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RESEARCH ARTICLE Open Access

Comparison of postmenopausal endogenous sex hormones among Japanese, Japanese Brazilians, and non-Japanese Brazilians

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Abstract

Background: Differences in sex hormone levels among populations might contribute to the variation in breast cancer incidence across countries. Previous studies have shown higher breast cancer incidence and mortality among Japanese Brazilians than among Japanese. To clarify the difference in hormone levels among populations, we compared postmenopausal endogenous sex hormone levels among Japanese living in Japan, Japanese Brazilians living in the state of São Paulo, and non-Japanese Brazilians living in the state of São Paulo.

Methods: A cross-sectional study was conducted using a control group of case-control studies in Nagano, Japan, and São Paulo, Brazil. Participants were postmenopausal women older than 55 years of age who provided blood samples. We measured estradiol, estrone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), testosterone and free testosterone by radioimmunoassay; bioavailable estradiol by the ammonium sulfate precipitation method; and sex hormone-binding globulin (SHBG) by immunoradiometric assay. A total of 363 women were included for the present analyses, comprising 185 Japanese, 44 Japanese Brazilians and 134 non-Japanese Brazilians.

Results: Japanese Brazilians had significantly higher levels of estradiol, bioavailable estradiol, estrone, testosterone and free testosterone levels, and lower SHBG levels, than Japanese. Japanese Brazilians also had significantly higher levels of bioavailable estradiol, estrone and DHEAS and lower levels of SHBG and androstenedione than non-Japanese Brazilians. Levels of estradiol, testosterone and free testosterone, however, did not differ between Japanese Brazilians and non-Japanese Brazilians. These differences were observed even after adjustment for known breast cancer risk factors. We also found an increase in estrogen and androgen levels with increasing body mass index, but no association for most of the other known risk factors.

Conclusions: We found higher levels of estrogens and androgens in Japanese Brazilians than in Japanese and levels similar to or higher than in non-Japanese Brazilians. Our findings may help explain the increase in the incidence and mortality rate of breast cancer among Japanese Brazilians.

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Background

The incidence and mortality rate of breast cancer vary considerably across countries and regions [1]. Although Japan has a lower risk for female breast cancer than Western countries, the incidence has gradually increased over the past 30 years [2,3]. The incidence and mortality rates in Japanese immigrants living in the United States and Brazil have approximated those in the host country [4-8]. For example, the mortality rate of first-generation Japanese immigrants to São Paulo, Brazil, increased from 1979 to 2001, with rates being intermediate between Japanese living in Japan and Brazilians living in the state of São Paulo [6].

Many epidemiologic studies have indicated that endogenous sex hormones, particularly estrogens, play an important role in the etiology of breast cancer [9]. A pooled analysis of nine prospective studies showed that higher estrogens and their androgen precursors were associated with a higher risk of breast cancer in postmenopausal women [9]. Differences in sex hormone levels among populations might therefore contribute to the variation in breast cancer incidence across countries and regions. Clarification of the difference in sex hormone levels among populations and their determinants might help our understanding of the etiology and prevention of breast cancer.

A relatively large number of epidemiological studies have examined sex hormone levels among ethnic groups and factors associated with sex hormone levels [10-16]. To our knowledge, however, no study has investigated sex hormone levels among Japanese Brazilians. In addition, although previous studies consistently showed that body weight and obesity were associated with higher estrogen levels in postmenopausal women [10-12,15], findings regarding other factors that influence circulating sex hormone levels have been inconsistent [10-14,16].

We have conducted a cross-sectional study using a control group of case-control studies in Nagano, Japan, and São Paulo, Brazil. The present study compared postmenopausal endogenous sex hormone levels among Japanese living in Japan, Japanese Brazilians living in São Paulo and non-Japanese Brazilians living in São Paulo, and examined factors associated with these levels.

Methods

Study participants

Participants were postmenopausal women who were enrolled as controls in multicenter, hospital-based, case-control studies of breast cancer. In addition to determining lifestyle factors and genetic susceptibility to the risk of breast cancer, the protocols of these studies were also designed to compare potential risk factors among Japanese living in Nagano, Japan, and Japanese Brazilians and non-Japanese Brazilians living in the state of São Paulo, Brazil. Details of this study have been described previously [17]. The study protocol was approved by Comissão Nacional de Ética em Pesquisa, Brasília, Brazil, and by the institutional review board of the National Cancer Center, Tokyo, Japan.

Briefly, eligible cases were a consecutive series of female patients ages 20 to 74 years with newly diagnosed and histologically confirmed invasive breast cancer. Inhabitants of the state of São Paulo were recruited and asked their ethnicity. Japanese and their descendants were defined as Japanese Brazilians, and Caucasian, black and mixed ethnicity populations were defined as non-Japanese Brazilians. A total of 405 individuals (98%) participated in Nagano, and 83 Japanese Brazilians (91%) and 389 non-Japanese Brazilians (99%) participated in São Paulo. In the study in Nagano, eligible controls were selected from among medical checkup examinees in two of the four hospitals and were confirmed not to have cancer. One control was matched for each case by age (within 3 years) and by residential area. Among potential controls, one examinee refused to participate and two refused to provide blood samples. In the study in São Paulo, eligible controls were preferentially selected from among cancer-free patients who visited the same hospital as the index cases. One control was matched for each case by age (within 5 years) and by ethnicity. Among potential controls, 22 patients refused to participate (participation rate, 96%). Consequently, we obtained written, informed consent from a total of 877 matched pairs (405 for Japanese, 83 for Japanese Brazilians and 389 for non-Japanese Brazilians).

Of 877 controls, we selected postmenopausal women over 55 years of age who provided blood samples and reported an energy intake between 500 and 4,000 kcal. Menopausal status was determined by self-report, and energy intake was assessed using a food frequency questionnaire (FFQ). The present study included a total of 382 women comprising 185 Japanese, 46 Japanese Brazilians and 151 non-Japanese Brazilians.

