Coffee Consumption and *CYP1A2*1F* Genotype Modify Age at Breast Cancer Diagnosis and Estrogen Receptor Status

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Abstract

CYP1A2 plays a key role in the metabolism of both estrogen and coffee. Women with higher coffee intake and the *CYP1A2*1F* A/A genotype have a ratio of high 2-hydroxyestrone (2-OHE1) to 16α-OHE1. 2-OHE1 is a weak estrogen and may even block the estrogen receptor (ER), whereas 16α-OHE1 is procarcinogenic. We hypothesized that moderate to high coffee consumption (≥2 cups per day) combined with the *CYP1A2*1F* A/A genotype would be associated with a later age at diagnosis and a greater proportion of ER-negative (ER−) tumors among patients with breast cancer. We genotyped 458 patients with breast cancer (age, 25-99 years) in Lund, Sweden, for *CYP1A2*1F*. Information on lifestyle factors and tumor characteristics were obtained from preoperative questionnaires and pathology reports. Among patients with *CYP1A2*1F* A/A (51.3%), moderate to high consumption was associated with a later age at diagnosis compared with low coffee consumption (59.8 versus 52.6 years, $P = 0.0004$). These patients were also more likely to have ER− tumors than patients with low consumption (14.7% versus 0%, $P = 0.018$). Coffee was not associated with ER status or age at diagnosis in patients with at least one C allele. Age at diagnosis was not associated with ER status in patients with *CYP1A2*1F* A/A, but younger patients (<50 years) with at least one C allele were more likely to have ER− tumors compared with older patients (odds ratio, 4.2; 95% confidence interval, 1.9-9.3; $P = 0.0002$). These findings raise the hypothesis that coffee slows the growth of ER-positive tumors in patients with *CYP1A2*1F* A/A and may have implications for breast cancer if confirmed. (Cancer Epidemiol Biomarkers Prev 2008;17(4):895–901)

Introduction

Some studies (1-3), but not all (3-7), have reported that moderate to high coffee consumption confers protection against breast cancer. One small study of 101 women with breast cancer investigated the effect of coffee consumption on breast cancer prognostic factors and found that a high coffee consumption was associated with moderately differentiated to well-differentiated tumors (8).

Coffee may protect against breast cancer by altering estrogen metabolism. Although coffee induces, and is metabolized by, the CYP1A2 enzyme (9), no association between the inducible *CYP1A2*1F* genotype (10) and coffee consumption has been observed in the general population (11). CYP1A2 is also a key enzyme in the 2-hydroxylation of the main estrogens, estrone and estradiol (12). 2-Hydroxylation and 16α-hydroxylation are two mutually exclusive pathways in estrogen metabolism (13). Whereas 2-hydroxyestrone (2-OHE1) acts as a weak estrogen or anti-estrogen (14), 16α-OHE1 acts as a procarcinogen (15) with effects similar to those of estradiol when binding to the estrogen receptor (ER; ref. 16). Some (17-20), but not all studies (21), have reported that a high urinary 2-OHE/16α-OHE1 ratio is associated with a reduced risk of breast cancer. A higher urinary 2-OHE/16α-OHE1 ratio has also been associated with an improved prognosis (22). In plasma, a higher 2-OHE/16α-OHE1 ratio has been associated with an increasing coffee consumption in healthy young women (23, 24) and in a previously published pilot study of 59 patients with breast cancer from the current cohort (25) but not in women using exogenous hormones (23, 24). Moreover, in our pilot study of patients with breast cancer, the highest 2-OHE/16α-OHE1 ratios were observed in women with the combination of the inducible *CYP1A2*1F* A/A genotype and a moderate to high coffee consumption (25).

In a study of several single nucleotide polymorphisms in estrogen-metabolizing genes, the only polymorphism associated with a higher 2-OHE/16α-OHE1 ratio was the *CYP1A2*1F* A/A genotype (26). The effect of coffee consumption on breast cancer may thus be limited to

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patients with the *CYP1A2*1F* A/A genotype. However, a recent study of *BRCA1* mutation carriers reported that coffee consumption was protective against breast cancer only in women with at least one *CYP1A2*1F* C allele (27). Because *BRCA1* mutation carriers have a large proportion of early-onset ER-negative (ER−) tumors (28, 29), this finding may or may not be applicable to sporadic cases. The combined effect of coffee consumption and *CYP1A2*1F* in relation to ER status and age at diagnosis was not reported in that study (27). In sporadic patients with breast cancer, ER− tumors are more common in younger than in older patients (30).

