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

Table 3. Odds ratios and 95% confidence intervals of breast cancer for combinations of dietary intake of isoflavones and polymorphisms in estrogen receptor genes among Japanese

	All subjects				Premenopausal women				Postmenopausal women			
	Isoflavone intake (mg/day), tertile category			*P* for trend	Isoflavone intake (mg/day), tertile category			*P* for trend	Isoflavone intake (mg/day), tertile category			*P* for trend
	1	2	3		1	2	3		1	2	3	
Estrogen receptor alpha gene (rs9340799)												
AA												
No.†	109/83	76/90	88/83		54/41	30/31	33/19		55/42	46/59	55/64	
OR‡	1	0.73	0.78	0.32	1	0.68	1.13	0.96	1	0.75	0.64	0.15
(95% CI)		(0.45–1.18)	(0.47–1.29)			(0.34–1.35)	(0.53–2.39)			(0.41–1.34)	(0.36–1.15)	
AG + GG												
No.†	42/46	42/39	31/47		25/25	22/12	12/7		17/21	20/27	19/40	
OR‡	**0.52**	0.68	0.51	0.75	0.64	1.38	1.13	0.54	0.59	0.56	**0.38**	0.15
(95% CI)	**(0.27–0.99)**	(0.37–1.24)	(0.26–1.01)		(0.31–1.35)	(0.59–3.23)	(0.39–3.30)		(0.26–1.32)	(0.26–1.20)	**(0.18–0.79)**	
	P for interaction = 0.39				*P* for interaction = 0.15				*P* for interaction = 0.87			
Estrogen receptor alpha gene (rs1913474)												
CC												
No.†	41/38	32/42	27/33		20/16	16/12	13/4		21/22	16/30	14/29	
OR‡	1	0.68	0.76	0.62	1	1.15	2.39	0.08	1	0.60	0.47	0.09
(95% CI)		(0.34–1.36)	(0.37–1.59)			(0.40–3.26)	(0.61–9.30)			(0.24–1.47)	(0.18–1.21)	
CT + TT												
No.†	110/91	86/87	92/97		59/50	36/31	32/22		51/41	50/56	60/75	
OR‡	0.97	0.97	0.84	0.33	0.91	0.86	0.97	0.99	1.20	1.08	0.80	0.14
(95% CI)	(0.54–1.74)	(0.55–1.72)	(0.45–1.55)		(0.41–2.02)	(0.37–2.04)	(0.39–2.44)		(0.56–2.59)	(0.51–2.29)	(0.38–1.68)	
	P for interaction = 0.69				*P* for interaction = 0.58				*P* for interaction = 0.73			
Estrogen receptor alpha gene (rs2234693)												
TT												
No.†	58/36	38/41	48/38		33/21	12/16	21/11		25/15	26/25	27/27	
OR‡	1	0.55	0.68	0.54	1	0.41	1.15	0.77	1	0.79	0.58	0.28
(95% CI)		(0.26–1.16)	(0.32–1.43)			(0.15–1.12)	(0.43–3.10)			(0.32–1.92)	(0.24–1.40)	
TC + CC												
No.†	93/93	80/88	71/92		46/45	40/27	24/15		47/48	40/61	47/77	
OR‡	**0.51**	**0.52**	**0.42**	0.46	0.64	0.99	0.86	0.39	0.54	**0.42**	**0.35**	0.14
(95% CI)	**(0.28–0.96)**	**(0.28–0.98)**	**(0.21–0.82)**		(0.31–1.34)	(0.45–2.16)	(0.35–2.15)		(0.24–1.21)	**(0.19–0.92)**	**(0.16–0.76)**	
	P for interaction = 0.37				*P* for interaction = 0.08				P for interaction = 0.97			
Estrogen receptor beta gene (rs4986938)												
GG												
No.†	115/86	88/96	86/99		57/46	39/32	32/21		58/40	49/64	54/78	
OR‡	1	0.74	0.65	0.06	1	0.96	1.03	0.94	1	0.60	**0.47**	**0.01**
(95% CI)		(0.47–1.16)	(0.39–1.07)			(0.51–1.83)	(0.49–2.15)			(0.33–1.07)	**(0.27–0.84)**	
GA + AA												
No.†	36/43	30/33	33/31		22/20	13/11	13/5		14/23	17/22	20/26	
OR‡	0.57	0.78	0.90	0.23	0.80	0.91	1.99	0.20	0.47	0.80	0.62	0.49
(95% CI)	(0.31–1.08)	(0.40–1.50)	(0.45–1.82)		(0.37–1.72)	(0.35–2.34)	(0.62–6.46)		(0.21–1.06)	(0.36–1.75)	(0.28–1.35)	
	P for interaction = 0.17				*P* for interaction = 0.48				*P* for interaction = 0.11			
Estrogen receptor beta gene (rs1256049)												
GG												
No.†	85/62	59/62	59/58		43/32	28/20	23/12		42/30	31/42	36/46	
OR‡	1	0.74	0.82	0.16	1	1.05	0.98	0.80	1	0.56	0.51	0.08
(95% CI)		(0.43–1.27)	(0.46–1.48)			(0.49–2.27)	(0.39–2.47)			(0.28–1.13)	(0.26–1.01)	
GA + AA												
No.†	66/67	59/67	60/72		36/34	24/23	22/14		30/33	35/44	38/58	
OR‡	0.70	0.74	0.61	0.93	0.79	0.78	1.31	0.20	0.50	0.60	**0.41**	0.35
(95% CI)	(0.41–1.19)	(0.43–1.27)	(0.35–1.07)		(0.39–1.58)	(0.36–1.69)	(0.55–3.08)		(0.24–1.03)	(0.31–1.18)	**(0.21–0.80)**	
	P for interaction = 0.63				*P* for interaction = 0.65				*P* for interaction = 0.31			

