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Coffee Intake, Variants in Genes Involved in Caffeine Metabolism and the Risk of Epithelial Ovarian Cancer

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Abstract

We evaluated whether genetic variability, as well as menopausal status, modify the association between coffee intake and risk of ovarian cancer. Risk factor information and biologic specimens from three large epidemiological studies, the Nurses' Health Study (NHS), NHSII, and the New England based Case-Control study of ovarian cancer (NECC) were pooled resulting in 1354 ovarian cancer cases and 1851 controls for analysis. Odds ratios (ORs) and 95% confidence intervals (CI) were estimated using conditional (NHS/NHSII) and unconditional (NECC) logistic regression. Coffee consumption was not associated with risk overall (OR = 0.99; 95% CI 0.77–1.28); however, there was a suggested increased risk of ovarian cancer among premenopausal women in the NECC only and an inverse association among postmenopausal women. Carrying one or both of the variant *CYP19013 A* or *CYP19027 G* alleles was associated with an 18% increased (*P* for trend = 0.02) and 15% decreased (*P* for trend = 0.05) risk of ovarian cancer, respectively. Variation in *CYP1A1*, *CYP1A2*, or *CYP2A6*, did not explain the inconsistent reports of coffee intake and risk. Furthermore, we did not observe any clear gene-environment interactions between caffeine metabolizing genes and ovarian cancer. Future studies evaluating mechanisms by which coffee mediates this relationship are warranted.

Keywords

ovarian cancer; coffee; CYP1A1; CYP1A2; CYP2A6; CYP19

INTRODUCTION

Increasing parity, oral contraceptive use and tubal ligation protect against ovarian cancer; however, primary prevention through potentially modifiable exposures including diet remains unclear (1). Caffeine has garnered attention due its possible inverse association with breast, liver, and colon cancer, as well as cancer of the ovary (2). Several epidemiological studies have evaluated a role of coffee, tea and other caffeine-containing beverages in the etiology of ovarian cancer, albeit, with conflicting findings (3). A meta-analysis suggested a positive, although not

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statistically significant, association between coffee consumption and ovarian cancer risk that was strongest among the cohort studies (3).

We recently reported an inverse association of increasing intakes of both caffeine and coffee with ovarian cancer risk in the prospective analysis of the Nurses' Health Study cohort (*P* for trend =0.03) (4). The inverse association was limited to postmenopausal women and the data were suggestive of a positive association among premenopausal women. These data are similar to those from the New England based Case-Control study of ovarian cancer (NECC), which reported an increased risk of ovarian cancer with coffee or caffeine consumption among premenopausal women (5).

In an attempt to elucidate why reports of caffeine and ovarian cancer have been inconsistent to date, we assessed whether menopausal status, as well as variability in certain *cytochrome P450* genes, may modify this association. Specifically, we evaluated the *CYP19, CYP1A1, CYP1A2*, and *CYP2A6* genotypes since these enzymes are involved in the metabolism of caffeine, estrogen, or both. An additional objective was to determine if these genotypes or associated haplotypes were independently associated with risk. By pooling three large studies (NECC, NHS and NHSII), the role of caffeine in the etiology of ovarian cancer may be clarified.

MATERIALS AND METHODS

Study Population

New England Case-Control Study—The New England based Case-Control Study (NECC) includes 1,231 epithelial ovarian cancer cases and 1,244 controls from Massachusetts and New Hampshire. Participants were enrolled in the study in two phases, corresponding to the time periods 1992–97 (563 cases, 523 controls) and 1998–2003 (668 cases, 721 controls). Recruitment methods and eligibility criteria are described elsewhere (6). Briefly, trained interviewers asked participants about potential ovarian cancer risk factors occurring more than one year prior diagnosis for cases or interview date for controls. Controls were identified using random digit dialing, driver license records, and town resident lists and were frequency-matched to cases by age and state. The institutional review boards of the Brigham and Women's Hospital and Dartmouth Medical School approved both phases of the study, and all participants provided written informed consent. Over 95% of study participants provided a blood specimen at enrollment, and the heparinized samples were separated into plasma, red blood cell, and buffy coat components.

Nurses' Health Study (NHS) and Nurses' Health Study II (NHSII)—The NHS cohort was established in 1976 among 121,700 U.S. female registered nurses, ages 30 to 55 years, and the NHSII was established in 1989 among 116,609 female registered nurses, ages 25 to 42 years. Women in both cohorts completed an initial questionnaire and have been followed biennially by questionnaire to update exposure status and disease diagnoses.

