The *CYP1A2* Genotype Modifies the Association Between Coffee Consumption and Breast Cancer Risk Among *BRCA1* Mutation Carriers

Joanne Kotsopoulos,[1,2] Parviz Ghadirian,[3] Ahmed El-Sohemy,[2] Henry T. Lynch,[4] Carrie Snyder,[4] Mary Daly,[5] Susan Domchek,[6] Susan Randall,[7] Beth Karlan,[8] Phil Zhang,[1] Shiyu Zhang,[1] Ping Sun,[1] and Steven A. Narod[1]

[1]Centre for Research in Women's Health, Women's College Hospital, University of Toronto, Canada; [2]Department of Nutritional Sciences, University of Toronto, Ontario, Canada; [3]Epidemiology Research Unit, Research Centre, Centre Hospitalier de l'Universitaire Montréal, CHUM Hôtel Dieu, Département de Nutrition, Faculte du Medicine, Quebec, Canada; [4]Department of Preventive Medicine and Public Health, Creighton University School of Medicine, Ohama, Nebraska; [5]Division of Population Science, Fox Chase Cancer Center, Philadelphia, Pennsylvania; [6]Departments of Hematology and Oncology, University of Pennsylvania, Philadelphia, Pennsylvania; [7]Department of Gynecologic Oncology, Princess Margaret Hospital, Toronto, Ontario, Canada; and [8]Gynecology Oncology, Cedars Sinai Medical Center, Los Angeles, California

Abstract

We have recently reported that, among *BRCA1* mutation carriers, the consumption of caffeinated coffee was associated with a significant reduction in breast cancer risk. Because the metabolism of caffeine is primarily by CYP1A2, we examined whether or not the *CYP1A2* genotype modifies the association between a history of coffee consumption and the risk of breast cancer. A common A to C polymorphism in the *CYP1A2* gene is associated with decreased enzyme inducibility and impaired caffeine metabolism. Information regarding coffee consumption habits and the *CYP1A2* genotype was available for 411 *BRCA1* mutation carriers (170 cases and 241 controls). We estimated the odds ratios (ORs) and 95% confidence intervals (95% CIs) for breast cancer associated with the *CYP1A2* genotype and a history of coffee consumption before age 35, adjusting for potential confounders. The *CYP1A2* genotype did not affect breast cancer risk. Among women with at least one variant C allele (AC or CC), those who consumed coffee had a 64% reduction in breast cancer risk, compared with women who never consumed coffee (OR, 0.36; 95% CI, 0.18-0.73). A significant protective effect of coffee consumption was not observed among women with the *CYP1A2* AA genotype (OR, 0.93; 95% CI, 0.49-1.77). Similar results were obtained when the analysis was restricted to caffeinated coffee. This study suggests that caffeine protects against breast cancer in women with a *BRCA1* mutation and illustrates the importance of integrating individual genetic variability when assessing diet-disease associations. (Cancer Epidemiol Biomarkers Prev 2007;16(5):912–6)

Introduction

We recently evaluated the association between coffee consumption and the risk of breast cancer among women harboring a deleterious mutation in one of the two breast cancer susceptibility genes, *BRCA1* or *BRCA2* (1). We observed a statistically significant reduction in the risk of breast cancer among women who drank six or more cups of coffee per day, compared with those who never drank coffee [odds ratio (OR), 0.51; 95% confidence interval (95% CI), 0.26-0.98]. The association was significant for women with a *BRCA1* mutation (OR, 0.25; 95% CI, 0.09-0.71) but not for *BRCA2* mutation carriers (OR, 0.40; 95% CI, 0.09-1.73). The protective effect was only seen with caffeinated coffee.

Ninety-five percent of caffeine is metabolized by the cytochrome P450 (CYP) 1A2, and caffeine is an inducer of the enzyme (2). A common A to C polymorphism at position −163 in the *CYP1A2* gene has been associated with decreased enzyme inducibility and enzymatic activity, resulting in the slower metabolism of caffeine (3-5). Two groups have investigated the association between breast cancer risk and this CYP1A2 polymorphism among women from the general population (6, 7). One group reported a protective effect with the presence of the variant C allele and the risk of postmenopausal breast cancer (6), and the other reported no association (7). In these studies, the authors did not evaluate the role of coffee consumption. In a recent publication, Cornelis et al. reported an increased risk of myocardial infarction with increasing coffee consumption among carriers of the variant C allele of the *CYP1A2* gene (8). The authors attributed this to the prolonged presence of caffeine in the circulation among the 'slow' metabolizers, due to lower enzyme inducibility (5).

