed the individual food items (frequency of consumption), as well as relative sizes compared to standard portions. Daily food intake was calculated by multiplying frequency by standard portion and relative size for each food item in the FFQ. Daily intakes of genistein and daidzein were calculated using a food composition table of isoflavones developed previously.[12,13] Isoflavone intake was defined for this study as the sum of genistein and daidzein intake. Other nutrients were calculated using the Japanese Standard Tables of Food Composition for the Japanese version,[14] and the United States Department of Agriculture (USDA) food composition tables for the Brazilian version.[15] For some Japanese-specific foods in the Brazilian version, the Japanese Standard Tables of Food Composition[14] was used.

The validity of isoflavone intake estimated from the Japanese version of the FFQ was evaluated in a subsample of the Japan Public Health Center-based Prospective Study by comparing the estimated intake according to the FFQ to that in four consecutive seven-day dietary records, one conducted in each of the four seasons. Spearman's correlation coefficients between energy-adjusted genistein and daidzein intake estimated from the FFQ and from dietary records to be 0.59 for genistein and 0.60 for daidzein.[10] For the Brazilian version, the validity of isoflavone intake estimated from the FFQ was evaluated in a subsample of the control group of this case-control study by comparing the estimated intake according to the FFQ to that in two consecutive four-day dietary records, one each in two seasons. Spearman's correlation coefficients between energy-adjusted genistein and daidzein intake estimated from the FFQ and from dietary records were 0.76 for genistein and 0.76 for daidzein.[11]

Genotyping. Genomic DNA samples were extracted from the peripheral blood using FlexiGene® DNA kits (Qiagen K.K., Tokyo, Japan) according to the manufacturer's protocol. We selected five single nucleotide polymorphisms (SNPs) in the estrogen receptor alpha (*ESR1*) gene (rs9340799, rs1913474, and rs2234693) and estrogen receptor beta (*ESR2*) gene (rs4986938 and rs1256049), which were the most frequently studied SNP in relation to breast cancer risk.[16–20] Genotyping of the five SNPs was performed by a commercial laboratory (Genetic Laboratory, Inc., Sapporo, Japan) using TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) (Table 1). Patients with cancer and matched controls were analyzed in the same well by laboratory personnel unaware of the case-control status. For quality control assessment, we genotyped six SNPs of four genes (N-acetyltransferase 2 [*NAT2*], cytochrome P450c17α [*CYP17*], aromatase [*CYP19*], and cytochrome P450 2E1 [*CYP2E1*]) in our laboratory using about 24% of the samples in the present study. However, SNPs used in the present study were not included. The concordance rates between Genetic Laboratory Inc. and our laboratory varied between 97.6 and 99.5% among the six SNPs.

Statistical analysis. Comparison of baseline characteristics between cases and controls was evaluated by the Mantel–Haenszel test using matched-pair strata in each population. Genotype frequencies were tested for deviation from the Hardy–Weinberg equilibrium with the χ^2-test. Dietary intake of isoflavones was adjusted for total energy intake by the residual method and divided into median or tertile categories based on control distribution for Japanese and Japanese Brazilians, respectively. Because of the small proportion of consumers, non-Japanese Brazilians were categorized into non-consumers and consumers of isoflavones. Using a conditional logistic regression model, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer for isoflavone intake, SNPs, and the joint effect between isoflavone intake and genotypes. An unconditional logistic regression model was used for stratified analyses according to menopausal status. Linear trends for ORs were tested in the logistic regression model using the exposure categories as ordinal variables. Tests for the interaction were performed based on the difference between two likelihood ratios of the models with and without the interaction terms between isoflavone intake and the SNP of interest. Adjustments were made for the following variables, selected mainly on the basis of comparison of baseline characteristics between patients with cancer and controls, as potential confounders: menopausal status, number of births, family history of breast cancer, smoking status, moderate physical activity in the past 5 years and vitamin

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Table 2. Odds ratios and 95% confidence intervals of breast cancer according to polymorphisms in estrogen receptor genes