In 1989–90, 32,826 NHS participants submitted a blood sample; details of the collection are described elsewhere (7). Follow-up of the NHS blood study cohort was 98% in 2004. In 2001–04, 33,040 additional women provided a buccal cell specimen using a mouthwash protocol. Between 1996 and 1999, 29,611 NHSII participants (ages 32–54 years) provided blood samples and completed a short questionnaire (8). Briefly, premenopausal women (n = 18,521) who had not taken hormones, been pregnant, or lactated within 6 months provided blood samples drawn on the 3rd to 5th day of their menstrual cycle (follicular) and 7 to 9 days before the anticipated start of their next cycle (luteal). Other women (n = 11,090) provided a single 30-mL untimed blood sample. Follow-up of the NHSII blood study cohort was 98% in 2003. These studies were approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

NHS/NHSII Nested Case-Control Study—We collected information on new diagnoses of ovarian cancer and confirmed each diagnosis using methods described previously (9). For this analysis, we included all cases with a DNA specimen submitted prior to diagnosis (incident cases), plus cases who submitted a DNA specimen within four years after diagnosis (prevalent cases) (10). All cases were diagnosed prior to June 1, 2004 (NHS) and June 1, 2003 (NHSII). We selected three controls per case from participants with DNA available, no prior bilateral oophorectomy, and no history of cancer, other than non-melanoma skin cancer, at the time of case diagnosis. We excluded 27 controls due to unavailability of genotyping data (n = 25) or because the participant was later diagnosed with ovarian cancer and was included in the analysis as a case (n = 2). Cases and controls were matched on month/year of birth, DNA source, and menopausal status at diagnosis (see (11) for details).

Dietary Assessment

In each study, diet was assessed through a validated self-administered, semi-quantitative food frequency questionnaire (FFQ) (12). Dietary intake was assessed in 1980, 1984, 1986, 1990, 1994, 1998 and 2002 for the NHS, and in 1991, 1995, 1999, and 2003 for the NHSII, allowing dietary intake at various intervals prior to the disease diagnosis to be assessed. In the NECC, cases were asked to avoid giving information about recent changes since the diagnosis of their cancer by focusing on intake in the year preceding their diagnosis. Similarly, controls were asked to avoid reporting recent changes in the previous year.

Caffeine consumption was calculated using US Department of Agriculture food composition sources (13–15). Respondents were asked about frequency of use of caffeine-containing beverages (coffee, tea and soda). The estimated caffeine content is 137 mg per cup of coffee, 47 mg per cup of tea, 46 mg per can or bottle of caffeinated soda, and 7 mg per chocolate serving. In 1980, the NHS questionnaire asked about any tea consumption; all other FFQs asked about non-herbal tea (i.e. caffeinated). The frequency of consumption was converted to cups per day for coffee and tea, and mg per day for caffeine. To maintain comparability with the NECC on the time period of exposure, we used the cumulative average intake of caffeine, coffee or tea, up to the cycle prior to the case diagnosis, and the comparable cycle for the matched controls for the NHS/NHSII. Average intake has been previously reported to be a better representation of overall long-term intake versus one FFQ (16).

We created dichotomous variables for caffeine, coffee and tea intake based on the distribution in the control subjects of the NHS, using the 75^{th} percentiles (< 409.5 and ≥ 409.5 mg/day for caffeine, < 2.5 cups/day and ≥ 2.5 cups/day for coffee, < 0.83 and ≥ 0.83 cups/day for tea, and < 1.19 and ≥ 1.19 cups/day for decaffeinated coffee). These cut-points were chosen to maximize power and match cut-points at which associations were observed in our previous study (4). Tests for trend were conducted by modeling the quintile median levels (caffeine, coffee and tea) or the continuous measure (decaffeinated coffee) and calculating the Wald statistic.

Genotyping Methods

DNA was extracted from the buffy coat or cheek cells using a Qiagen DNA extraction kit (Qiagen Inc., Valencia, CA). Genotyping was performed at the Dana Farber/Harvard Cancer Center High Throughput Genotyping Core. All samples were genotyped for single nucleotide polymorphisms (SNPs) in *CYP1A1, CYP1A2, CYP2A6,* and each of the 21 previously identified *CYP19* htSNPs (see Table 2 for RS numbers) (17). The selection of the *CYP19* htSNPs has been conducted by the National Cancer Institute Breast and Prostate Cancer Cohort Consortium[1] and the methods have been described in detail (17). Briefly, four regions of strong LD were identified and tagged by 21 htSNPs (mean R^2 of 0.92). Genotyping was performed

[1]http://www.uscnorris.com/MECGenetics/CohortGCKView.aspx

on whole genome amplified DNA using the 5' nuclease assay (Taqman) on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format. Laboratory personnel were blinded to case-control status, and each plate included blinded replicate samples for quality control purposes. The quality control replicate samples were 100% concordant for all genotypes. Over 90% of the samples were successfully genotyped for each of the polymporphisms and genotyping failures were considered as missing data.

Statistical Analysis

Due to ethnic variation in the allelic distribution of these genes, our analysis was limited to white women (excluded 22 {2%}, 5 {4%}, and 100 {4%} from the NHS, NHSII, and NECC, respectively). We evaluated Hardy-Weinberg equilibrium using a chi-square test, in each study separately, as well as by sample type for the NHS (cheek versus white blood cell). Unconditional logistic regression (NECC) and conditional regression (NHS, NHSII) were used to estimate the multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) associated with the main effects of caffeine, coffee and tea, and the main effect of each gene variant.

We adjusted for known and suspected risk factors for ovarian cancers. For both studies, we adjusted all the analyses for parity (continuous), oral contraceptive use (never, ≤3, >3–5, >5–8, >8 years), postmenopausal hormone use (premenopausal and postmenopausal never, past, or current use), tubal ligation (yes, no), smoking history (never, past, current/1–15 cigarettes per day, current/15+ cigarettes per day), and body mass index (<21, 21-<23, 23-<25, 25-<30, ≥30 kg/m2), family history of breast cancer (yes, no), and family history of ovarian cancer (yes, no). For the NECC, we additionally adjusted for the matching factors: age (continuous) and study center (Massachusetts or New Hampshire).

