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ORIGINAL ARTICLE

Genetic polymorphisms in estrogen metabolism and breast cancer risk in case–control studies in Japanese, Japanese Brazilians and non-Japanese Brazilians

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Although many studies have examined associations between single nucleotide polymorphisms (SNPs) in the *CYP1A1*, *CYP1A2* and *CYP1B1* genes and breast cancer risk, no study has examined functional SNPs in the *CYP3A5* gene and only a small number of studies have been investigated in Japanese populations. To examine the association between six SNPs, *CYP1A1*2A*, *CYP1A1*2C*, *CYP1A2*1F*, *CYP1B1* Arg^{48}Gly, *CYP1B1* Leu^{432}Val and *CYP3A5*3* and breast cancer risk, therefore, we conducted hospital-based case–control studies in Nagano, Japan and São Paulo, Brazil including 873 pairs (403 Japanese (JJ), 81 Japanese Brazilians (JB) and 389 non-Japanese Brazilians (NJB)). Although we found no significant association in the three populations combined, subgroup analyses revealed statistically significant associations of *CYP1A2*1F* in NJB, and *CYP1B1* Leu^{432}Val and *CYP3A5*3* in JJ with breast cancer risk. Compared to women with the AA genotype in *CYP1A2*1F*, the odds ratio (OR) (95% confidence interval (CI)) for NJB with the CC genotype was 0.54 (0.32–0.90); that for JJ with Leu/Val+Val/Val versus Leu/Leu genotype in *CYP1B1* Leu^{432}Val was 0.68 (0.48–0.97); and that for JJ with *3/*1+*1/*1 versus *3/*3 genotype in *CYP3A5*3* was 1.49 (1.10–2.04). Our findings provide further evidence that genetic polymorphisms related to estrogen metabolism may play a role in the development of breast cancer.
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Keywords: breast cancer; case–control study; cytochrome P450; immigrants; single nucleotide polymorphism

INTRODUCTION

Circulating levels of endogenous estrogens, such as estradiol and estrone have been associated with an increased risk of breast cancer.[1] An association between circulating levels of estrogen metabolites and risk has also been hypothesized, on the basis that these are potentially both estrogenic and genotoxic.[2,3] In particular, the association between the urinary ratio of 2-hydroxy (2-OHEs) to 16α-hydroxy estrogens (16α-OHEs) and breast cancer risk has been extensively examined,[4–8] whereas many studies have also investigated associations between single nucleotide polymorphisms (SNPs) related to estrogen metabolism and risk.[2]

Although many branch pathways in estrogen metabolism have been demonstrated after hydroxylation, the biological properties of the metabolites are determined mainly by the position of the hydroxylation. Estrogen hydroxylation is mediated by cytochrome P450 (CYP) enzymes in the liver, breast tissue or other tissues. Although the sites of localization of metabolism have not been precisely determined, postulated pathways demonstrated in previous expression analyses in human tissues and biochemical experiments are as follows:[9,10] 2-OHEs formation is likely catalyzed predominantly by CYP1A2 in the liver and CYP1A1 in the breast; 4-hydroxy estrogens (4-OHEs) formation is likely catalyzed predominantly by CYP1A2 in the liver and CYP1B1 in the breast; and 16-OHEs formation is likely catalyzed predominantly by CYP3A5 in the liver and CYP1A1 and CYP3A5 in the breast. Several functional SNPs in these genes have been identified, consisting of variant alleles with higher (*CYP1A1*2A*, *CYP1A1*2C*, *CYP1B1* Arg^{48}Gly and *CYP1B1* Leu^{432}Val)[2] or lower or deficient activities (*CYP1A2*1F* and *CYP3A5*3*)[11,12] (Table 1). Although a relatively large number of studies have examined associations between these SNPs and the risk of breast cancer,[2,13,15,24,25] no study has examined the association between functional SNPs in the *CYP3A5* gene and the risk of breast cancer, notwithstanding that *CYP3A5*3*

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Table 1 SNPs in genes involved in estrogen metabolism and their properties in previous studies