Data collection

Participants in Nagano were asked to complete a self-administered questionnaire, while in-person interviews were conducted in São Paulo by trained interviewers using a structured questionnaire. The two questionnaires contained closely similar questions concerning demographic characteristics, medical history, family history of cancer, menstrual and reproductive history, anthropometric factors, physical activity, smoking habits and dietary factors assessed by FFQ.

Participants in Nagano provided blood samples at the time they returned their self-administered questionnaire, and those in São Paulo provided blood samples at the time of the interview. Blood samples were divided into

serum aliquots in Nagano and into plasma aliquots and buffy coat layers in São Paulo. All blood samples were shipped to the Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan, and stored at -80°C until analysis.

Laboratory analysis

We used a radioimmunoassay method to measure estradiol, estrone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), testosterone and free testosterone in serum for the Nagano participants and in plasma for the São Paulo participants. The following kits were used: estradiol (DSL-4800 Ultra-Sensitive Estradiol Radioimmunoassay Kit; Diagnostic System Laboratories, Inc., Webster, TX, USA), estrone (DSL-8700 Estrone Radioimmunoassay Kit; Diagnostic System Laboratories, Inc.), androstenedione (DPC · Androstenedione; Diagnostic Products Corporation, Llanberis, UK), DHEAS (DPC · DHEA-S Kit, Diagnostic Products Corporation), testosterone (DPC · Testosterone Kit; Diagnostic Products Corporation) and free testosterone (DPC · Free Testosterone Kit; Diagnostic Products Corporation). Bioavailable estradiol (free and albumin-bound estradiol) was measured using the ammonium sulfate precipitation method. Sex hormone-binding globulin (SHBG) was measured by immunoradiometric assay (IRMA) using Spectria SHBG IRMA (Orion Diagnostica, Espoo, Finland). The kit for estrone was applicable to serum samples only, although other kits or methods were applicable to both serum and plasma samples. We therefore measured estrone levels in both serum and plasma from the same women over 50 years of age (n = 38) and calibrated estrone levels in plasma on the basis of a regression function, although the two levels were highly correlated (correlation coefficient = 0.94) and the percentage difference was relatively small (mean = -4%; 95% confidence interval, -9% to 1%). Lower detection limits (LODs) were 5 pg/mL for estradiol, 15 pg/mL for estrone, 6.25 nM/L for SHBG, 0.1 ng/mL for androstenedione, 5 μg/dL for DHEAS, 0.05 ng/mL for testosterone and 0.4 pg/mL for free testosterone. Measurement values below the LOD were assigned half the value of the LOD if measurable values below the LOD were not available. The intra-assay coefficients of variation were 6.5% for estradiol at a mean concentration of 24.9 pg/mL (n = 12), 10.6% for bioavailable estradiol at a mean concentration of 48.1% (n = 10), 5.6% for estrone at a mean concentration of 101.7 pg/mL (n = 10), 4.7% for SHBG at a mean concentration of 104.6 nM/L (n = 10), 9.4% for androstenedione at a mean concentration of 1.33 ng/mL (n = 10), 5.2% for DHEAS at a mean concentration of 75 μg/dL (n = 10), 4.5% for testosterone at a mean concentration of 0.83 ng/mL (n = 10) and 11.6% for free testosterone at a mean concentration of 5.4 pg/mL (n = 10). Interassay coefficients of variation were 9.7% for estradiol at a mean concentration of 28.0 pg/mL (n = 8), 11.9% for bioavailable estradiol at a mean concentration of 52.3% (n = 9), 11.1% for estrone at a mean concentration of 90.1 pg/mL (n = 8), 5.5% for SHBG at a mean concentration of 124.0 nM/L (n = 10), 9.8% for androstenedione at a mean concentration of 1.10 ng/mL (n = 20), 5.3% for DHEAS at a mean concentration of 92.5 μg/dL (n = 20), 7.7% for testosterone at a mean concentration of 0.90 ng/mL (n = 20) and 9.0% for free testosterone at a mean concentration of 6.4 pg/mL (n = 10). All hormone assays were performed by a commercial laboratory (Mitsubishi Kagaku Bio-Clinical Laboratories, Tokyo, Japan).

Statistical analysis

We excluded a total of 19 participants with estrone values >125 pg/mL, estradiol values >75 pg/mL or testosterone values >125 ng/dL (indicating postmenopausal hormone use), leaving 185 Japanese, 44 Japanese Brazilians and 134 non-Japanese Brazilians for inclusion in the present analyses.

All hormone values were natural log-transformed to produce approximately normal distributions. Geometric mean hormone levels according to the three populations, known breast cancer risk factors and lifestyle factors were calculated using multivariate regression analysis. The following variables were used for adjustment: age, ethnic group, age at first menarche, age at menopause, number of births, age at first birth, height, body mass index (BMI), smoking status, alcohol drinking habits and physical activity during the past 5 years. Analysis of covariance was used to test for differences in mean hormone levels across the three populations, known breast cancer risk factors and lifestyle factors. For comparisons among the three populations, Japanese Brazilians living in São Paulo were used as the reference group. Linear trends for mean hormone levels were tested in the multivariate regression model using categories of each factor as ordinal or continuous variables. All P values reported are two-sided, and the statistical significance level was set at $P < 0.05$. All statistical analyses were performed using SAS software version 9.1 (SAS Institute, Inc., Cary, NC, USA).