For each SNP, we compared the homozygous wild type (WT) genotype to both the heterozygote (Het) and homozygous (Hom) variant genotype; however, in the gene-environment interactions (see below) we combined the heterozygotes and homozygous variants into one category (Het + Hom) to maximize power. We calculated the *P* for trend for each unit increase in the number of minor alleles (log-additive model) using the Wald test.

The expectation-maximization (E–M) algorithm was used to predict the haplotype frequencies among the cases and controls across the four LD blocks in *CYP19* (18). This was estimated in the NHS/NHSII and NECC combined to increase power. We used unconditional logistic regression to estimate the risk of ovarian cancer associated with each common haplotype (frequency of more than 5%) compared with the most common haplotype in each LD block. Because of additional stratification by haplotypes resulting in smaller subgroups, we could not use the fully adjusted model for the NECC analysis. Thus, the NECC haplotype analysis only was adjusted for key risk factors (age, study center, parity, oral contraceptive use, and tubal ligation). A likelihood ratio test was used to evaluate the global association between all common haplotypes in each LD block and risk of ovarian cancer.

Because we previously observed that the association of coffee, caffeine and ovarian cancer varied by menopausal status (4,5), all the gene-environment interactions were stratified, *a priori*, by menopausal status. To assess the presence of effect modification of the association between caffeine, coffee or tea and the risk of ovarian cancer by genotype (WT or Het + Hom), stratum-specific ORs for coffee or caffeine intake and multivariate interaction terms were estimated across each SNP using unconditional logistic regression, giving separate multivariate ORs and 95% CIs for ovarian cancer among women with high versus low intake of caffeine, coffee or tea.

Data analyses were conducted separately for the NHS/NHSII and NECC and then pooled using a random effects model (19). For the main effects of caffeine and coffee, the *P* value for heterogeneity comparing the NHS/NHSII and NECC was significant (*P* = 0.03) among the premenopausal women. This is likely attributed to the small number of premenopausal cases in the NHS/NHSII (n = 44). Thus, the results for the gene-environment interactions among the premenopausal women were not pooled and are presented only for the NECC. Nevertheless, in a secondary analysis including the premenopausal cases from the NHS/NHSII did not substantially alter the final results (data not shown).

All tests of statistical significance were two-sided. The SAS version 9.1 (SAS Institute, INC., Cary, NC) was used for all the statistical analyses.

RESULTS

Study Population

The characteristics of the NECC and NHS/NHSII study populations have been previously described ((10)in press). Briefly, the current study included 234 cases and 691 matched controls identified from the NHS/NHSII, and 1120 cases and 1160 frequency-matched controls identified from the NECC. The mean age of the cases and controls was 60 and 59 years for the NHS/NHSII and 52 and 51 years for the NECC, respectively. In the NHS/NHSII, the average daily intake of coffee and decaffeinated coffee was identical among the cases and controls (1.7 and 1.7 cups per day, respectively) while caffeine intake and tea consumption were slightly lower in cases (caffeine: 275.1 versus 286.5 mg; tea: 0.5 versus 0.6 cups). In the subjects from the NECC, mean daily intake of caffeine, coffee, decaffeinated coffee and tea was slightly higher in the cases versus the controls (234.6 versus 219.9 mg, 1.4 versus 1.3, 0.4 versus 0.3, and 0.6 versus 0.5 cups).

Dietary Exposures

There was no association between coffee intake of ≥ 2.5 versus < 2.5 cups/day and the risk of ovarian cancer in the NHS/NHSII or NECC, or in the pooled analysis (OR = 0.99; 95% CI 0.77–1.28) (Table 1). Similarly, we observed no association between caffeine intake of ≥ 409.5 versus < 409.5 mg/day (OR = 1.06; 95% CI 0.66–1.72), decaffeinated coffee intake of ≥ 1.19 versus < 1.19 cups/day (OR = 0.93; 95% CI 0.72–1.20) or tea intake of ≥ 0.83 versus < 0.83 cups/day (OR = 0.99; 95% CI 0.72–1.37) and the risk of ovarian cancer (data not shown). Following stratification by menopausal status, there was a 53% (95% CI 1.06–2.21; *P* for trend = 0.007)(data not shown) and 35% (95% CI 1.03–1.78; *P* for trend = 0.003)(Table 1) increased risk of ovarian cancer with increasing caffeine and coffee intake, respectively, among the premenopausal women in the NECC. The opposite, but non-significant, trend was observed for premenopausal women in the NHS/NHSII; however, this was based on 44 cases and in the analysis of the entire NHS/NHSII cohort. In a previous analysis of the NHS/NHSII, there was a suggestion of an increased risk of premenopausal ovarian cancer with caffeine intake (4). Among postmenopausal women, heavy coffee consumption was non-significantly inversely associated with risk (pooled OR = 0.83; 95% CI 0.66–1.04; *P* for trend = 0.51)(Table 1). There was no association with caffeine, decaffeinated coffee or tea consumption on ovarian cancer risk among in this population (data not shown).