Genes	*dbSNP ID*	*Polymorphic nucleotide/ Amino acid change*	*Allele (trivial name)*	*Variant allele*	*Reported variant allele frequency %*[a]	*Presumed variant properties*[a]
CYP1A1	rs4646903	T3801C/3′-UTR	*CYP1A1*2A* (m1)	3801C	Japanese 36–43%,[13,14] Caucasian 5.2–11.7%,[15] African American 22.9–24.6%[15]	increased activity,[2] no effect on urinary ratio of 2-OHE1/16a-OHE1[16]
	rs1048943	A2455G/Ile^{462}Val	*CYP1A1*2C* (m2)	462Val	Japanese 25.4%,[13] Caucasian 3.9–8.6%,[15] African American 0–2.2%[15]	increased activity and inducibility[2]
CYP1A2	rs762551	−A163C/intron 1	*CYP1A2*1F*	163C	Japanese 36%,[14] Caucasian 28%,[14] African American 39%[14]	decreased activity and inducibility,[11] lower urinary ratio of 2-OHE1/16a-OHE1 and lower circulating E2 level[17]
CYP1B1	rs10012	C142G/Arg^{48}Gly		48Gly	Japanese 9.8%,[18] Caucasian 28%[19]	increased activity[2]
	rs1056836	G4326C/Leu^{432}Val	*CYP1B1*3*	432Val[b]	Japanese 20%,[14] Caucasian 43%,[14] African American 76%[14]	three-fold increase in activity[2] and lower urinary ratio of 2-OHE1/16 a-OHE1[16]
CYP3A5	rs776746	A6986G/intron3	*CYP3A5*3*	6986G	Japanese 74–77%,[20–23] Caucasian 91%,[23] African American 34%[23]	splicing defect[12]

Abbreviations: SNP, single nucleotide polymorphism; UTR, untranslated region.
[a]Numbers in superscript indicate reference numbers.
[b]Other studies regard the 433Leu allele as a variant allele,[24] but we follow Le Marchand L *et al.*[14] in this paper.

causes a splicing defect and decrease in the expression of functional CYP3A5 protein.[12]

Here, to investigate the associations between breast cancer risk and six functional SNPs (*CYP1A1*2A*, *CYP1A1*2C*, *CYP1A2*1F*, *CYP1B1* Arg^{48}Gly, *CYP1B1* Leu^{432}Val and *CYP3A5*3*) in genes related to estrogen hydroxylation, we analyzed data from three populations within hospital-based case–control studies in Nagano, Japan and São Paulo, Brazil: Japanese living in Nagano, Japan (JJ), Japanese Brazilians living in São Paulo (JB) and non-Japanese Brazilians living in São Paulo (NJB).

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 JJ, JB and NJB.[26] Eligible cases were a consecutive series of female patients aged 20–74 years with newly diagnosed and histologically confirmed invasive breast cancer. Cases 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 JJ cases (98%) participated in Nagano, and 83 JB (91%) and 389 NJB (99%) in São Paulo. In the study in Nagano, 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. Finally, 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 case by age (within 5 years) and ethnicity. Among potential controls, 22 patients refused to participate (participation rate=96%). Finally, we obtained written informed consent from 472 matched pairs (83 for JB and 389 for NJB). 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, whereas those in São Paulo were interviewed 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 and progesterone receptor 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.

Genotyping

Genomic DNA samples were extracted from the peripheral blood using QIAGEN FlexiGene DNA Kits according to the manufacturer's protocol. Genotyping of six SNPs, namely *CYP1A1*2A*, *CYP1A1*2C*, *CYP1A2*1F*, *CYP1B1* Arg^{48}Gly, *CYP1B1* Leu^{432}Val and *CYP3A5*3*, was performed by a commercial laboratory (Genetic Lab. Inc., Sapporo, Japan) using the TaqMan SNP Genotyping Assays developed by Applied Biosystems, USA (Table 1). Cases and matched controls were analyzed in the same well by laboratory personnel who did not know the case–control status.