We hypothesized that moderate to high coffee consumption (≥2 cups per day) induces the CYP1A2 enzyme in women carrying the inducible *CYP1A2*1F* A/A genotype, thereby leading to an increased 2-OHE/16 α-OHE1 ratio and a better prognosis. We also predicted a higher proportion of ER− tumors and later age at diagnosis among these women, provided that a high 2-OHE/16α-OHE1 ratio may have weak estrogenic or even antiestrogenic effects on the ER in the breast tissue and that ER-positive (ER+) tumors may thus go through necrosis or may be too small to be detected. We aimed to study whether moderate to high coffee consumption (≥2 cups per day) combined with the *CYP1A2*1F* A/A genotype is associated with a later age at diagnosis and a greater proportion of ER− tumors among patients with breast cancer in a population-based series of incident cases in the Lund area of southern Sweden.

Materials and Methods

All women assessed preoperatively at the Lund University Hospital, Sweden, for a primary breast cancer were invited to take part in an ongoing study regarding genetic and nongenetic factors that could be associated with breast cancer prognosis. A total of 458 patients were included between October 2002 and March 2007. Women were invited to participate regardless of ethnic background, age, and stage. Patients who had recently been diagnosed and treated for another type of cancer were not eligible to participate. The study was approved by the Ethics Committee of the Lund University. Written informed consents were collected during the preoperative visit at the Department of Surgery at the Lund University Hospital. At the same visit, the research nurse collected blood samples (EDTA-plasma and serum) and recorded the time and date when the blood samples were drawn. Serum, EDTA-plasma, and blood cells were stored at −70°C. All samples were labeled with serial codes to enable blinded analyses.

Body measurements and breast volumes were measured at the preoperative visit. All patients filled out a preoperative questionnaire, including questions on birth date, coffee consumption, smoking, alcohol intake, use of exogenous hormones and concomitant medications, reproductive history, and family history of cancer. There was no question regarding ethnicity. However, most women included were ethnic Swedes. Coffee consumption was reported as average daily consumption during the last week (0 to ≥8 cups of coffee per day). We had no question on tea consumption.

Patients who had not experienced a menstrual period during the last year were defined as postmenopausal. However, postmenopausal patients who used hormone replacement therapy may have had hormone replacement therapy–induced bleeding and may therefore have been misclassified as premenopausal. Patients who had had their uterus removed before menopause but not their ovaries may also have been misclassified as postmenopausal. We therefore classified patients according to age (<50 or ≥50 years) instead of reported menopausal status.

Additional baseline information, including type of surgery, sentinel node biopsy, and axillary node dissection, were obtained from each patient's chart. Tumor size, histologic type and grade, axillary node involvement, signs of distant metastases, and ER and progesterone receptor (PR) status were obtained from each patient's pathology report. ER and PR status were determined by immunohistochemistry using the Dako LSAB kit system (Dako) and the antibodies M7047 (ER) and M3569 (PR; Dako). Tumors with >10% positive nuclear staining were considered ER+ or PR+. All tumors were analyzed at the Department of Pathology at Lund University Hospital. HER-2/neu status was routinely analyzed as of November 2005.

Lund University Hospital is one of the nine hospitals in the South Swedish Health Care Region performing breast cancer surgery. The Lund University Hospital catchment area serves almost 300,000 inhabitants. Patients with breast cancer are not referred to other hospitals for surgery. We consider this study population based.