SNPs

There were no significant differences in the genotype distributions between the cases and controls and all of the distributions were in Hardy-Weinberg Equilibrium in each study (data not shown). The minor allele frequencies in our study populations were similar to what has been reported previously for white women (17).[2]

There was an increased risk of ovarian cancer among women who were heterozygous or homozygous for the variant *CYP19013 A* allele compared to women with the *CYP19013 GG* genotype (OR = 1.18; 95% CI 1.00–1.39; *P* for trend =0.02) (Table 2). Carriers of one or both of the *CYP19027 G* allele were at a 15% decreased risk compared to women with the *CYP19027 TT* genotype (OR = 0.85; 95%CI 0.72–0.99; *P* for trend = 0.05). No other significant associations between polymorphic variants in *CYP19*, *CYP1A1*, *CYP1A2*, or *CYP2A6*, and ovarian cancer risk were observed. There was no significant heterogeneity in risk between the NHS/NHSII and NECC for any of the SNPs that we evaluated (all *P* for heterogeneity > 0.05).

CYP19 Haplotypes

We observed five haplotypes in block 1, two haplotypes in block 2, three haplotypes in block 3, and four haplotypes in block 4 occurring with a frequency of > 5% in the *CYP19* gene. There were no global associations between any of the common haplotypes and risk (*P* values of the LRT were 0.79 for block 1, 0.61 for block 2, 0.25 for block 3, and 0.12 for block 4). Only one of the individual haplotypes in LD blocks 3 was associated with a decreased risk of ovarian cancer (OR = 0.85; 95%CI 0.74–0.99). We did not observe any significant associations between the other individual haplotypes in LD blocks 1–4 and ovarian cancer risk (data not shown).

Gene-Environment Interactions

We did not observe any significant interactions with caffeine, coffee, decaffeinated coffee or tea consumption for the SNPS in the *CYP1A1*, *CYP1A2*, or *CYP2A6* genes ($P \geq 0.17$) (Table 3). We observed a significant interaction between htSNP *CYP19011* and caffeine among the premenopausal women in the NECC (*P* for interaction = 0.04). Among women who were wild type for the *A* allele, those with a high caffeine intake were at a significantly increased risk of ovarian cancer compared to those with a low intake (OR = 1.62; 95% CI 1.10–2.40). There was no significant association with caffeine among women who were carriers of at least one variant *G* allele. The association was in the same direction for coffee, although the *P* for interaction was not significant (*P* = 0.12). There was also a significant interaction between *CYP19018* and high intake of caffeine (*P* = 0.004). Women who carried one or both of the variant *C* allele and had high intakes of caffeine were at two-fold increased risk of ovarian cancer compared with the women with the same genotype and had low intakes of caffeine (OR = 2.87; 95% CI 1.66–4.96). The direction of the association was similar with coffee intake, but the interaction was not statistically significant (*P* = 0.11). High intake of caffeine and carrying the variant *C* allele in *CYP19034* was associated with a two-fold increased risk of breast cancer (OR = 2.2; 95%CI 1.3–3.8; *P* for interaction = 0.03), although this association was not observed with high coffee consumption (*P* = 0.74). The gene-environment interactions were similar when the premenopausal women from the NHS/NHSII were included in the analysis (data not shown).

Among postmenopausal women, there were no significant interactions between caffeine, coffee or tea, and *CYP1A1*, *CYP1A2*, or *CYP2A6* (Table 3), or any of the *CYP19* htSNPs (*P* > 0.06) (data not shown).

DISCUSSION

Our results suggest that high intake of caffeine or coffee was associated with an increased risk of ovarian cancer among premenopausal women, but a lower risk among postmenopausal women. We did not observe any association with tea or decaffeinated coffee intake. Two SNPs in *CYP19*, but no variants in *CYP1A1*, *CYP1A2*, and *CYP2A6* were associated (one positively

[2]http://www.uscnorris.com/Core/DocManager/DocumentList.aspx?CID=13

and one inversely) with ovarian cancer risk. Several gene-environment interactions were observed among premenopausal but not postmenopausal women.

Our observations regarding a modifying role of menopausal status in the association between coffee and ovarian cancer risk is consistent with what has been described previously for the NHS/NHSII and NECC (4,5). We found that the inverse association with coffee was limited to postmenopausal women. The small sample size of the nested-case control in the NHS/NHSII may have limited our ability to detect any significant differences in this analysis; however, in our previous study of the entire NHS/NHSII cohort (n =507 cases), we reported a significant reduction in risk among those in the highest versus lowest quintile of caffeine intake (OR = 0.69; 95% CI 0.50–0.95; *P* for trend = 0.02) (4). Among premenopausal women from the NECC, increasing intake of both caffeine and coffee were positively associated with ovarian cancer (*P* for trend = 0.007 and 0.003, respectively). These results are similar to those from the earlier NECC publication (n = 549 cases) (5).

To date, five prospective studies and 12 case-control studies have investigated whether coffee is associated with ovarian cancer risk, with conflicting results (3,4,20). Only three studies have specifically evaluated caffeine (20–22). In the first, the authors observed an increased risk of ovarian cancer with caffeine intake among both menopausal groups (21). However, Jordan *et al.* reported a significant inverse association that was limited to postmenopausal women (22). More recently, Song *et al.* reported no association between caffeine consumption and ovarian cancer risk (20). Despite the uncertainty in the literature regarding the role of coffee in etiology of ovarian cancer, the consistency observed between the Jordan *et. al* study, the retrospective assessment of the NECC, and the prospective analysis of the NHS collectively suggest that coffee consumption may influence ovarian cancer risk differentially by menopausal status.