Statistical analysis

We excluded subjects whose DNA samples were not available, leaving a total of 873 pairs (403 Japanese (JJ), 81 Japanese Brazilians (JB) and 389 non-Japanese Brazilians (NJB)). 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. Using a conditional logistic regression model, we calculated odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer for SNPs. An unconditional logistic regression model was used for stratified analyses according to menopausal status. In addition to matching factors, the following variables selected mainly based on a comparison of baseline characteristics between cases and controls, were adjusted for as potential confounders: menopausal status (premenopausal women, age at menopause for post-menopausal women (−43, 44–47, 48–51, 52+) for the three populations combined, (−47, 48–49, 50–51, 52+) for JJ, (−47, 48–49, 50–52, 53+) for JB, and (−43, 44–47, 48–50, 51+) for NJB), number of births (0, 1, 2, 3, 4+) and smoking status (never, ever smokers). All reported *P*-values are two-sided, and significance level was set at $P<0.05$. All

Table 2 Characteristics of case and matched control subjects

	Japanese living in Nagano, Japan (JJ)			*Japanese Brazilians living in São Paulo, Brazil (JB)*			*Non-Japanese Brazilians living in São Paulo, Brazil (NJB)*		
	Case (n=*403*)	*Control* (n=*403*)	P[a]	*Case* (n=*81*)	*Control* (n=*81*)	P[a]	*Case* (n=*389*)	*Control* (n=*389*)	P[a]
Age (years), mean (s.d.)	53.7	53.9	—	56.9	56.6	—	52.5	52.6	—
Premenopausal women (%)	45	35	<0.01	28	31	0.59	42	38	0.06
Age at menopause (years), mean[b]	48.9	49.4	0.14	49.9	50.4	0.73	49.2	48.2	0.07
Age at menarche (years), mean[b]	13.4	13.2	0.31	12.9	13.0	0.26	13.2	13.1	0.70
Nulliparous women (%)	14	14	0.91	23	16	0.24	11	10	0.73
Number of births (⩾4 births) (%)	2	2	0.09	7	20	0.02	28	35	0.09
Age at first birth (years), mean[b,c]	26.8	26.4	0.58	28.4	27.5	0.39	23.2	22.5	0.29
Breast feeding (yes) (%)[c]	91	96	0.03	92	91	0.56	88	90	0.79
Oral contraceptives user (%)	3	3	0.85	28	36	0.24	62	65	0.52
Family history of breast cancer (%)	11	6	0.03	15	12	0.65	6	6	0.77
History of benign breast diseases (%)	12	7	0.04	12	6	0.17	7	7	0.89
Height (cm), mean[b]	155.4	155.6	0.54	153.8	153.9	1.00	158.2	158.4	0.79
Body mass index (kg m^{-2}), mean[b]	22.7	23.0	0.07	24.4	24.7	0.37	26.6	26.1	0.07
Smoking (ever smoker) (%)	21	8	<0.01	23	15	0.18	43	35	0.03
Alcohol drinking (regular drinker) (%)	26	30	0.23	2	5	0.41	6	5	0.65
Physical activity past 5 years (moderate to strenuous) (%)	34	42	0.03	35	41	0.41	11	16	0.02

Abbreviations: JB, Japanese Brazilians living in São Paulo, Brazil; JJ, Japanese living in Nagano, Japan; NJB, Non-Japanese Brazilians living in São Paulo, Brazil.
[a]*P* for Mantel–Haenszel test with matched-pair strata.
[b]Adjusted for age.
[c]Among parous women.

statistical analyses were performed with SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Characteristics of cases and controls are shown in Table 2. For JJ, cases were more likely to be premenopausal and ever smokers, and 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 JB, cases were less likely than controls to have given birth. For NJB, cases were more likely than controls to be ever smokers and less likely to be physically active.

Allele frequencies of the SNPs among controls in each population are presented in Table 3. Genotype frequencies of each SNP were consistent with the Hardy–Weinberg equilibrium, except for *CYP1B1* Leu^{432}Val in NJB (*P*=0.01). The minor allele frequencies of all SNPs were similar between JJ and JB, and similar allele frequencies of *CYP1A2*1F* and *CYP3A5*3* were seen among all three populations.