We examined whether the *CYP1A2* genotype modifies the association between a history of coffee consumption and the risk of breast cancer among *BRCA1* mutation carriers. We also investigated whether the *CYP1A2* genotype was associated with breast cancer risk, irrespective of coffee intake.

Materials and Methods

Study Population and Data Collection. The study population was drawn from a registry of *BRCA1* and *BRCA2* mutation carriers established at the Centre for Research in Women's Health in Toronto, Ontario. Eligible subjects included women who were currently alive and were known to be carriers of a deleterious mutation in the *BRCA1* gene. These women were participants in prior and ongoing clinical research protocols and received counseling and provided written informed consent for genetic testing.

Study subjects completed a standardized questionnaire that asked for all relevant information regarding family history, reproductive and medical histories, and selected lifestyle factors including smoking history and use of oral

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Requests for reprints: Steven A. Narod, Centre for Research in Women's Health, 790 Bay Street, Room 750, Toronto, Ontario, Canada M5G 1N8. Phone: 416-351-3765; Fax: 416-351-3767. E-mail: steven.narod@wchospital.ca

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Table 1. Characteristics of case and control subjects

Variable	Control (N = 241)	Case (N = 170)	P*
Date of birth, mean year (SD)	1,956.4 (14.2)	1,946.4 (12.5)	0.00004
Current age, mean (SD)	43.1 (13.2)	52.4 (12.3)	0.00004
Breast cancer, mean age of diagnosis (SD)		41.3 (9.5)	Not applicable
Age at menarche, mean (SD)	12.7 (1.4)	12.7 (1.5)	0.95
Parity†			
Nulliparous, n (%)	77 (32.1)	23 (13.5)	0.00004
Parity‡, mean (SD)	2.5 (1.2)	2.7 (1.4)	0.23
Age at first birth, mean (SD)	24.2 (4.9)	23.5 (4.4)	0.19
Menopausal status, n (%)			
Premenopausal	123 (51.0)	38 (22.4)	
Postmenopausal	118 (49.0)	132 (77.7)	0.00004
Timing of menopause, n (%)	118 (49.0)	132 (77.7)	
Prior to breast cancer diagnosis	Not applicable	49 (28.8)	
At breast cancer diagnosis	Not applicable	26 (15.3)	
After breast cancer diagnosis	Not applicable	55 (32.4)	
Missing	1 (0.9)	2 (1.2)	
Oral contraceptive use, n (%)			
Never	56 (23.3)	50 (29.6)	
Ever	184 (76.7)	119 (70.4)	
Missing	1	1	0.19
Smoking history, n (%)			
Never	133 (55.2)	100 (58.8)	
Ever	108 (44.8)	70 (41.2)	0.46
Alcohol consumption history, n (%)			
Never	81 (33.8)	54 (31.8)	
Ever	159 (66.3)	116 (68.2)	0.67
Missing	1		
Coffee consumption, n (%)			
Never	71 (29.5)	66 (38.8)	
Ever	170 (70.5)	104 (61.2)	0.05
Type of coffee consumed, n (%)			
Caffeinated	113 (49.9)	72 (42.4)	
Decaffeinated	9 (3.7)	9 (5.3)	
Both	19 (7.9)	9 (5.3)	
Missing	29 (12.0)	15 (8.8)	Not applicable
Age started drinking coffee, mean (SD)	20.5 (4.3)	20.3 (4.9)	0.70
CYP1A2 genotype, SNP rs762551, n (%)			
AA	122 (50.6)	85 (50.0)	
AC	107 (44.4)	68 (40.0)	
CC	12 (5.0)	17 (10.0)	0.13
Country of residence§, n (%)			
Canada	87 (36.4)	49 (28.8)	
United States	154 (63.9)	121 (71.2)	0.12
Ethnicity, n (%)			
Other White	138 (57.3)	105 (61.8)	
Jewish	81 (33.6)	52 (30.6)	
French Canadian	15 (6.2)	7 (4.1)	
Other	7 (2.9)	6 (3.5)	0.67