Tea consumption did not influence risk of ovarian cancer in this group of women which is in accordance with the majority of published case-control studies; whereas, prospective studies including a study of the entire NHS/NHSII cohort generally have found an inverse association between tea and ovarian cancer (3,4,20). The lack of an association with decaffeinated coffee supports a role of caffeine as the component of coffee mediating the association with ovarian cancer risk.

The biological rationale explaining why the relationship between coffee/caffeine and risk is modified by menopausal status is unclear; however, caffeine may differentially modulate hormone levels among pre- and postmenopausal women. Furthermore, Tworoger *et al.* reported that inverse association with caffeine was limited to women who did not use exogenous hormones (4). Synthetic hormones found in OCs and estrogen replacement therapy have been shown to inhibit CYP1A2-mediated caffeine metabolism in both pre- and postmenopausal women (23,24). Consequently, use of exogenous hormones may obscure the possible decrease in risk conferred by caffeine. Thus, it is possible that caffeine or coffee may only exert a protective effect in a low hormonal environment; this possible association should be re-evaluated in future studies.

Coffee or caffeine also may influence endogenous hormone levels. Both have been directly associated with estrone and SHBG levels, and inversely with testosterone (25) among postmenopausal women whereas in premenopausal women, caffeine appears to be associated with higher SHBG (26,27) and 2-hydroxyestrone (OHE) to 16α-OHE ratio (28,29), as well as decreased menstrual cycle length (30). Associations with other forms of estrogen are unclear (27,31). Future studies should evaluate whether intakes of caffeine and caffeine-containing beverages are associated with sex hormone levels in pre- and postmenopausal women and among non-exogenous hormone users.

We initially hypothesized that the contrasting associations between coffee and cancer risk in pre- versus postmenopausal women might be explained by variants in genes involved in caffeine or sex hormone metabolism pathways. The *cytochrome P450* (*CYP*) genes are a large superfamily of genes that encode Phase I enzymes involved in the oxidative metabolism of numerous exogenous and endogenous compounds including various steroid hormones and caffeine (32). CYP19, or aromatase, is the key enzyme mediating the conversion of testosterone to estradiol and androstenedione to estrone. Since these CYP enzymes are involved in the metabolism of caffeine, estrogen, or both, we evaluated whether or not polymorphisms in these genes may modify the association between coffee or caffeine intake and risk of ovarian cancer. The *CYP1A1, CYP1A2*, or *CYP2A6* genotypes were not associated with risk, nor was there any evidence for modification by these genotypes among women with high versus low levels of caffeine, coffee, decaffeinated coffee or tea intake.

CYP1A2 was the most likely mediator of risk with coffee intake since this enzyme is responsible for the metabolism of more than 95% of caffeine, is itself induced by caffeine (33), and is involved in estrogen metabolism (34). A common *A* to *C* polymorphism in the *CYP1A2* gene decreases enzyme inducibility and activity, resulting in the slower metabolism of caffeine (35–37). We did not see any evidence for effect modification by this genotype. Only one other study evaluated this association and observed an increased risk of ovarian cancer with coffee and caffeine intake only among women carrying both *AA* alleles (21). Nonetheless, these results cannot be directly compared to ours since most women in the Goodman *et al.* case-control study were Asian or Pacific Islander whereas our analysis was limited to Caucasian women.

CYP2A6 is involved in the biotransformation of nicotine (38), coumarin (39) and the metabolic activation of various carcinogens (40). CYP2A6 also metabolizes 1,7-dimethylxanthine, the primary metabolite of caffeine (41,42). Many variants in the *CYP2A6* gene have been identified that explain the inter-individual variation in enzymatic activity; however, their frequency in Caucasians is low (43,44). Five prior studies have examined the association between ovarian cancer and the *Msp1* polymorphism in *CYP1A1* and all reported no association, similar to our study (45–49). However, Terry *et al.* did report an elevated risk of ovarian cancer among women with the *Ile/Val* variant who consumed > 204.5 mg of caffeine per day (47). Comparable with our findings, they did not report such an interaction with the *Msp1* variant and caffeine intake.

Only one of the *CYP19* haplotypes estimated from the htSNPs was associated with ovarian cancer risk. Since there were no significant associations overall or with the other individual haplotypes, the significant inverse association in block 3 likely is due to chance. Interestingly, the two *CYP19* htSNPs *CYP19013* and *CYP19027*, which were independently associated with ovarian cancer risk in our study, have been previously shown to be significantly associated with endogenous estradiol and estrone levels among postmenopausal women (50). Levels of circulating estradiol and estrone were 12 to 17% higher among individuals who were homozygous variant for either SNP compared to wildtype (P for trend $\geq 1.3 \times 10^{-10}$). Furthermore, a significant 10% to 20% increase in postmenopausal estrogen levels were noted for heterozygous and homozygous carriers of the two-SNP haplotype compared with non-carriers (P for trend = 4.4×10^{-15}). Because the latter study was comprised of a multi-ethnic cohort and we limited our study population to white women, the minor allele frequency of *CYP19027* differed between the two groups. Thus, we repeated our analysis such that the reference allele of the two SNPs corresponded to that of the Haiman *et al.* paper (due to differing ethnic distributions) and showed a higher risk of ovarian cancer among women who were homozygous variant for the *A* alelle (OR = 1.12; 95% CI 0.90–1.38; P for trend = 0.05) for the *CYP19027* SNP. We also examined the association with the two-SNP haplotype and found that women with both the *A* risk alleles had a 13% increased risk of ovarian cancer compared