ORs for breast cancer by SNPs are shown in Table 4. We found no statistically significant association between any SNP examined and breast cancer risk in the three populations combined; however, we found a slightly decreased risk of breast cancer among women with the Val allele of *CYP1B1* Leu^{432}Val in comparison with those with the Leu/Leu genotype. Adjusted ORs (95% CI) for women with the Leu/Val and Val/Val versus Leu/Leu allele were 0.84 (0.67–1.05) and 0.82 (0.56–1.19), respectively. No substantial change was seen after further adjustment for other potential confounders, such as age at menarche, age at first birth, body mass index, physical activity in the past 5 years or family history of breast cancer. Further analysis examining the association between the six SNPs and hormone receptor-defined breast cancers showed no remarkable difference in risk by hormone receptor-defined subtype (data not shown).

In the subgroup analyses, we found that three SNPs (*CYP1A2*1F*, *CYP1B1* Leu^{432}Val and *CYP3A5*3*) were significantly associated with breast cancer risk. *CYP1A2*1F* was significantly associated with risk in NJB but not in the other two populations. Compared to women with the AA genotype in *CYP1A2*1F*, the adjusted OR (95% CI) for women with the CC genotype was 0.54 (0.32–0.90). We found a decreased risk of breast cancer among JJ and JB with at least one Val allele of *CYP1B1* Leu^{432}Val in comparison with those with the Leu/Leu genotype, but not among NJB. The adjusted ORs (95% CI) for women with the Leu/Val+Val/Val versus Leu/Leu genotype were 0.68 (0.48–0.97) for JJ and 0.57 (0.25–1.30) for JB. *CYP3A5*3* was significantly associated with breast cancer risk in both JJ and JB, but not in NJB. The adjusted OR (95% CI) for JJ with the *3/*1+*1/*1 versus *3/*3 genotype was 1.49 (1.10–2.04). In contrast, the adjusted OR (95% CI) for JB with the *1/*1 versus *3/*3 genotype was 0.10 (0.02–0.66).

Stratified analyses according to menopausal status showed an elevated breast cancer risk among premenopausal but not post-menopausal women with minor alleles in *CYP1B1* Arg^{48}Gly and *CYP3A5*3* in the three populations combined, although their associations were not statistically significant (data not shown in the Table). The adjusted ORs (95% CI) for women with the Arg/Gly and Gly/Gly genotypes in *CYP1B1* Arg^{48}Gly were 1.22 (0.86–1.73) and 1.51 (0.78–2.93) in premenopausal women, and 1.01 (0.76–1.35) and 0.99 (0.58–1.67) in post-menopausal women, respectively, compared with those with the Arg/Arg genotype. The adjusted ORs (95% CI) for women with the *1/*3 and *1/*1 genotypes in *CYP3A5*3* were 0.94 (0.68–1.30) and 1.53 (0.84–2.78) in premenopausal women, and 1.14 (0.87–1.50) and 0.88 (0.53–1.44) in post-menopausal women, respectively, compared with those with the *3/*3 genotype. This pattern was found predominantly among JJ. Associations between the four other SNPs

Table 3 Minor allele frequencies of SNPs among control groups

Genes	*SNP*	*Minor allele frequency (HWE* P*-value)*		
		JJ	*JB*	*NJB*
CYP1A1	*CYP1A1*2A*	0.35 (0.71)	0.32 (0.86)	0.20 (0.57)
	*CYP1A1*2C*	0.22 (0.72)	0.21 (0.70)	0.10 (0.63)
CYP1A2	*CYP1A2*1F*	0.37 (0.93)	0.38 (0.81)	0.34 (0.08)
CYP1B1	*CYP1B1* $Arg^{48}Gly$	0.12 (0.25)	0.10 (0.13)	0.34 (0.84)
	CYP1B1 $Leu^{432}Val$	0.15 (0.31)	0.16 (0.45)	0.44 (0.01)
CYP3A5	*CYP3A5*3*	0.21[a] (0.62)	0.30[a] (0.31)	0.27[a] (0.15)

Abbreviations: HWE, Hardy–Weinberg Equilibrium; JB, Japanese Brazilians living in São Paulo, Brazil; JJ, Japanese living in Nagano, Japan; NJB, Non-Japanese Brazilians living in São Paulo, Brazil.
[a]For *CYP3A5*, a minor allele means a wild type allele (*CYP3A5*1*).

and breast cancer risk did not substantially differ by menopausal status.