According to data from the Regional Tumor Registry as of February 19, 2007, a total of 4,593 primary female breast cancers were registered between October 1, 2002, and June 30, 2006, in the South Swedish Health Care Region. During the same period, 601 breast tumors were registered in Lund, of which 567 were primary and received surgery. In our study, 396 (70%) of them were included. In the region and in Lund, women are registered whether they have been operated on or not, whereas we only included women who came to the preoperative visit and who had not been diagnosed with other cancers during the past 10 years. The mean age at diagnosis was 63.3 years in the region, 59.1 years in Lund, and 58.3 years in our study. ER and PR status were only recorded in the Regional Tumor Registry between January 1, 2005, and June 30, 2006. ER status was registered for 80% of the tumors from the region, for 82% of the tumors from Lund, and for 97% of the patients in the current study. Of the registered tumors, 85% were ER+ in the region and 85% were ER+ in Lund compared with 89% of the tumors included in the current study.

Genetic Analysis. Genomic DNA was extracted from 300 μL of peripheral blood using Wizard, Genomic DNA Purification Kit (Promega). *CYP1A2*1F* is an A to C transversion in intron 1, also denoted rs762551.[5] PCR primers 5′-AGGTATCAGCAGAAAGCCAGCAC-3′ and 5′-GCTGAGGGTTGAGATGGAGACAT-3′ yielded a 380-bp nucleotide sequence. PCR was done in 25 μL

[5] http://www.imm.ki.se/CYPalleles/

reactions using 25 ng of DNA, 0.2 μmol/L of each primer, 0.1 mmol/L of each deoxynucleotide (Amersham Biosciences), 1.5 mmol/L $MgCl_2$ (Applied Biosystems), 1× PCR Gold Buffer (Applied Biosystems), and 0.5 units AmpliTaq Gold (Applied Biosystems). The PCR product was sequenced (Big Dye, Terminator Cycle Sequencing, Applied Biosystems) according to the manufacturer's instructions and was run on an ABI 3100 Genetic Analyzer (Applied Biosystems). In April 2006, the system was upgraded to an ABI Prism 3130*xl* Genetic Analyzer (Applied Biosystems). Seventy-one samples were run on the upgraded system. For quality control, every fourth sample was run in duplicate in separate PCR and sequencing reactions. The concordance rate was 100%.

Statistical Analyses. Data analysis was done with the statistical software SPSS 13.0 (SPSS, Inc.). For univariate analyses, χ^2 analysis was used for dichotomous variables and Student's *t* test was used for continuous variables. Spearman rank correlation (r_s) was used to study the correlation between reported coffee consumption preoperatively and at 1st, 2nd, and 3rd year follow-up visits.

Age at diagnosis was classified as being diagnosed <50 years of age (yes/no) or as a continuous variable. ER status was classified as being ER+ (yes/no) and *CYP1A2*1F* genotype was classified as carrying the A/A genotype or at least one C allele. The median consumption was 3 cups per day, and we first calculated the odds ratio, which is the interaction in a case-only study, using a χ^2 analysis between coffee ≥3 cups per day and *CYP1A2*1F* genotype. Because only a few patients consumed no coffee at all, we then chose to categorize coffee consumption into low consumption (0-1 cup per day) and moderate to high consumption (≥2 cups per day). Coffee was also classified as 0 to ≥6 cups per day. Alcohol was classified as how often the patient consumed alcohol (never, ≤1/mo, 2-4/mo, 2-3/wk, or ≥4/wk).

A logistic regression model was used to study the association between age at diagnosis and ER status and how this association was modified by the patient's *CYP1A2*1F* genotype. Multivariate linear and multivariate regression analyses were done to evaluate the interaction between the *CYP1A2*1F* genotype (A/A or any C) and coffee consumption (0-1 or ≥2 cups per day) on age at breast cancer diagnosis. An interaction term was calculated between *CYP1A2*1F* and coffee consumption. The interaction was also adjusted for other breast cancer risk factors such as alcohol, body mass index of <25 kg/m^2, ever hormone replacement therapy, and parity. The magnitude of the interactions was presented as exp(β) in the logistic regression models (i.e., odds ratio) and as β in the linear regression models.

The interaction term between coffee and genotype on ER status was calculated using the exact logistic regression procedure in SAS 9.1 because there were zero patients in one group. *P* values were obtained from conditional exact tests. We were unable to perform further adjustment of the interaction term due to limited RAM on available computer servers.