*All P values are univariate and were derived using the Student's t test.
†Parity includes liveborn and stillborn.
‡Among parous women.
§Country of residence at the time of testing.

contraceptives. Questionnaires were administered by each of the individual centers at the time of a clinic appointment or at their home at a later date. Additional variables of interest included information on demographics, ethnicity, as well as alcohol and coffee consumption. The questionnaire was completed at the time blood was drawn for genetic testing, or within a year of receiving the test result. The study subjects were asked if they ever consumed coffee, if they currently consumed coffee, at what age they first began coffee consumption, at what age they stopped drinking coffee and their average daily coffee consumption over this period. Questions were asked separately for caffeinated and decaffeinated coffee. The information on smoking history was collected similarly to coffee use. The questionnaire also asked for information regarding each pregnancy (year of pregnancy and outcome).

Due to the small number of women in this study, we limited the coffee exposure to a history of coffee consumption (having initiated coffee drinking prior to age 35). Based on this information, two categorical variables were created for each study subject: (*a*) coffee consumption, which included intake of either caffeinated or decaffeinated coffee, prior to age 35 (coded as ever or never), and (*b*) intake of caffeinated coffee prior to age 35 (coded as ever or never).

Genotyping. The *CYP1A2* A to C (rs 762551) polymorphism was detected by restriction fragment-length polymorphism-PCR. The primers were synthesized by Integrated DNA Technologies, Inc. The following set of primers was used to amplify a 385-bp fragment—forward, 5′-GGT ATA TGG AAG GTA TCA GC-3′; and reverse, 5′-GGG TTG AGA TGG AGA CAT TC-3′. Forty nanograms of DNA was amplified with PCR conditions of an initial denaturation at 94°C for 12 min followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 1 min. PCR products were digested with 4 units of *Apa*I restriction enzyme (Roche Diagnostics) incubated at 30°C overnight; *CYP1A2* A allele was not digested by the *Apa*I restriction enzyme, whereas the *CYP1A2* C allele was cut into 262 and 123 bp fragments. The fragments were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistical Analysis. A DNA sample and a research questionnaire was available for 492 *BRCA1* mutation carriers from Canada or the Unites States. A total of 81 women were excluded because information regarding coffee consumption was missing ($n = 65$), if they started drinking coffee at or after age 35 ($n = 14$), or if they started drinking coffee after the diagnosis of breast cancer ($n = 2$). After these exclusions, there was a total of 411 eligible women, including 170 women with a history of invasive breast cancer (potential cases) and 241 women who never had breast cancer and who were carriers of a mutation in the *BRCA1* (controls). We combined the AC and CC genotypes for this analysis because previous genotype-phenotype studies have shown similar rates of caffeine metabolism in these two groups (3-5).

The Student's *t*-test was used to compare continuous variables between case and control subjects. The χ^2 test was used to test for differences in categorical variables. ORs and 95% CIs for breast cancer associated with a history of coffee consumption were estimated using unconditional logistic regression. A multivariate analysis was carried out to control for the potential confounding effects of year of birth, parity (0, 1, 2, 3, or ≥4), and smoking history (ever/never). Similar analyses were done to evaluate the association between *CYP1A2* genotype and breast cancer risk. To evaluate the statistical significance of the observed gene-exposure interaction, a multivariable unconditional logistic regression was conducted using a model which contained terms for the main effects of the *CYP1A2* variants and coffee as well as an interaction term. We also examined the association between coffee consumption and breast cancer risk following stratification by *CYP1A2* genotype. Because only 9% of case and control subjects reported a history of decaffeinated coffee consumption, we include only the results for total coffee consumption. All tests of statistical significance were two-sided.

Results

Subject characteristics are presented in Table 1 by case or control status. Cases were similar to controls with respect to oral contraceptive use and smoking history (Table 1). Case subjects were significantly older than the control subjects (52.4 versus 43.4 years; $P < 0.01$). Fourteen percent of the cases compared with 32% of the controls had never been pregnant ($P < 0.01$). Because of these differences between the case and control subjects, we adjusted for year of birth and parity in the multivariate analysis.