DISCUSSION

In these case–control studies, we found no statistically significant association between any of the six SNPs examined and breast cancer risk among the three populations combined. On subgroup analysis, however, statistically significant associations with the risk of breast cancer were seen between *CYP1A2*1F* in NJB, and *CYP1B1* $Leu^{432}Val$ and *CYP3A5*3* in JJ. These findings suggest that genetic polymorphisms related to estrogen metabolism were associated with breast cancer risk, notwithstanding that no overall consistent findings in the three populations were obtained.

The two genetic polymorphisms of *CYP1A1* most frequently studied for their association with breast cancer risk are *CYP1A1*2A* and *CYP1A1*2C*.[13-15,27–29] A recent meta-analysis showed no overall association between these two SNPs and the risk of breast cancer,[15] which is in general agreement with our findings. However, a subgroup analysis in this meta-analysis showed that the Val/Val genotype of *CYP1A1*2C* was associated with a decreased risk of breast cancer in east Asian women,[15] which supports the proposed mechanism that women with at least one Val allele may be expected to have higher concentrations of intrinsic 2-OHE1/2 than those with the wild type and to have a decreased risk of breast cancer. However, our present results showed no significant association between *CYP1A1*2C* and breast cancer risk.

CYP1A2 is considered as one of the most important enzymes in the 2-hydroxylation of estrogens,[3] and accumulating evidence indicates an association between *CYP1A2*1F* and breast cancer risk.[14,30–33] Findings have been inconsistent, however, showing no association for Chinese,[30] Caucasian[31] or American-African women;[31] a positive association for Russian women;[33] and an inverse association for a multiethnic group (Multiethnic Cohort Study).[14] In the present study, we found a significantly decreased risk among NJB with the CC genotype of *CYP1A2*1F*. Given suggestions that *CYP1A2*1F* has two opposite effects on the development of breast cancer,[17] a growth-promoting effect due to the lower urinary ratio of 2-OHE1/16α-OHE1 and a growth inhibitory effect due to lower levels of serum E2, the decreased risk might reflect a mechanism which involves estrogen level rather than estrogen metabolites.

The remarkable enhancement of enzymatic activity in Val allele carriers has led to a relatively large number of investigations of the association between *CYP1B1* $Leu^{432}Val$ and breast cancer risk.[2,24] A recent meta-analysis demonstrated that Val allele carriers have a lower risk of breast cancer than Leu/Leu carriers among women of mixed/African ethnicity, but that there is no difference in Asians.[24] In the present study, Val allele carriers tended to have a decreased risk in the three populations combined and a significantly decreased risk among JJ. These results are inconsistent with those reported for Asians in the meta-analysis.[24] Previous studies have demonstrated that the Val allele contributes to an increase in 4-hydroxylation activity and a lower urinary 2-OHEs/16α-OHEs ratio (Table 1), whereas the Leu allele contributes to an increase in procarcinogen activation.[2,16,34] Our results might be explained by the latter mechanism. On the other hand, we found no overall association between *CYP1B1* $Arg^{48}Gly$ and breast cancer risk in the three populations combined, which was in good accordance with the majority of previous studies.[19,35,36]