The statistical analyses were based on *a priori* hypotheses; that is, that women with the *CYP1A2*1F* genotype and moderate to high coffee consumption would have higher 2-OHE/16α-OHE1 ratios that would lead to a slower growth of ER+ tumors and a higher age at diagnosis, as well as a higher proportion of ER− tumors. All *P* values were calculated based on two-tailed statistical tests and are presented without Bonferroni adjustment for multiple comparisons unless otherwise indicated.

Results

Characteristics of the Patients. The baseline characteristics for the 458 patients are shown in Table 1. Age at breast cancer diagnosis ranged from 25 to 99 years, with a mean age of 59.0 years. The median reported coffee consumption was 3 cups a day. A total of 235 (51.3%) of the patients carried the *CYP1A2*1F* A/A genotype; 182 patients (39.7%) carried the A/C genotype; and 41 patients (9.0%) carried the C/C genotype.

Characteristics of the Breast Tumors. A total of 422 tumors were untreated; 11 tumors were treated preoperatively with intestinal laser thermotherapy; and 24 tumors were treated with neoadjuvant treatment before surgery. In the case of one patient, it was unclear whether she actually had received intestinal laser thermotherapy. Information on ER and PR status were available for the tumors of 451 patients, and 67% were ER+PR+, 19% were ER+PR−, 13% were ER−PR−, and 1% were ER−PR+. Of the previously untreated tumors, 74% were

Table 1. Baseline Characteristics of the 458 Patients

	Median or %	IQR	Missing
Age at diagnosis (y)	59.6	51.0-66.5	—
Height (cm)	165	161-170	1
Weight (kg)	68.0	60.5-76.0	1
Body mass index (kg/m^2)	24.5	22.4-27.6	2
Waist-to-hip ratio	0.83	0.78-0.88	4
Total breast volume (cm^3)	1,000	688-1,575	9
Age at menarche (y)	13	12-14	4
Premenopausal (%)	23		2
Age at menopause (y)	50	47-52	123
Age at first full-term pregnancy* (y)	25	22-28	77
Parity	2	1-3	1
Ever oral contraceptive use (%)	69		—
Ever hormone replacement therapy use (%)	45		—
Abstainer of alcohol (%)	12		2
Current smoker (%)	22		—
Daily coffee consumption (cups) (%)			1
None	10		
1	7		
2	19		
3	24		
4	23		
5	8		
≥6	10		
First-degree relative with breast cancer (%)	19		13
First- or second-degree relative with breast cancer (%)	33		16

Abbreviation: IQR, interquartile range.
*Among parous women only.

≤2 cm (414 pT_1, 7 pT*is*, and 1 unknown). Of the patients, 64% had no lymph node involvement (pN_0). Of the tumors, 27% were grade 1, 55% were grade 2, and 18% were grade 3.

All analyses have been done with and without the eight women with pT*is* or unknown pT stage. Because the results were essentially identical, we present the data on all 458 women.

ER Status According to Age at Diagnosis and *CYP1A2*1F* Genotype. Patients diagnosed before the age of 50 years were more likely to have ER− tumors compared with patients diagnosed at the age of 50 years or older (odds ratio, 2.0; 95% confidence interval, 1.1-3.6; $P = 0.017$; Table 2A). We then stratified patients according to *CYP1A2*1F* genotype. Age at diagnosis was not associated with ER status in patients carrying the *CYP1A2*1F* A/A genotype (Table 2B). On the other hand, patients carrying at least one *CYP1A2*1F* C allele and who were diagnosed before the age of 50 years were more than four times as likely to have ER− tumors than patients diagnosed at the age of 50 years or older (odds ratio, 4.2; 95% confidence interval 1.9-9.3; $P = 0.0002$; Table 2C). This finding was also statistically significant after Bonferroni correction. The association between age at diagnosis and ER status differed depending on the patients' *CYP1A2*1F* genotype [$\exp(\beta) = 0.197$, $P_{interaction} = 0.010$]. This interaction was even stronger when age was used as continuous variable [$\exp(\beta) = 1.073$, $P_{interaction} = 0.004$]. The interaction remained essentially the same after adjustment for alcohol, body mass index of <25 kg/m^2, ever hormone replacement therapy use, and parity [$\exp(\beta) = 1.070$, $P_{interaction} = 0.005$].