Results

The characteristics of the study populations are presented in Table 1. Japanese participants had a later menarche, fewer births and lower BMI, and they smoked less, drank more and were physically more active than the other two populations. On the other hand, non-Japanese Brazilians had earlier ages at menopause and at first birth, more births and greater BMI,

Table 1 Characteristics of study populations

	Japanese living in Nagano, Japan	Japanese Brazilians living in São Paulo, Brazil	Non-Japanese Brazilians living in São Paulo, Brazil	*P* for difference
Number of participants	185	44	134	
Mean age (±SE), yr	62.8 (0.40)	63.8 (0.82)	63.6 (0.47)	0.37
P[a]	0.31	Reference	0.84	
Family history of breast cancer, *n* (%)	17 (9.2)	5 (11.4)	14 (10.5)	0.92
P[a]	0.68	Reference	0.98	
History of benign breast disease, *n* (%)	11 (6.0)	4 (9.1)	8 (6.0)	0.46
P[a]	0.65	Reference	0.62	
Mean age at first menarche (±SE), yr	13.9 (0.12)	13.2 (0.26)	13.3 (0.15)	<0.01
P[a]	<0.01	Reference	0.75	
Mean age at menopause (±SE), yr	50.0 (0.34)	50.8 (0.69)	48.2 (0.40)	<0.01
P[a]	0.29	Reference	<0.01	
Nulliparous, *n* (%)	17 (9.2)	5 (11.4)	15 (11.2)	0.55
P[a]	0.58	Reference	0.81	
Number of births (more than four births), *n* (%)[b]	6 (3.6)	12 (30.8)	57 (47.9)	<0.01
P[a]	<0.01	Reference	<0.01	
Mean age at first birth (±SE), yr[b]	26.2 (0.34)	26.5 (0.72)	23.6 (0.41)	<0.01
P[a]	0.65	Reference	<0.01	
Breast feeding (yes), *n* (%)[b]	154 (93.3)	35 (89.7)	107 (89.9)	0.72
P[a]	0.27	Reference	0.61	
Mean height (±SE), cm	152.9 (0.43)	151.8 (0.89)	157.1 (0.52)	<0.01
P[a]	0.29	Reference	<0.01	
Mean body mass index (±SE), kg/m^2	23.4 (0.28)	24.7 (0.57)	27.0 (0.34)	<0.01
P[a]	0.04	Reference	<0.01	
Smoking (ever smoker), *n* (%)	6 (3.3)	7 (15.9)	38 (28.4)	<0.01
P[a]	<0.01	Reference	<0.01	
Alcohol drinking (drinker), *n* (%)	67 (36.2)	5 (11.4)	25 (18.7)	<0.01
P[a]	<0.01	Reference	0.63	
Physical activity in past 5 years (yes), *n* (%)	85 (46.5)	19 (43.2)	26 (19.4)	<0.01
P[a]	<0.01	Reference	<0.01	

[a]*P* values for comparison with Japanese Brazilians living in São Paulo, Brazil; [b]Among parous women only.

and they smoked more and were taller and physically less active than the other two populations. Japanese Brazilians had an earlier menarche, more births and greater BMI, and they smoked more, drank less and were physically less active than Japanese, but they had later ages at menopause and first birth, fewer births and lower BMI, and they smoked less and were shorter and physically more active than non-Japanese Brazilians.

Because of an insufficient amount of sampled blood, we did not measure the levels of the following hormones: estradiol for 17 participants; bioavailable estradiol, estrone or SHBG for two participants each; or androstenedione for one participant. The proportion of participants with levels below the LOD were 0.9% for estradiol, 3.6% for estrone, 0% for bioavailable estradiol and SHBG, 0.6% for androstenedione and DHEAS, 24% for testosterone and 69% for free testosterone.

Adjusted hormone levels varied significantly across the three populations for all hormones (Table 2). Japanese Brazilians had significantly higher levels of estradiol, bioavailable estradiol, estrone, testosterone and free testosterone, and lower SHBG levels, than Japanese, whereas levels of androstenedione and DHEAS did not differ between the two populations (Table 2). Similar results were seen for analyses stratified by BMI (under and over 25), except for androstenedione level, which did not differ between Japanese Brazilians and Japanese whose BMI was under 25, but androstenedione level was significantly lower among Japanese Brazilians than among Japanese whose BMI was over 25 (Table 3).

Table 2 Adjusted geometric mean hormone levels in three populations[a]

	Japanese living in Nagano, Japan	Japanese Brazilians living in São Paulo, Brazil	Non-Japanese Brazilians living in São Paulo, Brazil	*P* for difference
Estradiol, pg/mL				
Age-adjusted	9.0	13.8	15.5	<0.01
(95% CI)	(8.6 to 9.4)	(12.5 to 15.3)	(14.6 to 16.5)	
P[a]	<0.01	Reference	0.052	
Multivariate[b]	9.7	14.3	15.5	<0.01
(95% CI)	(8.7 to 10.9)	(12.5 to 16.4)	(14.0 to 17.1)	
P[a]	<0.01	Reference	0.28	
Bioavailable estradiol, %				
Age-adjusted	23.1	30.6	22.9	<0.01
(95% CI)	(22.1 to 24.1)	(28.0 to 33.4)	(21.7 to 24.1)	
P[a]	<0.01	Reference	<0.01	
Multivariate[b]	23.7	30.2	20.6	<0.01
(95% CI)	(21.6 to 26.0)	(27.0 to 33.8)	(19.0 to 22.3)	
P[a]	<0.01	Reference	<0.01	
Estrone, pg/mL				
Age-adjusted	23.0	40.3	34.1	<0.01
(95% CI)	(22.0 to 24.0)	(36.8 to 44.1)	(32.4 to 35.9)	
P[a]	<0.01	Reference	<0.01	
Multivariate[b]	23.8	41.1	33.3	<0.01
(95% CI)	(21.5 to 26.3)	(36.5 to 46.3)	(30.6 to 36.4)	
P[a]	<0.01	Reference	<0.01	
Sex hormone-binding globulin, nM/L				
Age-adjusted	74.1	54.3	60.2	<0.01
(95% CI)	(69.4 to 79.1)	(47.5 to 62.0)	(55.8 to 65.1)	
P[a]	<0.01	Reference	0.18	
Multivariate[b]	68.4	53.0	70.7	0.01
(95% CI)	(59.5 to 78.5)	(44.9 to 62.4)	(62.6 to 79.7)	
P[a]	<0.01	Reference	<0.01	
Androstenedione, ng/mL				
Age-adjusted	0.65	0.56	1.04	<0.01
(95% CI)	(0.60 to 0.70)	(0.47 to 0.66)	(0.95 to 1.15)	
P[a]	0.12	Reference	<0.01	
Multivariate[b]	0.73	0.60	1.00	<0.01
(95% CI)	(0.61 to 0.88)	(0.48 to 0.76)	(0.85 to 1.18)	
P[a]	0.06	Reference	<0.01	
DHEAS, µg/dL				
Age-adjusted	50.6	58.0	44.5	0.03
(95% CI)	(46.3 to 55.4)	(48.2 to 69.8)	(40.0 to 49.4)	
P[a]	0.19	Reference	0.01	
Multivariate[b]	57.2	63.1	46.7	0.04
(95% CI)	(46.6 to 70.2)	(49.4 to 80.6)	(39.2 to 55.8)	
P[a]	0.38	Reference	0.02	
Testosterone, ng/mL				
Age-adjusted	0.02	0.11	0.18	<0.01
(95% CI)	(0.02 to 0.03)	(0.07 to 0.17)	(0.14 to 0.24)	
P[a]	<0.01	Reference	0.06	
Multivariate[b]	0.03	0.10	0.14	<0.01
(95% CI)	(0.02 to 0.04)	(0.06 to 0.20)	(0.09 to 0.22)	
P[a]	<0.01	Reference	0.38	
Free testosterone, pg/mL				