to women with both the wild-type alleles (OR = 1.13; 95% CI 1.00–1.27). Despite the numerous statistical comparisons in our study, these are important preliminary results since these variants exert a functional effect on endogenous estrogen levels, and thus elevated estrogen levels may explain the increased risk of ovarian cancer we observed. While this provides biological plausibility for our observations, replication is required in future studies.

One study evaluated whether allelic variants of *CYP19*, *CYP1A2* and *CYP1A1* were associated with the risk of hormone-dependent cancers among Caucasian women from Russia (48). The authors reported no association between the *CYP19* and *CYP1A1 Msp1* polymorphisms and risk, and an inverse association between the *CYP1A2 AA* genotype and risk. A major limitation of this study is that the authors did not distinguish between endometrial and ovarian tumor types in their analysis. Among premenopausal women, we reported significant interactions between caffeine intake and three of the *CYP19* htSNPs; however, it is unclear by what mechanism these variants in *CYP19* may alter the association between caffeine and ovarian cancer risk. Thus, our results should be interpreted with caution.

There are several limitations to the present study. There were very few premenopausal cases in the NHS/NHSII due to the age of the cohort. Nonetheless, there were a sufficient number of premenopausal cases in the NECC (n = 510) for the analysis of the gene-environment interactions. In the NECC, the FFQ was completed in the cases following their diagnosis. This retrospective assessment of coffee intake is subject to recall bias and misclassification of the exposure variables; however, this is unlikely since the results of the NECC were similar to those in the prospective analysis of the NHS/NHSII (4). We used cumulative updating for the NHS/NHSII but could not take into account duration of intake among women in the NECC. The current analysis was limited to white women and may not generalizable to other populations of different ancestries. Given the large number of SNPs and gene-environment interactions that were evaluated, our positive findings may be attributed to chance.

A major strength of our study was the ability to evaluate the association between a common exposure and ovarian cancer risk using a large study population that included 1352 cases and 1847 controls. Furthermore, we controlled for the majority of the known or suspected risk factors for ovarian cancer thus decreasing the influence of confounding. The prospective nature of the NHS/NHSII allowed for the detailed collection of unbiased dietary and risk factor information.

These data further suggest that menopausal status modifies the association between coffee consumption and ovarian cancer risk and that genetic variation in *CYP19013* and *CYP19027* may be implicated in the etiology of ovarian cancer; however few, if any, diet-genotype interactions exist. Because coffee is such a common exposure, clarifying its role is critical especially since the data supporting nutritional or lifestyle factors in the prevention of ovarian cancer is scarce. Moreover, our results could have important public health implications especially since the inverse association in postmenopausal women was seen with consumption of at least 2.5 cups of coffee per day. Additional studies evaluating mechanisms by which coffee mediates risk are warranted before recommendations are implemented.

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Table 1

Association between coffee intake and ovarian cancer risk in the NHS and NECC, among all women and stratified by menopausal status

	NHS/NHSII[1]			NECC[1]			Pooled
All Women	**Cases (n = 232) [3] n (%)**	**Controls (n = 687) [3] n (%)**	**OR (95% CI)[2]**	**Cases (n = 1120) [3] n (%)**	**Controls (n = 1160) [3] n (%)**	**OR (95% CI)**	**OR (95% CI)**
< 2.5 cups/day	151 (70%)	411 (66%)	1.00 (ref.)	645 (62%)	696 (63%)	1.00 (ref.)	1.00 (ref.)
≥ 2.5 cups/day	66 (30%)	214 (34%)	0.82 (0.57–1.19)	400 (38%)	401 (37%)	1.08 (0.90–1.30)	0.99 (0.77–1.28)
P - trend			0.68			0.11	0.34
P - heterogeneity							0.27
Premenopausal							
< 2.5 cups/day	26 (62%)	71 (55%)	1.00 (ref.)	266 (55%)	328 (63%)	1.00 (ref.)	1.00 (ref.)
≥ 2.5 cups/day	16 (38%)	59 (45%)	0.60 (0.26–1.41)	217 (45%)	194 (37%)	1.35 (1.03–1.78)	1.00 (0.47–2.15)
P - trend			0.20			0.003	0.96
P - heterogeneity							0.03
Postmenopausal							
< 2.5 cups/day	120 (73%)	314 (69%)	1.00 (ref.)	347 (68%)	341 (65%)	1.00 (ref.)	1.00 (ref.)
≥ 2.5 cups/day	44 (27%)	144 (31%)	0.79 (0.51–1.24)	167 (32%)	184 (35%)	0.84 (0.64–1.10)	0.83 (0.66–1.04)
P - trend			0.98			0.43	0.51
P - heterogeneity							0.67

[1] Conditional (NHS/NHSII) and unconditional (NECC) logistic regression adjusted for age, study center (NECC only), parity, oral contraceptive use, postmenopausal hormone use, tubal ligation, smoking history, and body mass index, family history of breast or ovarian cancer.