Because we aimed to study the *CYP1A2*1F* genotype in combination with coffee consumption, we first explored whether coffee intake was associated with any of the *CYP1A2*1F* genotypes. The highest mean intake was observed in patients with the *CYP1A2*1F* A/A genotype (3.3 cups per day). The mean coffee consumption did not differ between patients with the A/C or C/C genotypes (2.9 versus 2.9 cups, $P = 0.84$). The average daily coffee consumption in patients carrying the *CYP1A2*1F* A/A genotype was higher than in patients with at least one C allele (3.3 versus 2.9 cups, $P = 0.008$). There was an interaction between consuming ≥3 cups of coffee per day and the *CYP1A2*1F* genotype in this case-only series (odds ratio, 1.5; 95% confidence interval, 1.0-2.3; $P = 0.027$), in which patients with the *CYP1A2*1F* A/A genotype were more likely to drink ≥3 cups of coffee per day. However, there was no statistically significant association between ER status and age at diagnosis from consuming ≥3 cups of coffee per day in patients with either the *CYP1A2*1F* A/A genotype or any C allele ($P \geq 0.08$). Because the previous report (27) on a protective effect from coffee in *BRCA1* carriers with at least one C allele was based on ever coffee consumption, we reanalyzed our data with a lower cutoff (i.e., ≥2 cups of coffee per day). Too few women drank no coffee to allow for a comparison of no coffee versus any coffee.

Table 2. Age at Diagnosis and ER Status

	<50 y	≥50 y		
A. Patients with known ER status ($n = 451$)				
ER−	21	41	62	
ER+	79	310	389	
	100	351	451	χ^2 $P = 0.017$
B. Patients carrying the *CYP1A2*1F* A/A genotype ($n = 230$)				
ER−	6	23	29	
ER+	48	153	201	
	54	176	230	χ^2 $P = 0.70$
C. Patients carrying at least one *CYP1A2*1F* C allele ($n = 221$)				
ER−	15	18	33	
ER+	31	157	188	
	46	175	221	χ^2 $P = 0.0002$

Table 3. Coffee Consumption and ER Status

	0-1 cup/d	≥2 cups/d		
A. Patients with known coffee consumption and ER status ($n = 450$)				
ER−	6	56	62	
ER+	71	317	388	
	77	373	450	χ^2 $P = 0.09$
B. Patients carrying the *CYP1A2*1F* A/A genotype ($n = 230$)				
ER−	0	29	29	
ER+	33	168	201	
	33	197	230	χ^2 $P = 0.018$
C. Patients carrying at least one *CYP1A2*1F* C allele ($n = 220$)				
ER−	6	27	33	
ER+	38	149	187	
	44	176	220	χ^2 $P = 0.78$

ER Status According to Coffee Consumption and *CYP1A2*1F* Genotype. Moderate to high coffee consumption (≥2 cups of coffee per day) alone was not statistically significantly associated with ER status when all patients were included in the analysis (Table 3A). Among carriers of the *CYP1A2*1F* A/A genotype, patients with moderate to high coffee consumption were more likely to have ER− tumors than patients with low coffee consumption (14.7% versus 0%, $P = 0.018$; Table 3B). There was no trend in increasing number of ER+ tumors with increasing coffee consumption from 2 to ≥6 cups of coffee per day ($P = 0.52$). In fact, no patient with low coffee consumption had an ER− tumor.

Because smoking has been shown to induce the CYP1A2 enzyme (10), we explored the positive association between consuming ≥2 cups of coffee per day and ER− status in nonsmokers with the *CYP1A2*1F* A/A genotype, and the association remained essentially the same (13.3% versus 0%, $P = 0.029$). Only one smoker had a low coffee consumption. The positive association between a moderate to high coffee consumption and ER− tumor status was also similar in patients who had never used hormone replacement therapy (16.2% versus 0%, $P = 0.043$) and not statistically significant in patients who had ever used hormone replacement therapy ($P = 0.20$). Coffee consumption was not associated with ER status in patients carrying at least one *CYP1A2*1F* C allele (Table 3C). The association between coffee consumption and ER status differed depending on the patients' *CYP1A2*1F* genotypes, although not significantly ($P_{interaction} = 0.09$).