Table 2 Adjusted geometric mean hormone levels in three populations[a] *(Continued)*

Age-adjusted	0.21	0.39	0.44	<0.01
(95% CI)	(0.19 to 0.23)	(0.33 to 0.46)	(0.40 to 0.48)	
P^a	<0.01	Reference	0.18	
Multivariate[b]	0.22	0.39	0.39	<0.01
(95% CI)	(0.19 to 0.26)	(0.32 to 0.47)	(0.34 to 0.45)	
P^a	<0.01	Reference	0.92	

DHEAS, dehyroepianrosterone sulfate; 95% CI, 95% confidence interval; [a]*P* values for comparison with Japanese Brazilians living in São Paulo, Brazil; [b]Adjusted for age (continuous), age at first menarche (continuous), age at menopause (continuous), number of births (0, 1, 2 or 3, 4+), age at first birth (≤22, 23 to 26, ≥27 yr, nulliparous), height (continuous), body mass index (continuous), smoking (never smokers, past smokers, current smokers), alcohol drinking (nondrinkers, occasional drinker, regular drinkers), and physical activity in past 5 years (no, ≤2 days/wk, ≥3 days/wk).

Japanese Brazilians had significantly higher levels of bioavailable estradiol, estrone and DHEAS, and lower levels of SHBG and androstenedione, than non-Japanese Brazilians. Levels of estradiol, testosterone and free testosterone, however, did not differ between Japanese Brazilians and non-Japanese Brazilians (Table 2). Similar results were obtained when analyses were stratified by BMI (under and over 25), except for estrone and DHEAS. Levels of estrone were significantly higher among Japanese Brazilians than among non-Japanese Brazilians in individuals with a BMI under 25, but estrone levels did not differ between the two populations in individuals whose BMI was over 25, while DHEAS level did not differ regardless of BMI (under or over 25) (Table 3).

We further examined associations between endogenous sex hormone levels and known breast cancer risk factors or lifestyle factors (Table 4). BMI was significantly associated with higher estradiol, bioavailable estradiol, estrone, androstenedione, testosterone and free testosterone levels, as well as lower SHBG levels, but was not associated with DHEAS levels. Stratified analyses by study site (that is, the study in Nagano vs. the study in São Paulo) showed similar results for the two study sites. No statistically significant associations were observed between sex hormone levels and family history of breast cancer, history of benign breast disease, age at first menarche, age at menopause, parity, number of births, age at first birth, breast-feeding, height, smoking, alcohol drinking or physical activity during the past 5 years except for the following. We found a significantly higher level of SHBG among women who had a later age at menopause and among shorter women. We also observed a significantly higher level of DHEAS among women who had more births and a significantly lower level of testosterone among physically more active women. In stratified analyses by study site, however, we did not observe any findings which were consistent between the sites.

Discussion

In this cross-sectional study among postmenopausal Japanese, Japanese Brazilian and non-Japanese Brazilian women, we found significant differences in endogenous sex hormones among the three populations even after adjustment for known breast cancer risk factors. In particular, levels of estrogen and androgen in Japanese Brazilians were higher than levels in Japanese and were similar to or higher than levels in non-Japanese Brazilians. This pattern was observed for women with BMI values under and over 25. We also confirmed an increase in estrogen and androgen levels and a decrease in SHBG levels with increasing BMI.

As an initial comment, several methodological limitations of this study should be considered. First, our findings might be subject to the difference in study methods between Japan and Brazil, albeit that the two studies were conducted under a similar protocol. For example, we used serum samples for Japanese and plasma samples for both Japanese Brazilians and non-Japanese Brazilians. In this regard, we measured estrone levels in both serum and plasma from the same participants (n = 38). Although both levels were highly correlated (correlation coefficient = 0.94) and the percentage difference was relatively small (mean = -4%; 95% confidence interval, -9% to 1%), we used corrected values for the present study because the kit for estrone was applicable to serum samples only. Concurrently, we compared estrone levels among the three populations using crude values and observed the same results. The difference in blood samples is therefore unlikely to have affected the difference in sex hormone levels between the two populations. Given that blood collection methods also differed between the Japan and Brazil study sites, in addition to the types of blood samples used, we cannot exclude the possibility that our findings were affected by these differences. Another example is the difference in questionnaire data and data collection methods between Japan and Brazil. If such differences led to exposure misclassification, this might explain the observed absence of associations between sex hormone levels and known breast cancer risk factors or lifestyle factors. Second, although at least more than 96% of participants had detectable levels of estradiol, estrone, bioavailable estradiol, SHBG, androstenedione and DHEAS, the