[2] Odds ratio (95% confidence intervals).

[3] Frequencies do not add up to total n due to missing data on coffee intake.

Table 2

Association between *CYP1A1*, *CYP1A2*, *CYP2A6*, and *CYP19* htSNPs genotypes and ovarian cancer risk in the NECC, NHS and NHSII (pooled results)[1]

RS Number	SNP	Wt	Het	Hom	*P* – trend[2]	Het + Hom	*P*[3]
rs4646903	*CYP1A1 MSP1 (T,C)*	1.00 (ref)	1.06 (0.87–1.29)	0.62 (0.33–1.14)	0.74	1.01 (0.84–1.22)	0.87
rs762551	*CYP1A2 A154C (A,C)*	1.00 (ref)	1.10 (0.94–1.30)	0.97 (0.57–1.66)	0.75	1.09 (0.92–1.29)	0.17
rs1801272	*CYP2A6 L160H (T,A)*	1.00 (ref)	1.05 (0.52–2.11)	n/a[4]	n/a	1.05 (0.53–2.09)	0.90
rs2446405	*CYP19001 (T,A)*	1.00 (ref)	1.09 (0.92–1.30)	1.18 (0.74–1.88)	0.25	1.10 (0.93–1.30)	0.68
rs2445765	*CYP19002 (G,C)*	1.00 (ref)	1.05 (0.88–1.24)	1.23 (0.76–1.97)	0.38	1.06 (0.90–1.25)	0.56
rs2470144	*CYP19003 (G,A)*	1.00 (ref)	0.96 (0.79–1.16)	0.94 (0.75–1.16)	0.56	0.95 (0.80–1.14)	0.52
rs1004984	*CYP19004 (C,T)*	1.00 (ref)	1.01 (0.86–1.20)	0.99 (0.77–1.27)	0.96	1.01 (0.86–1.18)	0.50
rs1902584	*CYP19005 (A,T)*	1.00 (ref)	1.08 (0.86–1.35)	1.39 (0.53–3.64)	0.39	1.09 (0.88–1.35)	0.66
rs28566535	*CYP19006 (A,C)*	1.00 (ref)	0.81 (0.41–1.62)	1.07 (0.35–3.28)	0.59	0.83 (0.43–1.59)	0.07
rs2445759	*CYP19007 (G,T)*	1.00 (ref)	0.98 (0.78–1.23)	1.39 (0.31–6.17)	0.95	0.99 (0.79–1.23)	0.62
rs936306	*CYP19010 (C,T)*	1.00 (ref)	0.92 (0.71–1.20)	1.35 (0.81–2.26)	0.84	0.98 (0.83–1.17)	0.58
rs1902586	*CYP19011 (G,A)*	1.00 (ref)	0.86 (0.49–1.49)	1.29 (0.45–3.73)	0.68	0.87 (0.51–1.50)	0.12
rs749292	*CYP19013 (G,A)*	1.00 (ref)	1.14 (0.95–1.36)	1.30 (1.04–1.62)	0.02	1.18 (1.00–1.39)	0.92
rs1008805	*CYP19014 (T,C)*	1.00 (ref)	0.90 (0.75–1.07)	0.82 (0.66–1.03)	0.08	0.88 (0.74–1.03)	0.81
rs4646	*CYP19018 (C,A)*	1.00 (ref)	0.87 (0.74–1.02)	1.02 (0.75–1.39)	0.32	0.89 (0.76–1.04)	0.99
rs700519	*CYP19023 (G,A)*	1.00 (ref)	0.96 (0.71–1.31)	n/a	n/a	0.95 (0.70–1.27)	0.82
rs10046	*CYP19025 (C,T)*	1.00 (ref)	1.16 (0.85–1.59)	1.21 (0.97–1.51)	0.09	1.18 (0.90–1.54)	0.48
rs727479	*CYP19027 (T,G)*	1.00 (ref)	0.85 (0.72–1.01)	0.82 (0.65–1.04)	0.05	0.85 (0.72–0.99)	0.43
rs2414096	*CYP19028 (C,T)*	1.00 (ref)	1.02 (0.85–1.23)	1.12 (0.90–1.39)	0.30	1.06 (0.95–1.18)	0.35
rs17601241	*CYP19031 (G,A)*	1.00 (ref)	1.09 (0.88–1.36)	1.82 (0.80–4.17)	0.21	1.12 (0.90–1.38)	0.41
rs6493494	*CYP19032 (G,A)*	1.00 (ref)	1.11 (0.93–1.32)	1.21 (0.96–1.51)	0.09	1.13 (0.96–1.34)	0.71
rs28757184	*CYP19033 (G,A)*	1.00 (ref)	0.96 (0.66–1.41)	n/a	n/a	0.96 (0.66–1.41)	0.85
rs2445762	*CYP19034 (T,C)*	1.00 (ref)	0.95 (0.81–1.12)	0.98 (0.73–1.31)	0.66	0.96 (0.82–1.12)	0.73
rs3751591	*CYP19036 (A,G)*	1.00 (ref)	0.98 (0.77–1.24)	0.62 (0.24–1.65)	0.45	0.93 (0.70–1.23)	0.11

[1] Results shown are the pooled odds ratio (95% confidence intervals) for the main effect of the heterozygous (Het) or homozygous (Hom) variant alleles compared to the wild–type (WT) genotype. Conditional (NHS/NHSII) and unconditional (NECC) logistic regression adjusted for age, study center (NECC only), parity, oral contraceptive use, postmenopausal hormone use, tubal ligation, smoking history, and body mass index, family history of breast or ovarian cancer.