Age at Diagnosis According to Coffee Consumption and *CYP1A2*1F* Genotype. Although the mean coffee consumption did not differ between patients diagnosed before the age of 50 years or older (3.1 versus 3.1 cups,

P = 0.74), patients with a low coffee consumption were more likely to be diagnosed before the age of 50 years compared with patients with a moderate to high coffee consumption (odds ratio, 1.8; 95% confidence interval, 1.1-3.1; P = 0.029). Overall, *CYP1A2*1F* was not associated with age at diagnosis. We then stratified patients according to *CYP1A2*1F* genotype. Patients carrying the *CYP1A2*1F* A/A genotype who had low coffee consumption were more likely to be diagnosed before the age of 50 years compared with patients with moderate to high coffee consumption (odds ratio, 3.2; 95% confidence interval, 1.5-6.8; P = 0.002). In fact, low coffee consumption was associated with a 7.2-year earlier age at diagnosis compared with moderate to high coffee consumption (52.6 versus 59.8 years, P = 0.0004). This was limited to patients who had never used hormone replacement therapy (48.0 versus 57.7 years, P = 0.001). In ever hormone replacement therapy users, coffee was not associated with age at diagnosis (62.2 versus 62.1 years). In patients with at least one *CYP1A2*1F* C allele, coffee consumption was not associated with age at diagnosis irrespective of ER status or ever hormone replacement therapy use. The positive association between a moderate to high coffee consumption and mean age at diagnosis differed according to the *CYP1A2*1F* genotype (β = 7.35, $P_{interaction}$ = 0.009). The interaction remained essentially the same after adjustment for alcohol consumption, body mass index of <25 kg/m^2, ever hormone replacement therapy, and parity (β = 7.32, $P_{interaction}$ = 0.007).

Age at Diagnosis According to Coffee Consumption, *CYP1A2*1F* Genotype, and ER Status. We then stratified patients carrying the *CYP1A2*1F* A/A genotype according to ER status. Age at diagnosis in patients carrying the *CYP1A2*1F* A/A genotype who had ER+ tumors was lower among women with low coffee consumption than among women with moderate to high coffee consumption (51.9 versus 59.6 years, P = 0.0002). The association between coffee and age at diagnosis was again limited to patients who had never used hormone replacement therapy (46.8 versus 57.6 years, P < 0.0001), whereas no association was seen in ever hormone replacement therapy users. The mean age at diagnosis in patients carrying the *CYP1A2*1F* A/A genotype who had a moderate to high coffee consumption and ER− tumors was 61.3 years. No patient carrying the *CYP1A2*1F* A/A genotype with a low coffee consumption had an ER− tumor.

Discussion

The main findings of this study were that patients with a moderate to high coffee consumption and the highly inducible *CYP1A2*1F* A/A genotype were more likely to have a later age at diagnosis and to have ER− tumors. As expected, patients diagnosed before the age of 50 years were more likely to have ER− tumors than patients diagnosed at 50 years of age or older (30). Unexpectedly, this finding was limited to patients carrying at least one *CYP1A2*1F* C allele. To our knowledge, this is the first study to examine age at diagnosis and ER status in relation to coffee consumption and *CYP1A2*1F* genotype.

The patients in this study included 70% of the female breast cancers reported in Lund between October 1, 2002, and June 30, 2006. There was only a small difference in the mean age at diagnosis between the women included in our study (58.3 years) and all women (59.1 years) registered in Lund at the same period. However, tumors included in our study were in comparison with all the tumors reported in Lund, somewhat more ER+ (89% versus 85%) and PR+ (74% versus 71.5%). On the whole, women who chose to participate in our study may be considered representative for women registered with a primary breast cancer in Lund with the exception that women with metastatic disease who did not present for surgery were not included. Using population-based incident cases allows maximized generalizability of the findings (31).