Table 3 Adjusted geometric mean hormone levels[a] of three populations with stratification by body mass index[b]

	Japanese living in Nagano, Japan	Japanese Brazilians living in São Paulo, Brazil	Non-Japanese Brazilians living in São Paulo, Brazil	*P* for difference
Estradiol, pg/mL				
Low (BMI < 25)	9.5	14.2	15.0	<0.01
P[c]	<0.01	Reference	0.60	
High (BMI ≥25)	8.2	12.2	14.5	<0.01
P[c]	<0.01	Reference	0.06	
Bioavailable estradiol, %				
Low (BMI <25)	22.4	28.7	17.9	<0.01
P[c]	<0.01	Reference	<0.01	
High (BMI ≥ 25)	25.6	32.5	23.4	<0.01
P[c]	<0.01	Reference	<0.01	
Estrone, pg/mL				
Low (BMI < 25)	22.5	40.4	32.1	<0.01
P[c]	<0.01	Reference	<0.01	
High (BMI ≥25)	23.2	38.4	34.2	<0.01
P[c]	<0.01	Reference	0.19	
Sex hormone-binding globulin, nM/L				
Low (BMI < 25)	76.6	62.8	85.8	0.03
P[c]	0.04	Reference	<0.01	
High (BMI ≥25)	59.6	43.8	59.5	0.03
P[c]	0.02	Reference	0.02	
Androstenedione, ng/mL				
Low (BMI < 25)	0.64	0.63	0.91	0.03
P[c]	0.90	Reference	0.02	
High (BMI ≥25)	0.76	0.51	1.05	<0.01
P[c]	0.03	Reference	<0.01	
DHEAS, μg/dL				
Low (BMI < 25)	51.9	64.7	48.7	0.21
P[c]	0.13	Reference	0.11	
High (BMI ≥25)	54.6	52.2	43.4	0.29
P[c]	0.81	Reference	0.32	
Testosterone, ng/mL				
Low (BMI < 25)	0.01	0.07	0.13	<0.01
P[c]	<0.01	Reference	0.27	
High (BMI ≥25)	0.04	0.15	0.18	<0.01
P[c]	<0.01	Reference	0.69	
Free testosterone, pg/mL				
Low (BMI < 25)	0.18	0.32	0.31	<0.01
P[c]	<0.01	Reference	0.90	
High (BMI ≥25)	0.26	0.46	0.48	<0.01
P[c]	<0.01	Reference	0.85	

BMI, body mass index; DHEAS, dehyroepianrosterone sulfate; [a]Adjusted for age (continuous), age at first menarche (continuous), age at menopause (continuous), number of births (0, 1, 2 or 3, 4+), age at first birth (≤22, 23 to 26, ≥27, nulliparous), height (continuous), BMI (continuous), smoking (never smokers, past smokers, current smokers), alcohol drinking (nondrinkers, occasional drinkers, regular drinkers) and physical activity in the past 5 years (no, ≤2 days/wk, ≥3 days/wk); [b]The total participants in the low and high BMI groups were 199 and 156, respectively; [c]*P* values for comparison with Japanese Brazilians living in São Paulo, Brazil.

proportion of participants with levels below the LOD was relatively high for testosterone (24%) and free testosterone (69%). Our findings for testosterone and free testosterone should therefore be interpreted cautiously. Third, since our study included only a small number of Japanese Brazilians (n = 44), the findings might be due to chance and should be interpreted with caution.

We found higher circulating levels of estrogen and androgen in Japanese Brazilians than in Japanese, which were not accounted for by differences in the prevalence

Table 4 Adjusted geometric mean hormone levels by breast cancer risk factors and lifestyle-factors[a]

Breast cancer risk and lifestyle factors	Participants, *n*	Estradiol, pg/mL	Bioavailable estradiol, %	Estrone, pg/mL	Sex hormone-binding globulin, nM/L	Androstenedione, ng/mL	DHEAS, µg/dL	Testosterone, ng/mL	Free testosterone, pg/mL
Family history of breast cancer									
No	327	13.9	22.7	32.6	66.2	0.84	52.7	0.09	0.34
Yes	36	13.8	21.2	31.6	74.6	0.80	51.4	0.05	0.36
P for difference		0.90	0.18	0.57	0.12	0.66	0.83	0.08	0.40
History of benign breast disease									
No	339	13.9	22.6	32.5	66.9	0.84	52.9	0.09	0.34
Yes	23	14.3	22.0	33.5	69.0	0.78	52.1	0.08	0.31
P for difference		0.69	0.68	0.67	0.75	0.61	0.92	0.72	0.38
Age at first menarche, yr									
<12	101	13.7	22.9	31.6	66.7	0.83	49.5	0.08	0.33
13 or 14	166	13.9	22.2	32.4	65.2	0.83	54.8	0.09	0.34
15+	96	13.9	22.6	33.6	69.7	0.85	53.3	0.08	0.35
P for trend		0.81	0.81	0.18	0.51	0.78	0.43	0.99	0.60
P for trend[b]		0.70	0.47	0.30	0.24	0.68	0.29	0.83	0.39
Age at menopause, yr									
<48	116	14.0	23.0	32.6	64.5	0.89	57.0	0.08	0.34
49 to 51	108	14.0	22.0	33.1	70.2	0.78	51.6	0.09	0.34
52+	139	13.6	22.5	32.1	67.0	0.80	48.5	0.09	0.33
P for trend		0.47	0.65	0.68	0.57	0.20	0.05	0.66	0.75
P for trend[b]		0.80	0.06	0.93	0.02	0.32	0.51	0.59	1.00
Parity									
Parous	326	13.8	22.0	32.3	67.5	0.80	48.4	0.08	0.33
Nulliparous	37	13.7	23.3	32.9	67.2	0.87	58.0	0.10	0.34
P for difference		0.89	0.28	0.73	0.95	0.42	0.11	0.51	0.86
Number of births[c]									
1	32	13.7	20.6	32.8	69.6	0.77	43.7	0.10	0.30
2 or 3	219	13.4	22.2	31.6	67.8	0.79	43.9	0.08	0.32
4+	75	14.7	22.3	33.2	65.8	0.86	56.0	0.08	0.35
P for trend		0.27	0.26	0.71	0.55	0.38	0.046	0.76	0.20
Age at first birth[c], yr									
<22	79	13.2	21.3	31.5	70.9	0.80	44.0	0.09	0.31
23 to 26.9	138	13.9	21.5	33.1	68.1	0.78	46.7	0.07	0.33
27+	109	14.7	22.3	33.1	64.3	0.84	52.2	0.10	0.32
P for trend		0.09	0.29	0.52	0.16	0.47	0.11	0.40	0.89
P for trend[b]		0.10	0.32	0.53	0.37	0.58	0.39	0.47	0.81
Breast-feeding[c]									
No	27	14.3	23.2	33.5	63.4	0.82	46.9	0.09	0.33
Yes	296	13.7	21.9	32.2	67.6	0.81	47.2	0.08	0.32