†No. of patients with cancer/No. of controls.
‡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 smoker), 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). For stratified analyses according to menopausal status, an unconditional model adjusting for age, area, number of births (0, 1, 2, 3, 4, 5+), family history of breast cancer (yes, no), smoking status (never, past, current smoker), 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.

0.16–0.85) (*P* for interaction = 0.11, 0.08 and 0.21, respectively) (Tables 3 and 4). Moreover, we found no remarkable difference in the association between isoflavone intake and breast cancer risk by the four other polymorphisms.

Discussion

In these case-control studies of Japanese, Japanese Brazilians, and non-Japanese Brazilians, we found that a statistically significant

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Table 4. Odds ratios and 95% confidence intervals of breast cancer for combinations of dietary intake of isoflavones and polymorphisms in estrogen receptor genes among Japanese Brazilian and non-Japanese Brazilian subjects

	Japanese Brazilians living in São Paulo, Brazil		Non-Japanese Brazilians living in São Paulo, Brazil	
	Isoflavone intake (mg/day), median category		Isoflavone intake (mg/day)	
	1	2	Non-consumers	Consumers
Estrogen receptor alpha gene (rs9340799)				
AA				
No.†	31/21	23/29	145/157	16/25
OR‡	1	**0.36**	1	0.68
(95% CI)		**(0.14–0.95)**		(0.32–1.43)
AG + GG				
No.†	15/18	10/11	198/161	20/36
OR‡	0.44	0.34	1.23	0.61
(95% CI)	(0.14–1.45)	(0.09–1.32)	(0.89–1.68)	(0.33–1.13)
	P for interaction = 0.36		*P* for interaction = 0.52	
Estrogen receptor alpha gene (rs1913474)				
CC				
No.†	13/12	12/12	213/204	24/35
OR‡	1	0.76	1	0.65
(95% CI)		(0.18–3.18)		(0.36–1.19)
CT + TT				
No.†	33/27	21/28	129/114	12/26
OR‡	1.25	0.55	1.13	0.49
(95% CI)	(0.42–3.72)	(0.17–1.78)	(0.82–1.56)	(0.24–1.01)
	P for interaction = 0.52		*P* for interaction = 0.40	
Estrogen receptor alpha gene (rs2234693)				
TT				
No.†	17/12	8/10	97/106	10/16
OR‡	1	0.41	1	0.57
(95% CI)		(0.10–1.65)		(0.22–1.47)
TC + CC				
No.†	29/27	25/30	246/212	26/45
OR‡	0.65	0.36	1.20	0.65
(95% CI)	(0.23–1.84)	(0.12–1.08)	(0.84–1.71)	(0.37–1.15)
	P for interaction = 0.71		*P* for interaction = 0.94	
Estrogen receptor beta gene (rs4986938)				
GG				
No.†	38/30	21/30	156/148	13/28
OR‡	1	**0.31**	1	**0.37**
(95% CI)		**(0.12–0.78)**		**(0.16–0.85)**
GA + AA				
No.†	8/9	12/10	156/170	23/33
OR‡	0.62	0.97	0.97	0.68
(95% CI)	(0.16–2.35)	(0.31–3.01)	(0.70–1.35)	(0.37–1.24)
	P for interaction = 0.08		*P* for interaction = 0.21	
Estrogen receptor beta gene (rs1256049)				
GG				
No.†	27/23	20/25	308/286	34/59
OR‡	1	0.49	1	**0.55**
(95% CI)		(0.21–1.17)		**(0.35–0.90)**
GA + AA				
No.†	19/16	13/15	35/32	2/2
OR‡	0.97	0.53	1.10	0.84
(95% CI)	(0.36–2.58)	(0.18–1.58)	(0.64–1.87)	(0.10–6.97)
	P for interaction = 0.89		*P* for interaction = 0.78	