Sixty-one percent of the case subjects had consumed coffee prior to age 35 compared with 71% of the control subjects ($P = 0.05$). There was no significant difference in the distribution of *CYP1A2* genotype frequencies between the cases and the controls (AA = 50.0% versus 50.6%; AC = 40.0% versus 44.4%; and CC = 10.0% versus 5.0%; $P = 0.13$, for cases and controls, respectively; Table 1). Among the control subjects, the *CYP1A2* genotype distributions were in Hardy-Weinberg equilibrium.

A history of coffee consumption was associated with a significant reduction in breast cancer risk among *BRCA1* mutation carriers (OR, 0.58; 95% CI, 0.36-0.95; Table 2). Restricting the analysis to caffeinated coffee resulted in a similar finding (OR, 0.57; 95% CI, 0.36-0.90). There was no association between the presence of one or both of the C alleles and the risk of breast cancer among *BRCA1* mutation carriers compared with the *CYP1A2* AA genotype (OR, 1.00; 95% CI, 0.67-1.53).

We examined whether the *CYP1A2* genotype modifies the protective effect of coffee consumption (Table 3). There was a strong protective effect associated with coffee consumption among carriers of the *CYP1A2* C allele (OR, 0.36; 95% CI, 0.18-0.73), but not among women with the *CYP1A2* AA genotype (OR, 0.93; 95% CI, 0.49-1.77). The results did not differ when the analysis was limited to caffeinated coffee intake (data not shown). The interaction between the *CYP1A2* genotype, coffee consumption, and breast cancer risk was statistically significant ($P = 0.04$).

Discussion

We undertook this study to examine whether or not the *CYP1A2* genotype modifies the relationship between a history of coffee consumption and breast cancer risk among *BRCA1* mutation carriers. Having consumed coffee before age 35 was associated with a 39% reduction in breast cancer risk in this group of genetically susceptible women, consistent with our previous results (1). The current study is based on a subset of women from this earlier study (1). The *CYP1A2* genotype alone did not affect breast cancer risk; however, there was an interaction with a history of coffee consumption. Among women with one or both of the low-inducibility C alleles, coffee drinkers had a nearly 3-fold decreased risk compared with women who never drank coffee (OR, 0.36; 95% CI, 0.18-0.73).

Two previous studies of CYP1A2 and breast cancer included women from the general population and information regarding *BRCA1* mutation status was not reported (6, 7). Furthermore, they did not take into account the effect of coffee as an important exposure variable. Le Marchand et al. reported an inverse relationship between the presence of the variant C allele and the risk of postmenopausal breast cancer (6). The ORs (95% CI) for breast cancer associated with the AA, AC and CC genotypes were 1.0, 0.9 (0.70-1.0), and 0.7 (0.50-1.0), respectively. In another study, Long et al. reported that the *CYP1A2* genotype was not associated with breast cancer risk among either premenopausal or postmenopausal women; however, they also reported that the C allele was associated with an elevated caffeine metabolism (7). The latter observation is not in agreement with the majority of genotype-phenotype studies showing higher activity with the A allele as compared with the C allele (3).

In a recent case-control study, Baker et al. reported a significant inverse association between intake of caffeinated coffee and breast cancer risk among premenopausal women, but not postmenopausal women (9). Similar to our previous study (1), they reported that the protective effect of caffeinated coffee intake was limited to women with the highest levels of consumption (four or more cups of coffee per day). In the Nkondjock et al. study, the protective effect of high intakes of coffee (six or more cups of coffee per day) was limited to women who were diagnosed with breast cancer prior to age 50. In the current study, the average age of breast cancer diagnosis was 41.1 years. Collectively, the results from these three studies support a protective effect of high intakes of coffee against early onset breast cancer. This observation is particularly relevant with respect to genetically predisposed women because the majority of *BRCA1*-associated breast cancers occur

Table 2. Association between the CYP1A2 genotype or a history of coffee consumption and breast cancer risk in *BRCA1* mutation carriers, main effects

	Control, *n* (%)	Case, *n* (%)	OR (95% CI)*	*P*
Coffee use				
Never	71 (29.5)	66 (38.8)	1	
Ever, total†	170 (70.5)	104 (61.2)	0.61 (0.38-0.97)	0.04
CYP1A2 genotype				
AA	122 (50.6)	85 (45.0)	1	
AC or CC	119 (49.4)	95 (55.0)	1.02 (0.66-1.55)	0.94

*ORs and 95% CI adjusted for year of birth, age at menarche, parity (0, 1, 2, 3, or ≥4), and smoking history (ever/never).
† Total refers to consumption of either caffeinated or decaffeinated coffee.