Table 4 Adjusted geometric mean hormone levels by breast cancer risk factors and lifestyle-factors[a] *(Continued)*

P for difference		0.59	0.33	0.53	0.47	0.87	0.96	0.85	0.87
Height, cm									
<150.9	107	13.8	22.3	32.2	69.4	0.84	54.7	0.09	0.34
151 to 156.9	126	14.3	22.1	33.4	67.2	0.81	51.9	0.08	0.34
157+	124	13.7	23.2	32.2	63.8	0.85	51.7	0.09	0.34
P for trend		0.83	0.31	0.99	0.16	0.91	0.54	0.71	0.86
P for trend[b]		0.62	0.07	0.65	0.01	0.33	0.96	0.47	0.72
BMI, kg/m^2									
<24.9	199	13.3	20.9	31.1	75.3	0.77	51.1	0.07	0.30
25 to 29.9	116	14.5	24.2	32.2	60.2	0.79	48.4	0.09	0.34
30+	40	15.5	26.4	38.4	51.2	1.15	65.3	0.16	0.50
P for trend		0.01	<0.01	<0.01	<0.01	0.01	0.21	0.01	<0.01
P for trend[b]		<0.01	<0.01	<0.01	<0.01	<0.01	0.13	0.01	<0.01
Smoking									
Never smoker	310	13.2	24.3	32.0	62.9	0.80	53.5	0.09	0.35
Past smoker	37	13.6	23.7	32.4	62.3	0.77	51.4	0.06	0.38
Current smoker	14	14.9	20.0	33.2	76.3	0.94	52.8	0.12	0.29
P for difference		0.48	0.06	0.91	0.28	0.55	0.95	0.43	0.28
Alcohol drinking									
Nondrinker	266	14.0	22.0	32.7	69.9	0.85	49.4	0.10	0.34
Occasional drinker	39	14.1	23.5	32.4	63.7	0.82	59.1	0.08	0.34
Regular drinker	58	13.5	22.2	32.4	67.1	0.83	49.8	0.08	0.34
P for difference		0.76	0.48	0.97	0.42	0.89	0.29	0.48	0.98
Physical activity in past 5 years									
No	231	14.0	22.5	32.8	66.7	0.84	52.2	0.11	0.34
≤2 days/wk	63	13.8	22.1	32.1	67.5	0.79	50.6	0.05	0.33
≥3 days/wk	68	13.5	23.3	32.1	66.8	0.85	55.8	0.07	0.35
P for trend		0.46	0.48	0.58	0.95	0.97	0.56	0.02	0.60

DHEAS, dehyroepianrosterone sulfate; BMI, body mass index; [a]Adjusted for age (continuous), ethnic group (Japanese, Japanese Brazilians, non-Japanese Brazilians (Caucasian, mixed, Black), age at first menarche (continuous), age at menopause (continuous), number of births (0, 1, 2 or 3, 4+), age at first birth (≤22, 23 to 26, ≥27 yr, nulliparous), height (continuous), BMI (continuous), smoking (never smokers, past smokers, current smokers), alcohol drinking (nondrinkers, occasional drinkers, regular drinkers) and physical activity in the past 5 years (no, ≤2 days/wk, ≥3 days/wk); [b]Continuous variables; [c]Among parous women only.

of known breast cancer risk factors. This hormonal profile in Japanese Brazilians is consistent with the higher incidence and mortality rate of breast cancer in this population [4-6]. For instance, the age-adjusted incidence per 100,000 population for breast cancer among first-generation Japanese Brazilians from 1969 to 1978 was 24, while the incidences among Japanese from 1973 to 1977 were 12.7 in Osaka and 17.5 in Miyagi [4]. The standard mortality ratio for breast cancer among first-generation Japanese Brazilians from 1999 to 2001 on the basis of age-specific rates for Japanese in 2000 was 139 [5].

We also found higher circulating levels of bioavailable estradiol and estrone in Japanese Brazilians than in non-Japanese Brazilians, although levels of estradiol, testosterone and free testosterone did not significantly differ between the two populations. In the Multiethnic Cohort Study, Japanese Americans had significantly higher

estradiol levels than Caucasians and a slightly higher risk factor-adjusted incidence of breast cancer [10,18]. Although previous studies have shown lower incidence and mortality rates of breast cancer among Japanese Brazilians than among non-Japanese Brazilians [4-6], our findings suggest that the recent incidence and mortality rates among Japanese Brazilians might be similar to or higher than those of non-Japanese Brazilians.

The significant difference in sex hormone levels between Japanese Brazilians and Japanese might be determined by long-term exposure to environmental and lifestyle factors in Brazil. These differences were observed even after adjustment for known breast cancer risk factors, including BMI, which is a major determinant of estrogen levels in postmenopausal women. Although diet is one environmental factor that substantially differs between Japan and Brazil, the present study did not take into account dietary factors because we used different FFQ in the case-control studies in Nagano and São Paulo. Given that the report from the World Cancer Research Fund and American Institute for Cancer Research in 2007 showed no convincing or probable dietary risk factors for breast cancer [19], however, the difference in sex hormone levels between the two populations might not be explained by dietary factors only.