In our case-only study, ~50% of the patients carried the *CYP1A2*1F* A/A genotype, which is consistent with the frequencies reported in other studies (10, 32), and the distribution of the genotypes was in Hardy-Weinberg equilibrium. *CYP1A2*1F* is located in intron 1 of the *CYP1A2* gene. This polymorphism may not directly be associated with the activity of the enzyme but rather linked to other polymorphisms with regulatory properties (10). In this study, we did not phenotype our patients for CYP1A2 activity. However, in a Danish twin study, the heritability estimate for CYP1A2 activity was 0.725, which indicates that genetics play a major role in the activity of the enzyme (33). The remaining variation in CYP1A2 activity is dependent on lifestyle factors, and coffee has been reported to predict 4% of the variation in the enzyme activity (9).

Because others have shown no association between the *CYP1A2*1F* genotype and coffee consumption (i.e., no gene-environment interaction) in the general population (11), it is valid to use a case-only study design to assess a potential interaction between coffee consumption and *CYP1A2*1F* on breast cancer (31). In a large population-based Swedish cohort including >60,000 women ages 40 to 76 years, coffee intake decreased with age (34). Of the women in our study, 88% were 40 to 76 years old. In our study, the mean coffee consumption did not differ by age when studying all women. However, in patients carrying the *CYP1A2*1F* A/A genotype, a moderate to high coffee consumption was associated with a later age at diagnosis. Taken together, these findings suggest that had we had access to a matched control population; the relationship between coffee consumption and age at diagnosis may have been even stronger in women with the *CYP1A2*1F* A/A genotype.

Information on coffee consumption was obtained from the preoperative questionnaire. Coffee consumption was reported as the average daily consumption during the last week. Because this is a short latency time and all women were patients, recall bias is minimal. However, we had no question on previous coffee consumption, which is a limitation. Preoperative coffee consumption correlated strongly with the consumption reported during the 1st, 2nd, and 3rd yearly postoperative follow-up visits (r_s, 0.76-0.82, all P < 0.0001). This observation suggests that a woman's coffee consumption might remain constant during a substantial portion of her adult life. Moreover, it is important to point out that, in a Swedish work place, there is a mandatory coffee break in the morning and in the

afternoon (although alternative beverages may be consumed). The median coffee consumption of 3 cups a day is in line with the consumption among Swedish women reported by Michels et al. (4). The fact that Sweden has one of the highest coffee consumptions per capita in the world (35) limited our analysis because there were only a few women consuming no coffee at all. We therefore categorized coffee consumption as low (0-1 cups per day) or as moderate to high (≥2 cups per day). The categorization of what constitutes low, moderate, and high coffee intake varies considerably between studies (1-7). We found an association between coffee intake, *CYP1A2*1F* genotype, age at diagnosis, and ER status when we defined low coffee consumption as consisting of 1 or 0 cup of coffee a day but not when this category included 2 cups. This finding suggests that even moderate coffee consumption affects tumor characteristics.

Coffee is metabolized by CYP1A2 (9) and is even used as a probe when measuring CYP1A2 activity *in vivo* (36). Coffee also induces CYP1A2. Sachse et al. (10) found that smoking preferentially induced CYP1A2 in Caucasians with the *CYP1A2*1F* A/A genotype compared with the *CYP1A2*1F* A/C or C/C genotypes. In our study, we found that patients with the *CYP1A2*1F* A/A genotype consumed significantly more coffee than women with the A/C or C/C genotypes. In a recent study by Kotsopoulos et al. (27), *BRCA1* mutation carriers with the *CYP1A2*1F* A/C or C/C genotype consuming coffee had a 64% reduced risk of developing breast cancer, which is in line with our findings of a significantly lower coffee consumption in patients with breast cancer carrying the *CYP1A2*1F* A/C or C/C genotype. *BRCA1* mutation carriers have a large proportion of early-onset ER− tumors compared with sporadic patients (28, 29). Despite coffee being protective in women with at least one C allele, we found a 4-fold odds of having an ER− tumor before the age of 50 years in patients with at least one C allele compared with other patients. However, in women with the C allele who were diagnosed with breast cancer, we saw no modifying effect of coffee on the clinicopathologic features of their breast tumors. In women with the *CYP1A2*1F* A/A genotype, no protective effect of coffee was reported in the previous study (27); however, we observed a statistically significant interaction between the *CYP1A2*1F* genotype and a moderate to high coffee intake on age at diagnosis and an insignificant interaction between the *CYP1A2*1F* genotype and coffee intake on ER status.