To our knowledge, this is the first epidemiological study to investigate the association between a functional SNP in the *CYP3A5* gene and the risk of breast cancer. Although no significant association was found between *CYP3A5*3* and breast cancer risk among the three populations combined, subgroup analysis revealed a significantly increased risk of breast cancer in JJ women with the *CYP3A5*1* genotype compared with those with the *CYP3A5*3*/3* genotype. CYP3A5 may be considered one of the most important CYP enzymes for 16α-hydroxylation *in vivo*.[37–39] *CYP3A5*3*, a nonfunctional allele in *CYP3A5*, produces a cryptic splicing site leading to the inclusion of a novel exon and ultimately a premature stop codon. In contrast, *CYP3A5*1* creates no aberrant *CYP3A*5 mRNA splicing and is considered a functional allele.[12] Thus, *CYP3A5*1* carriers might be expected to have higher endogenous 16α-OHEs and higher breast cancer risk. Our finding among JJ supports this hypothesis. Stratified analyses according to menopausal status showed higher risk in premenopausal than post-menopausal women with the *1/*1 allele, in particular among JJ, and consequently suggest that this SNP may play a more important role in the development of breast cancer in premenopausal than post-menopausal women. In contrast, a decreased risk of breast cancer was observed in *CYP3A5*1/*1* carriers in both JB and NJB. The reason for this inconsistency is unclear, particularly for that between JJ and JB considering their common genetic background. Given the relatively small number of cases in JB, our findings in this population might merely be due to chance. Alternatively, the decreased risk might be partly explained by enhanced clearance activity of potential oncogenic substances, on the basis that CYP3A activity accounts for the majority of total body clearance for many drugs.[40,41] CYP3A5 also catalyzes the 6β-hydroxylation of testosterone, leading to the inactivation of testosterone. For this reason, *CYP3A5*1* is considered a protective genotype of prostate cancer, and an association between *CYP3A5*3* and prostate cancer risk has been demonstrated.[42] Therefore, a second possibility is that a portion of endogenous estradiol derived from testosterone might be modulated by the *CYP3A5*1* genotype to result in a lower breast cancer risk among *CYP3A5*1* carriers in JB and NJB. Further studies are required to confirm whether the *CYP3A5*3* mutation is associated with an increased or decreased risk of breast cancer.

Our study has a methodological advantage over previous studies of SNPs related to estrogen metabolism and the risk of breast cancer in that the substantially high participation rates among both eligible cases and controls minimized potential biases related to control selection. Although the use of controls from medical checkup examinees and cancer-free patients, whose lifestyles may have differed from the general population due to health consciousness or disease, might have lead to selection bias, it is less likely that allele frequencies among controls from medical checkup examinees and cancer-free patients differ from those of the general population. In this regard, allele

Table 4 Odds ratio and 95% confidence intervals for breast cancer categorized by genetic polymorphism