We observed an increase in estrogen and androgen levels and a decrease in SHBG levels with increasing BMI. Our findings are in general agreement with those of previous studies, and these associations have been consistently observed among both Asian and Western populations [10-13,15]. On the other hand, the determinants of sex hormone levels in postmenopausal women have not been firmly established, notwithstanding a relatively large number of epidemiological studies [10-14,16]. In the present study, we found a higher level of SHBG among women who had a later age at menopause and among shorter women. We also observed a higher level of DHEAS among women who had more births and a lower level of testosterone among physically more active women. In addition to the lack of consistency in these findings between the two study sites (that is, the study in Nagano vs. the study in São Paulo), our findings are inconsistent with those of previous studies, which found no significant associations among age at menopause, height and SHBG level, for example, or number of births and DHEAS level [12-14]. Higher physical activity levels were associated with lower levels of both estrogen and androgen [11,16], while another study reported no such association [10]. Given this lack of consistency with previous studies, our findings might be explained by multiple comparisons.

Conclusions

We found that levels of estrogen and androgen in Japanese Brazilians were higher than those in Japanese and similar to or higher than levels in non-Japanese Brazilians. Our findings may explain the previously observed increase in the incidence and mortality rate of breast cancer among Japanese Brazilians.

Abbreviations

BMI: body mass index; DHEAS: dehydroepiandrosterone sulfate; FFQ: food frequency questionnaire; IRMA: immunoradiometric assay; LOD: lower detection limit; SHBG: sex hormone-binding globulin.

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Authors' contributions

MI made substantial contribution to the conception and design of the study, as well as the analysis and interpretation of data, and was involved in drafting the manuscript. YK, SY, HO, HN, RK, GSH, INN, MSM, JM, FML and RA made substantial contributions to the study conception and design and the acquisition of data and were involved in critically revising the manuscript for important intellectual content. ST made substantial contributions to the study conception and design, as well as the analysis and interpretation of data, and was involved in critically revising the manuscript for important intellectual content. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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EPIDEMIOLOGY

Fragment c gamma receptor gene polymorphisms and breast cancer risk in case–control studies in Japanese, Japanese Brazilians, and non-Japanese Brazilians

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Abstract Previous studies showing the presence of antibodies against tumor-associated antigens in healthy individuals suggest that antibody-dependent cell cytotoxicity (ADCC) might play a role in the development of breast cancer. We hypothesized that functional polymorphisms in fragment c gamma receptor (FcgR) genes were associated with breast cancer risk. We conducted hospital-based case–control studies of patients aged 20–74 years with invasive breast cancer, and matched controls from medical checkup examinees in Nagano, Japan and from cancer-free patients in São Paulo, Brazil. A total of 869 pairs (403 Japanese, 80 Japanese Brazilians and 386 non-Japanese Brazilians) were genotyped for two single nucleotide polymorphisms (SNPs): a histidine (H)/arginine (R) polymorphism at position 131 of FcgRIIa (FcgRIIa H131R) and a valine (V)/phenylalanine (F) polymorphism at position 158 of FcgRIIIa (FcgRIIIa F158V). We found no statistically significant association between either of the two SNPs and breast cancer risk regardless of population. In analyses of the three populations combined, adjusted odds ratio (OR) was 0.93 [95% confidence interval (CI) 0.66–1.32] for women with the R/R versus H/H genotype of the FcgRIIa H131R polymorphism and 1.04 (95% CI 0.69–1.57) for the

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V/V versus F/F genotype of the FcgRIIIa F158V polymorphism. On combination of the two SNPs, compared to women with both the R/R genotype of the FcgRIIa H131R polymorphism and F/F genotype of the FcgRIIIa F158V polymorphism, the adjusted OR for women with both the H/H and V/V genotype was 0.68 (95% CI 0.37–1.27). In conclusion, our findings suggest that ADCC might not play a major role in the etiology of breast cancer.

Keywords Fragment c gamma receptor gene · Single nucleotide polymorphism · Breast cancer · Case–control study · Immigrants

Abbreviations

ADCC	Antibody-dependent cell cytotoxicity
CI	Confidence interval
FcgR	Fragment c gamma receptor
HER2	Human epidermal growth factor receptor 2
MUC1	Epithelial mucin
NK	Natural killer
OR	Odds ratio
SNP	Single nucleotide polymorphism

Introduction

Breast cancer is the most common malignancy in women in Japan and many other parts of the world [1, 2]. Although sex hormones, particularly estrogens, play an important role in the etiology of breast cancer [3], present knowledge has proved insufficient to allow the disease to be overcome, and the identification of other important etiological factors thus requires further study.

It has been hypothesized that the immune system recognizes malignant cells as foreign agents and eliminates them. Although several epidemiological studies have supported this hypothesis, only a few studies have investigated the role of the immune system in the etiology of breast cancer [4–6]. Natural killer (NK) cells are large granular lymphocytes that mediate innate immunity against pathogens and tumors. Natural cytotoxicity is believed to play an important role in host anti-cancer defense mechanisms. In their cohort study of 3,625 participants in Japan with 11-year follow-up, e.g., Imai and colleagues [5] showed that high cytotoxic activity of peripheral blood lymphocytes was associated with a decreased risk of total cancer, while Dewan et al. [6] recently reported that NK activities of peripheral blood mononuclear cells from breast cancer patients were significantly lower than those of healthy individuals.

NK cells are also capable of mediating antibody-dependent cell cytotoxicity (ADCC) against antibody-coated targets via the expression of a low-affinity receptor for IgG [fragment c gamma receptor (FcgR) III]. Many kinds of autoantibodies against tumor-associated antigens have been investigated, some of which are also detected in healthy control sera [7]. Anti-epithelial mucin (MUC1) antibodies, e.g., are frequently detected in healthy individuals, particularly in women during pregnancy and lactation [8, 9]. Notably, Forsman and colleagues [10] reported that serum from multiparous women, but not nulliparous women or men, contained antibodies which selectively mediated ADCC against established mammary carcinoma cell lines. Given that breast cancer risk is higher in nulliparous than multiparous women, this finding suggests that ADCC might play a role in the development of breast cancer.