	Japanese living in Nagano, Japan				Japanese Brazilians living in São Paulo, Brazil				Non-Japanese Brazilians living in São Paulo, Brazil			
	No.		OR†	95% CI	No.		OR†	95% CI	No.		OR†	95% CI
	Case	Control			Case	Control			Case	Control		
Estrogen receptor alpha gene (rs9340799)												
AA	273	256	1		54	50	1		161	182	1	
AG	103	119	0.68	(0.45–1.02)	22	26	0.75	(0.31–1.84)	175	161	1.16	(0.84–1.59)
GG	12	13	0.75	(0.28–1.98)	3	3	0.68	(0.10–4.57)	43	36	1.27	(0.78–2.07)
AG + GG	115	132	0.69	(0.47–1.02)	25	29	0.74	(0.31–1.79)	218	197	1.18	(0.88–1.59)
Estrogen receptor alpha gene (rs1913474)												
CC	100	113	1		25	24	1		237	239	1	
CT	192	176	1.19	(0.81–1.76)	39	34	1.24	(0.55–2.81)	127	122	1.09	(0.80–1.49)
TT	96	99	1.08	(0.70–1.66)	15	21	0.79	(0.28–2.20)	14	18	0.80	(0.38–1.67)
CT + TT	288	275	1.15	(0.80–1.64)	54	55	1.07	(0.51–2.27)	141	140	1.05	(0.78–1.42)
Estrogen receptor alpha gene (rs2234693)												
TT	144	115	1		25	22	1		107	122	1	
TC	180	196	**0.70**	**(0.49–0.995)**	39	43	0.66	(0.29–1.47)	187	194	0.99	(0.68–1.43)
CC	64	77	0.64	(0.40–1.02)	15	14	0.93	(0.31–2.86)	85	63	1.51	(0.98–2.31)
TC + CC	244	273	**0.68**	**(0.49–0.96)**	54	57	0.71	(0.32–1.54)	272	257	1.15	(0.83–1.61)
Estrogen receptor beta gene (rs4986938)												
GG	289	281	1		59	60	1		169	176	1	
GA	94	102	0.88	(0.59–1.31)	17	17	1.32	(0.53–3.31)	163	154	1.09	(0.78–1.51)
AA	5	5	1.53	(0.39–6.07)	3	2	0.71	(0.09–5.57)	47	49	0.93	(0.59–1.47)
GA + AA	99	107	0.91	(0.62–1.34)	20	19	1.22	(0.51–2.93)	210	203	1.05	(0.77–1.42)
Estrogen receptor beta gene (rs1256049)												
GG	203	182	1		47	48	1		342	345	1	
GA	161	178	0.79	(0.56–1.10)	26	30	0.95	(0.46–1.98)	36	32	1.21	(0.71–2.04)
AA	24	28	0.84	(0.44–1.60)	6	1	4.80	(0.50–46.19)	1	2	0.54	(0.04–6.53)
GA + AA	185	206	0.79	(0.57–1.09)	32	31	1.04	(0.50–2.13)	37	34	1.16	(0.70–1.94)

†Conditional model adjusting for menopausal status (premenopausal, postmenopausal), number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), 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). ORs and 95% CIs with statistical significance are written in bold letter. CIs, confidence intervals; OR, odds ratio.

supplement use. We did not include a history of benign breast disease as a covariate since we regarded it as an intermediate variable in the causal pathway between isoflavone intake and breast cancer. All P-values reported are two-sided, and significance level was set at $P < 0.05$. All statistical analyses were performed with SAS version 9.1 software (SAS Institute, Inc., Cary, NC, USA).

Results

We excluded subjects who reported extremely low or high total energy intake (<500 or ≥4000 kCal) or had no DNA sample, leaving 388 pairs of Japanese, 79 pairs of Japanese Brazilians and 379 pairs of non-Japanese Brazilians for inclusion in the present analyses.

Characteristics of patients with cancer and controls are shown in a previous report (data not shown in table).[8] For Japanese women, the proportion of premenopausal women, current smokers, and vitamin supplement users was higher in cases than in controls, and patients with cancer tended to have a family history of breast cancer and history of benign breast disease. Patients with cancer were less likely than controls to breast-feed, be physically active and eat vegetables. For Japanese Brazilians, patients with cancer were less likely than controls to give birth and be physically active, and more likely to eat vegetables and fruits. For non-Japanese Brazilians, the proportion of premenopausal women and current smokers was higher in patients with cancer than controls, while the proportion of physically active women and vitamin supplement users was lower. Isoflavone intake substantially varied among populations, with mean intakes in control subjects of 46.2 mg/day for Japanese, 23.5 mg/day for Japanese Brazilians, and 4.4 mg/day for non-Japanese Brazilians.

The distributions of SNPs in the *ESR1* gene (rs9340799, rs1913474 and rs2234693) and *ESR2* gene (rs4986938 and rs1256049) are shown in Tables 1 and 2. No deviation from the Hardy–Weinberg equilibrium was observed among the controls in any population. The prevalence of the minor allele in the rs9340799 and rs4986938 polymorphisms was lower in the control group of Japanese and Japanese Brazilians than in that of non-Japanese Brazilians, while that of the minor allele in the rs1913474 and rs1256049 polymorphisms was higher in the control group of Japanese and Japanese Brazilians. We found a decreased risk of breast cancer among Japanese women with at least one minor allele of the rs9340799 or rs2234693 polymorphism in comparison with those with the major allele homozygote, but not among Japanese Brazilian and non-Japanese Brazilian women. This decrease was statistically significant for the rs2234693 polymorphism but not for the rs9340799 polymorphism. Stratified analyses by menopausal status showed that this decreased risk occurred primarily among postmenopausal Japanese for both SNPs (data not shown). In contrast, no association was observed for the rs1913474, rs4986938, or rs1256049 polymorphisms in the three populations, regardless of menopausal status.

Analyses of combinations of isoflavone intake and the rs4986938 polymorphism in the *ESR2* gene revealed that the risk of breast cancer significantly decreased with increasing isoflavone intake only among women with the GG genotype among postmenopausal Japanese (OR for highest *versus* lowest tertile = 0.47; 95%CI 0.27–0.84; P for trend = 0.01), Japanese Brazilians (OR for highest *versus* lowest median = 0.31; 95%CI 0.12–0.78), and non-Japanese Brazilians (OR for consumers *versus* non-consumers = 0.37; 95%CI