Study population	Three populations combined				Japanese living in Nagano, Japan (JJ)				Japanese Brazilians living in São Paulo, Brazil (JB)				Non-Japanese Brazilians living in São Paulo, Brazil (NJB)			
Genotype	No.				No.				No.				No.			
	Case	Control	OR[a]	95% CI	Case	Control	OR[a]	95% CI	Case	Control	OR[a]	95% CI	Case	Control	OR[a]	95% CI
*CYP1A1*2A*																
TT	461	457	1.00		175	168	1.00		31	37	1.00		255	252	1.00	
TC	328	343	0.96	(0.78–1.19)	173	187	1.01	(0.73–1.40)	40	36	1.37	(0.64–2.90)	115	120	0.95	(0.69–1.31)
CC	84	73	1.13	(0.79–1.61)	55	48	1.09	(0.66–1.78)	10	8	1.95	(0.63–6.06)	19	17	1.06	(0.54–2.09)
CC+TC	412	416	0.99	(0.81–1.21)	228	235	1.02	(0.75–1.40)	50	44	1.49	(0.74–2.99)	134	137	0.97	(0.71–1.31)
*CYP1A1*2C*																
Ile/Ile	592	613	1.00		246	249	1.00		46	50	1.00		300	314	1.00	
Ile/Val	245	232	1.10	(0.88–1.38)	137	134	1.10	(0.80–1.52)	30	28	1.40	(0.66–2.94)	78	70	1.13	(0.77–1.65)
Val/Val	29	28	1.08	(0.63–1.85)	20	20	0.88	(0.44–1.77)	0	3	—	—	9	5	2.02	(0.66–6.24)
Ile/Val+Val/Val	274	260	1.10	(0.88–1.37)	157	154	1.07	(0.79–1.46)	30	31	1.27	(0.62–2.60)	87	75	1.19	(0.83–1.71)
*CYP1A2*1F*																
AA	362	370	1.00		151	163	1.00		30	32	1.00		181	175	1.00	
AC	414	383	1.12	(0.90–1.37)	202	186	1.21	(0.88–1.68)	33	37	0.96	(0.47–1.93)	179	160	1.09	(0.79–1.49)
CC	96	120	0.84	(0.61–1.15)	50	54	1.04	(0.66–1.64)	17	12	1.70	(0.55–5.24)	29	54	**0.54**	**(0.32–0.90)**
CC+AC	510	503	1.05	(0.86–1.28)	252	240	1.17	(0.86–1.58)	50	49	1.09	(0.57–2.09)	208	214	0.96	(0.71–1.29)
CYP1B1 Arg^{48}Gly																
Arg/Arg	536	553	1.00		307	316	1.00		61	67	1.00		168	170	1.00	
Arg/Gly	275	264	1.09	(0.87–1.35)	92	79	1.26	(0.88–1.83)	17	12	1.49	(0.65–3.42)	166	173	0.93	(0.67–1.26)
Gly/Gly	61	56	1.31	(0.85–2.00)	4	8	0.48	(0.13–1.75)	3	2	1.66	(0.26–10.75)	54	46	1.30	(0.81–2.10)
Arg/Gly+Gly/Gly	336	320	1.12	(0.90–1.38)	96	87	1.19	(0.83–1.69)	20	14	1.51	(0.69–3.31)	220	219	1.00	(0.75–1.33)
CYP1B1 Leu^{432}Val																
Leu/Leu	505	479	1.00		310	285	1.00		67	58	1.00		128	136	1.00	
Leu/Val	281	297	0.84	(0.67–1.05)	83	111	**0.65**	**(0.45–0.94)**	12	20	0.58	(0.24–1.40)	186	166	1.10	(0.80–1.52)
Val/Val	87	97	0.82	(0.56–1.19)	10	7	1.18	(0.36–3.83)	2	3	0.54	(0.07–4.16)	75	87	0.87	(0.57–1.32)
Leu/Val+Val/Val	368	394	0.84	(0.67–1.03)	93	118	**0.68**	**(0.48–0.97)**	14	23	0.57	(0.25–1.30)	261	253	1.03	(0.76–1.40)
*CYP3A5*3*																
*3/*3	491	506	1.00		210	251	1.00		44	42	1.00		237	213	1.00	
*3/*1	309	304	1.05	(0.85–1.31)	160	132	**1.43**	**(1.04–1.99)**	34	30	1.23	(0.60–2.53)	115	142	0.72	(0.51–1.01)
*1/*1	73	63	1.12	(0.77–1.65)	33	20	1.91	(0.97–3.76)	3	9	**0.10**	**(0.02–0.66)**	37	34	0.92	(0.54–1.56)
*3/*1+*1/*1	382	367	1.07	(0.87–1.31)	193	152	**1.49**	**(1.10–2.04)**	37	39	0.88	(0.46–1.68)	152	176	0.76	(0.55–1.04)

Abbreviations: CI, confidence interval; JB, Japanese Brazilians living in São Paulo, Brazil; JJ, Japanese living in Nagano, Japan; NJB, Non-Japanese Brazilians living in São Paulo, Brazil; OR, odds ratio.
[a]Conditional model adjusted for menopausal status, age at menopause, number of births and smoking status.
ORs and 95% CIs with statistical significance are written in bold letters.

frequencies among the individual populations (Table 3) were reasonably similar to those previously reported in that population (Table 1).

In contrast, a limitation of this study is that the stratified analyses involved a relatively small number of cases, meaning that the interpretability of our results might be limited.

In conclusion, we found no overall association between any SNP examined and breast cancer risk in the three populations combined. In contrast, subgroup analyses demonstrated significant associations with a risk of *CYP1A2*1F* in NJB, and *CYP1B1* Leu^{432}Val and *CYP3A5*3* in JJ. Our findings add further evidence to the idea that genetic polymorphisms related to estrogen metabolism may play a role in the development of breast cancer.

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