†No. of patients with cancer/No. of controls.
‡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.

inverse association between isoflavone intake and breast cancer risk appeared only among women with the GG genotype of the rs4986938 polymorphism in the *ESR2* gene, but the interaction was not statistically significant. Our findings support the hypothesis that polymorphisms in the *ESR2* gene may modify the association between isoflavone intake and breast cancer risk.

To date, many studies investigating the possible effect of SNPs in the *ESR2* gene on breast cancer risk have focused on the

rs4986938 and rs1256049 polymorphisms, although their functional importance has yet to be clarified. Here, we found no association between either SNP and the risk of breast cancer, which is in general agreement with most previous studies.[16,17] In contrast, we did see an inverse association between isoflavone intake and breast cancer risk with the rs4986938 polymorphism in three populations, but only among women with the GG genotype. We also saw a suggestive interaction in the case-control studies of Japanese and Japanese Brazilians but not in the case-control study of non-Japanese Brazilians. Although the reason for the inconsistency in interactions among populations remains unclear, it might reflect the amount of intake, on the basis that the findings were relatively consistent among the populations with a high intake (Japanese and Japanese Brazilians). Moreover, the prevalence of the GG genotype of the rs4986938 polymorphism among the control group was higher in Japanese (72.4%) and Japanese Brazilians (75.9%) than in non-Japanese Brazilians (46.4%). This might partly explain the previous inconsistencies in results for isoflavone exposure and breast cancer risk between Asian and Western populations.[4]

To our knowledge, only two studies have investigated interactions between phytoestrogen exposure and polymorphisms in the *ESR2* gene in the risk of hormone-related diseases.[21,22] Hedelin *et al*. reported a significant interaction between phytoestrogen intake and a promoter SNP in the *ESR2* gene (rs2987983) in the risk of prostate cancer in a population-based case-control study in Sweden.[22] Tsuchiya *et al*. reported a significant interaction between urinary genistein level and RsaI polymorphism in the *ESR2* gene in the risk of advanced endometriosis among infertile Japanese women.[21] These findings suggest that isoflavones may reduce the risk of hormone-related diseases via a mechanism that involves estrogen receptor beta. Considering that functional data are not presently available, our finding suggests that the rs4986938 polymorphism, or some other genetic variants in strong linkage disequilibrium with this SNP, modify the protective effect of isoflavones on breast cancer. In this regard, we provide further evidence for a role of isoflavones in the development of breast cancer.