Table 3. Association between a history of coffee consumption and breast cancer risk by *CYP1A2* genotype

Type of coffee/ *CYP1A2* genotype	Control, *n* (%)	Case, *n* (%)	OR (95% CI)*	*P*
Total, AA†				
Never	41 (33.6)	30 (35.3)	1	
Ever	81 (66.4)	55 (64.7)	0.93 (0.49-1.77)	0.82
Total, AC or CC				
Never	30 (25.2)	36 (42.4)	1	
Ever	89 (74.8)	49 (57.7)	0.36 (0.18-0.73)	0.005

*ORs and 95% CI adjusted for year of birth, age at menarche, parity (0, 1, 2, 3, or ≥4), and smoking history (ever/never).
† Total refers to consumption of either caffeinated or decaffeinated coffee.

prior to menopause. In addition, caffeine intake has been positively associated with CYP1A2 activity in premenopausal, but not in postmenopausal women (10).

Most of the evidence supporting a protective role of dietary constituents and the risk of breast cancer is based on studies involving women not known to have a genetic predisposition. Evidence is lacking to support the role of modifiable risk factors in the etiology of hereditary breast cancer. Moreover, the current prevention options for *BRCA* mutation carriers are based on preventive mastectomy and chemoprevention with tamoxifen, although many women forego these options, which suggests a need to pursue novel alternatives such as dietary or lifestyle strategies that may help reduce risk.

In the current study, the inverse association between coffee and breast cancer risk was observed among *BRCA1* mutation carriers who had one or two of the variant *CYP1A2* C alleles. *CYP1A2* was chosen for this study because this enzyme is required for the metabolism of both caffeine and estrogen. It is surprising that a genotype associated with lower enzyme inducibility could protect against breast cancer in response to coffee consumption. We hypothesized that coffee protected against breast cancer by inducing CYP1A2, and increasing the 2-hyroxyestrone (OHE)/16α-OHE1 ratio, a marker of lower breast cancer risk (11). We previously reported a significant positive correlation between daily coffee consumption and the plasma 2-OHE/16α-OHE1 ratio (12, 13). Lurie et al. reported a significantly lower urinary 2-OHE/16α-OHE1 ratio in women with the *CYP1A2* AC versus the AA or CC genotype (14). In a more recent study of patients with breast cancer, the number of C alleles was correlated with a significantly lower postoperative plasma 2-OHE/16α-OHE1 ratio; whereas coffee consumption was associated with a higher ratio (15). The tissue specificity of *BRCA1*-associated breast cancers along with the current modifiers of breast cancer risk that specifically interrupt the estrogen-signaling pathway (i.e., oophorectomy and tamoxifen, reviewed in ref. 16) suggest a critical role of estrogen in the course of *BRCA1*-associated breast cancer development. Because substrates of *CYP1A2* can increase enzyme induction, we initially proposed that a woman with the 'fast' genotype would have increased enzyme inducibility in response to coffee or caffeine intake, resulting in an increase in 2-hydroxylation of estradiol. In turn, this would translate into a beneficial promotion of estrogen metabolism and a higher 2-OHE/16α-OHE1 ratio. However, this does not seem to be the underlying protective mechanism because a history of coffee consumption was most protective among individuals who were carriers of the slow C allele with no effect among homozygous wild-type women.

There are other plausible mechanisms, other than the induction of CYP1A2, whereby caffeine might influence the risk of breast cancer. Coffee is a source of numerous biochemically active substances including caffeine, minerals and polyphenols such as phytoestrogens (i.e., flavonoids and lignans), phenols (i.e., chlorogenic acid), and other phytonutrients (i.e., tocopherols; refs. 17-19). The complexity of this mixture makes it difficult to isolate the compound that is associated with the beneficial properties of coffee. However, because caffeine is the only major compound in coffee that is known to be metabolized by CYP1A2, the decreased risk must be attributed to the prolonged exposure of caffeine among slow metabolizers. This can be further substantiated by the similar results we obtained when our analysis was limited to caffeinated coffee intake versus both decaffeinated and caffeinated combined.