Some studies (1-3), but not all (3-7), have reported that moderate to high coffee consumption confers protection against breast cancer. How coffee may modulate breast cancer risk is unclear. In Sweden, most coffee consumed is caffeinated. Caffeine has been shown to reverse cell cycle checkpoint function and p53 defect cells are forced through the cell cycle, which results in a lethal mitosis (37). Coffee contains not only caffeine but also phytoestrogens that can interact with and even block the ER (38). Furthermore, coffee contains caffeic and chlorogenic acid, which have been shown to inhibit DNA methylation *in vitro*, an important mechanism in the tumor development (39).

We hypothesize instead that coffee affects tumor characteristics by increasing the 2-OHE/16α-OHE1 ratio in women carrying the *CYP1A2*1F* A/A genotype. A high 2-OHE/16α-OHE1 ratio effects a weak stimulation or an inhibition of the ER (14). In a study of several polymorphisms in estrogen-metabolizing genes, the polymorphism associated with a higher 2-OHE/16α-OHE1 ratio was the *CYP1A2*1F* A/A genotype (26). Compared with patients with low coffee consumption or the *CYP1A2*1F* C/A or C/C genotypes, patients with moderate to high coffee consumption and the *CYP1A2*1F* A/A genotype had more ER− tumors and a later age at diagnosis, especially when the tumor was ER+. All three findings are compatible with the hypothesis that a higher 2-OHE/16α-OHE1 ratio in these women hinders the growth of ER+ tumors. We even found that patients with a moderate to high coffee consumption carrying the *CYP1A2*1F* A/A genotype and an ER+ tumor had a tumor size that was, on average, 4 mm smaller than those of patients with a low coffee consumption (data not shown). Pozner et al. (8) reported that a higher coffee intake was associated with moderately differentiated to well-differentiated breast tumors. In our study, coffee consumption was not associated with other prognostic factors such as grade or node status (data not shown). The effect of moderate to high coffee consumption and the *CYP1A2*1F* A/A genotype was not present in women who had consumed hormone replacement therapy. This observation is compatible with our previous reports that coffee does not increase the 2-OHE/16α-OHE1 ratio in women currently using exogenous hormones such as oral contraceptives (23, 24).

Le Marchand et al. (40) found that postmenopausal women carrying the *CYP1A2*1F* C/C genotype had a lower odds of developing breast cancer compared with women carrying the *CYP1A2*1F* A/A or A/C genotypes, but they did not take coffee consumption into account. Their finding was stronger in patients with ER− and PR− tumors. We observed that ER− tumors were less common in older patients with at least one C allele, which is compatible with their finding. In our study, no patients with the highly inducible *CYP1A2*1F* A/A genotype and a low coffee consumption had an ER− tumor. This finding may be as a result of a small sample size because only four ER− tumors were expected in this subgroup. One hypothesis that is compatible with both studies is that ER+ tumors have been transformed into ER− tumors via genetic or epigenetic events (41) in women carrying the *CYP1A2*1F* A/A genotype with a moderate to high coffee consumption. The alternative would be that coffee may initiate ER− tumors. Moreover, the relationship between coffee and other hormone-dependent female cancers is modified by the *CYP1A2*1F* genotype. Goodman et al. (42) reported that coffee consumption in women carrying the *CYP1A2*1F* A/A had a higher odds of developing ovarian cancer compared with women who did not consume any coffee at all.

In conclusion, we found that patients with moderate to high coffee consumption and the *CYP1A2*1F* A/A genotype had a significantly later age at diagnosis and a higher proportion of ER− tumors. This study is the first to report an association between coffee consumption, *CYP1A2*1F* genotype, and breast cancer characteristics, and our results warrants confirmation. Because coffee is widely consumed and the *CYP1A2*1F* A/A genotype is present in half of the population, our findings may have important implications with regard to breast cancer given that coffee consumption is a potentially modifiable factor.

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Coffee Consumption and *CYP1A2*1F* Genotype Modify Age at Breast Cancer Diagnosis and Estrogen Receptor Status

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