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. Although these are the most frequently studied SNPs, results have been inconsistent.[18–20] Most studies have shown no association between the rs2234693 polymorphism and breast cancer risk.[18–20] On the other hand, several but not all studies have reported that the G allele of the rs9340799 polymorphism was associated with a decreased risk of breast cancer,[18,20] which is consistent with our findings in Japanese women. Since we failed to observe an overall consistency of findings in our three populations, however, our findings in Japanese women might be merely due to chance.

Although interactions between phytoestrogen exposure and polymorphisms in the *ESR1* gene in the risk of breast cancer have not been investigated, we are aware of two studies examining interactions on circulating sex hormone levels.[23,24] In their study of 125 postmenopausal women in the European Prospective Investigation of Cancer and Nutrition–Norfolk cohort, Low *et al*. reported that urinary and serum isoflavones were negatively correlated with plasma estradiol among women with the CC genotype for PvuII polymorphism in the *ESR1* gene, but not those with other genotypes.[23] Moreover, they reported a significant interaction between urinary lignans and rs9340835 polymorphism in the *ESR1* gene, affecting plasma estrone levels in a cross-sectional study of 1988 healthy postmenopausal women from the same cohort.[24] Although these studies imply the presence of gene–nutrient interaction, we found no remarkable difference in the association between isoflavone intake and breast cancer risk by polymorphisms in the *ESR1* gene. Further studies based on a comprehensive evaluation of this gene would clarify this gene–nutrient interaction.

Our study has methodological advantages over studies conducted previously. First, and unique to this study, we assessed gene–nutrient interactions using three populations with substantially different isoflavone intakes and allele frequencies of SNPs. For example, isoflavone intake differed considerably among the three populations, with median levels (interquartile range) in the control group of (mg/day) 40.7 (25.8–61.4) among Japanese, 13.4 (7.9–31.1) among Japanese Brazilians, and 0 (0–0) among non-Japanese Brazilians. In addition, allele frequency also differed among the populations, such as that of the G allele of the rs4986938 polymorphism in the *ESR2* gene, at 0.86 for Japanese, 0.87 for Japanese Brazilians, and 0.67 for non-Japanese Brazilians. Second, the overall consistency of findings in the three populations could allow the results to be more generalized than those from a single population.

Several limitations of the study also warrant mention. First, dietary intake of isoflavones was assessed after the diagnosis of breast cancer, and therefore, is sensitive to recall bias. Second, although the substantially high participation rates among both eligible patients with cancer and controls minimized potential biases related to control selection, the use of controls from medical checkup examinees and cancer-free patients, whose dietary habits may differ from those of the general population due to health consciousness or disease, might have lead to selection bias. For example, isoflavone intake was higher among women aged 50–69 years in the control group of the Nagano study (median intake = 46.3 mg/day) than in participants aged 50–69 years living in Nagano in the 10-year follow-up survey of the Japan Public Health Center-based Prospective Study (median intake = 38.8 mg/day), which used a similar FFQ and had a high response rate. Third, the evaluation of gene–nutrient interactions was performed in a relatively small number of patients with cancer. The interpretability of our results might therefore be limited.

Allowing for these methodological issues, we found a suggestive interaction between isoflavone intake and the rs4986938 polymorphism of the *ESR2* gene in the risk of breast cancer in case-control studies of Japanese and Japanese Brazilians. Our findings support the hypothesis that polymorphisms in the *ESR2* gene may modify the association between isoflavone intake and breast cancer risk. Further, they provide additional evidence that the mechanisms by which isoflavones may reduce the risk of breast cancer might involve estrogen receptor beta.

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