Lurie et al. reported that among healthy premenopausal women, the *CYP1A2* CC genotype was associated with significantly lower serum estradiol levels compared with the homozygous or heterozygous common allele carriers combined (14). Epidemiologic studies have clearly shown an increased risk of breast cancer with higher circulating estradiol levels (20-22). Intake of caffeine and caffeine-containing beverages has been positively associated with sex hormone–binding globulin levels and inversely associated with bioavailable testosterone (23, 24). This data suggests that caffeine may mediate its protective effects by favorably altering endogenous hormone levels and not necessarily the 2-OHE/16α-OHE1 ratio.

Coffee seems to be a major contributor to the total *in vitro* antioxidant capacity of the diet (25) and has been found to have a higher antioxidant activity than tea on a per-cup basis (26). This is of particular relevance for *BRCA1* mutation carriers since Bae et al. showed a decrease in the expression of genes involved in the antioxidant response (including glutathione *S*-transferases and other antioxidant genes) in *BRCA1*-deficient cells (27). Coffee is the largest single source of caffeine in the diet (not in all countries). Upon ingestion, catabolism of caffeine (1,3,7-trimethylxanthine) yields predominantly paraxanthine (1,7-dimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine), which is further metabolized and excreted in the urine (28). Caffeine, along with its catabolic products, theobromine and xanthine, have been shown to have a quenching effect on the production of hydroxyl radicals, as well as on oxidative DNA breakage by hydroxyl radicals (29).

Along with its role as an antioxidant (17), recent studies have shown additional anticarcinogenic properties of caffeine, that include antimetastatic effects (30), the ability to inhibit cell proliferation (31), and enhance apoptosis (32). With respect to mammary tumorigenesis, one group reported that chronic caffeine exposure prior to the appearance of the tumor was associated with a significant reduction in tumor burden and metastasis in a transgenic mouse model (33). Furthermore, higher coffee and/or caffeine consumption was previously associated with significant tumor differentiation among women with breast cancer (34). Although unclear, the evidence collectively points towards a protective effect of caffeine with respect to the etiology of breast cancer.

There are several limitations to the present study. The sample size was relatively small and so our analyses were limited. There were too few BRCA2 carriers in the database to analyze these separately. We grouped women based on the presence or absence of a history of coffee consumption, and did not distinguish between those women who regularly consumed coffee versus those who were occasional drinkers. Nor did we take into account the amount and duration of intake. The questionnaire was completed in the cases following the diagnosis of breast cancer. It is possible that cases would systematically underestimate their intake of coffee, if they felt that this was a risk factor, but recall bias cannot explain the observed interaction with the *CYP1A2* genotype. Cases may decrease their coffee intake following disease diagnosis, therefore, we limited our exposure assessment to coffee intake before diagnosis. Information on caffeine intake was restricted to coffee, information was not collected on other dietary sources of caffeine, such as tea, soft drinks, and chocolate.

Cigarette smoking, certain drugs, cruciferous vegetables, and caffeine have all been shown to induce CYP1A1 and CYP1A2 activity (35, 36). Because of this effect, we adjusted for smoking, however, we did not have information on intake of cruciferous vegetables. Future studies with a larger population of *BRCA1*, as well as, *BRCA2* mutation carriers will allow for confirmation of our findings and the evaluation of a possible dose-effect relationship. Replication of these findings using a population of noncarriers may lend further support for our observations.

Conclusion. The current study is the first to evaluate how the *CYP1A2* genotype modifies the association between coffee intake and breast cancer risk among genetically predisposed women. We confirmed that ever having consumed coffee before age 35 was inversely associated with breast cancer among women with a *BRCA1* mutation, although the effect was only observed among women who carried at least one of the variant *CYP1A2* C alleles. The results of this study illustrate the importance of integrating individual genetic variability in the metabolism when assessing diet-disease associations. Although our results were based on women with a hereditary predisposition, future epidemiologic studies investigating the association between coffee or other caffeine-containing beverages and breast cancer risk should include the potential modifying role of the *CYP1A2* genotype.

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