Immune effector cells, including NK cells, recognize antibodies bound to target cells through FcgRs and elicit ADCC activity, which can be modulated by FcgR gene polymorphisms. At least two functional FcgR gene polymorphisms that may affect the killing function of immune effector cells have been identified: a histidine (H)/arginine (R) polymorphism at position 131 of FcgRIIa (FcgRIIa H131R) and a valine (V)/phenylalanine (F) polymorphism at position 158 of FcgRIIIa (FcgRIIIa F158V) [11–14]. The H allele of the FcgRIIa H131R polymorphism has higher binding efficiency to human IgG2 than the R allele, and confers enhanced phagocytic activity [13, 14]. The V allele of the FcgRIIIa F158V polymorphism has higher affinity for human IgG than the F allele and cells bearing this allele mediate ADCC more effectively than those with the F allele [11, 12]. In fact, ADCC is a potential anti-tumor mechanism behind targeted therapy with the humanized monoclonal antibody trastuzumab for human epidermal growth factor receptor 2 (HER2)-positive breast cancer [15]. FcgR gene polymorphisms have, therefore, been suggested to modulate the clinical efficacy of trastuzumab-based therapy in patients with metastatic HER2-positive breast cancer [16].

In addition to this putative effect of polymorphisms in the FcgR gene in modulating ADCC activity, roles in several other mechanisms in the immune system have been suggested, with FcgRs on leukocytes also modulating phagocytosis, clearance of immune complexes, superoxide generation, degranulation, cytokine production, and regulation of antibody production [17].

To better understand the role of the immune system in the etiology of breast cancer, we tested the hypothesis that polymorphisms in the FcgR gene are associated with the risk of breast cancer using data from hospital-based case–control studies in Nagano, Japan and São Paulo, Brazil.

Materials and methods

Study subjects

We conducted multicenter, hospital-based case–control studies of breast cancer in Japan and Brazil. In addition to determining lifestyle factors and genetic susceptibility to the risk of breast cancer, the protocols of these studies were also designed to compare potential risk factors among Japanese living in Nagano, Japan, and Japanese Brazilians and non-Japanese Brazilians living in São Paulo, Brazil [18, 19]. Eligible case patients were a consecutive series of female patients aged 20–74 years with newly diagnosed and histologically confirmed invasive breast cancer. Case patients were recruited between 2001 and 2005 at four hospitals in Nagano, and between 2001 and 2006 at eight hospitals in São Paulo. A total of 405 case patients (98%) participated in Nagano, and 83 Japanese Brazilian (91%) and 389 non-Japanese Brazilian case patients (99%) in São Paulo. In the Nagano study, eligible control subjects were selected from medical checkup examinees in two of the four hospitals who were confirmed not to have cancer. One control subject was matched for each case patient by age (within 3 years) and residential area during the study period. Among potential controls, one examinee declined participation and two declined the provision of blood samples. Consequently, we obtained written informed consent from 405 matched pairs. In the São Paulo study, eligible control subjects were preferentially selected from cancer-free patients who visited the same hospital as the index patients. One control was matched with each case by age (within 5 years) and ethnicity during the study period. Among potential control subjects, 22 patients declined participation (participation rate = 96%). Consequently, 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 CONEP (Comissão Nacional de Ética em Pesquisa), Brasília, Brazil and by the institutional review board of the National Cancer Center, Tokyo, Japan.

Data collection

Participants in Nagano were asked to complete a self-administered questionnaire, while those in São Paulo were given in-person interviews conducted by trained interviewers using a structured questionnaire. The two questionnaires contained closely similar questions concerning demographic characteristics, medical history, family history of cancer, menstrual and reproductive history, anthropometric factors, physical activity, and smoking habits.

Information on estrogen receptor (ER) and progesterone receptor (PR) status was obtained from medical records. Hormone receptor status was determined by either enzyme-linked immunoassay or immunohistochemical assay. Hormone receptor positivity values were determined either as specified by the laboratory that performed the assay or in accordance with the laboratory's written interpretation thereof, or both.

Participants in Nagano provided blood at the time they returned their self-administered questionnaire, and those in São Paulo at the time of interview. Blood samples were divided into serum aliquots in Nagano and into plasma aliquots and buffy layers in São Paulo. All blood samples were shipped to the Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan and stored at −80°C until analysis.

Genotyping

Genomic DNA samples were extracted from peripheral blood using Qiagen FlexiGene® DNA Kits (Qiagen K.K., Tokyo, Japan) according to the manufacturer's protocol. We genotyped two single nucleotide polymorphisms (SNPs), namely FcgRIIa H131R (rs1801274) and FcgRIIIa F158V (rs396991) by TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). Cases and matched controls were analyzed in the same well by laboratory personnel who did not know the case–control status. The quality of genotyping was assessed by duplicate quality control samples ($n = 140$), with concordance rates of 100% for FcgRIIa H131R and 99% for FcgRIIIa F158V.

Statistical analysis

We excluded subjects whose DNA samples were not available, leaving a total of 869 pairs (403 Japanese, 80 Japanese Brazilians and 386 non-Japanese Brazilians). 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 Chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer for SNPs and their combination were calculated using a conditional logistic regression model. Stratified analyses according to menopausal status and parity were calculated using an unconditional logistic regression model. Tests for interaction were performed based on the difference between two likelihood ratios of the models with and without the interaction terms. Associations between SNPs and hormone receptor-defined breast cancer were assessed by an unconditional polytomous logistic regression model. The Wald

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

Results

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

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

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

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

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

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

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

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

[a] Hardy–Weinberg equilibrium

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

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

[a] Crude OR

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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