[2] *P* - trend for a one-unit increase in the number of minor alleles and calculated using the Wald test.

[3] *P* - values for tests for heterogeneity comparing the NECC and NHS/NHSII results were all > 0.05.

[4] n/a = not applicable. There were no homozygous variants available for analysis.

Table 3

Association between high versus low caffeine, coffee or tea intake and ovarian cancer risk, stratified by genotype among premenopausal (NECC) and postmenopausal women (pooled) [1]

	OR (95% CI) for Caffeine < 409.5 vs ≥ 409.5 mg/day			OR (95% CI) for Coffee < 2.5 vs ≥ 2.5 cups/day			OR (95% CI) Tea < 0.83 vs ≥ 0.83 cups/day		
	WT	**Het + Hom**	***P*–int[2]**	**WT**	**Het + Hom**	***P*–int**	**WT**	**Het+ Hom**	***P*–int**
Premenopausal (only NECC)									
CYP1A1 MSP1 (T,C)	1.3 (0.9–2.1)	1.9 (0.8–4.3)	0.47	1.3 (0.9 –1.8)	1.5 (0.8–2.8)	0.64	1.1 (0.8–1.6)	1.1 (0.5–2.5)	0.94
CYP1A2 A154C (A,C)	1.4 (0.8–2.3)	1.6 (0.9–2.7)	0.77	1.2 (0.8–1.7)	1.5 (1.0–2.3)	0.33	0.9 (0.6–1.5)	1.2 (0.7–1.9)	0.53
CYP2A6 L160H (T,A)	1.4 (0.9–2.1)	2.7 (0.5–16.3)	0.48	1.3 (1.0–1.8)	1.1 (0.4–3.5)	0.75	1.1 (0.8–1.6)	1.6 (0.4–6.5)	0.64
CYP19011 (G,A)	1.6 (1.1–2.4)	0.3 (0.1–1.4)	0.04	1.5 (1.1–2.0)	0.7 (0.3–1.7)	0.12	1.0 (0.7–1.4)	1.6 (0.6–4.3)	0.44
CYP19013 (G,A)	1.3 (0.7–2.6)	1.6 (1.0–2.6)	0.64	1.2 (0.7– 2.0)	1.5 (1.1–2.1)	0.44	0.7 (0.4–1.4)	1.4 (0.9–2.0)	0.09
CYP19018 (C,A)	0.9 (0.5–1.6)	2.9 (1.7–5.0)	0.004	1.2 (0.8–1.7)	1.9 (1.2–2.8)	0.11	1.0 (0.7–1.6)	1.0 (0.6–1.7)	0.96
CYP19027 (T,G)	1.2 (0.6– 2.3)	1.6 (1.0–2.5)	0.45	1.1 (0.7–1.7)	1.6 (1.1–2.3)	0.16	1.1 (0.7–1.9)	1.0 (0.7–1.5)	0.75
CYP19034 (T,C)	1.0 (0.6–1.6)	2.2 (1.3–3.8)	0.03	1.3 (0.9–1.8)	1.4 (0.9–2.1)	0.74	0.9 (0.5–1.4)	1.4 (0.9–2.2)	0.17
Postmenopausal (NHS/NHSII+NECC)									
CYP1A1 MSP1 (T,C)	1.0 (0.4–2.1)	0.7 (0.4–1.5)	0.62	0.7 (0.5–1.1)	0.9 (0.6–1.5)	0.51	1.3 (1.0–1.7)	0.9 (0.5–1.6)	0.27
CYP1A2 A154C (A,C)	0.7 (0.3–1.7)	1.4 (0.9–2.3)	0.21	0.7 (0.4–1.0)	1.0 (0.7–1.5)	0.17	1.0 (0.4–2.4)	1.3 (0.8–2.1)	0.64
CYP2A6 L160H (T,A)	1.0 (0.7–1.5)	0.5 (0.2–1.7)	0.32	0.8 (0.6–1.1)	0.5 (0.1–1.7)	0.51	1.2 (0.9–1.6)	1.0 (0.3–3.0)	0.73
CYP19011 (G,A)	1.0 (0.7–1.3)	1.0 (0.7–1.3)	0.62	0.8 (0.6–1.0)	0.8 (0.6–1.0)	0.95	1.2 (0.9–1.5)	1.2 (0.9–1.5)	0.38
CYP19013 (G,A)	1.5 (0.5–5.0)	0.8 (0.6–1.2)	0.34	1.0 (0.4–2.5)	0.7 (0.5–0.9)	0.67	1.5 (0.9–2.2)	1.1 (0.8–1.6)	0.36
CYP19018 (C,A)	0.8 (0.4–1.4)	1.4 (0.9–2.1)	0.06	0.7 (0.4–1.0)	1.0 (0.7–1.4)	0.25	1.0 (0.6–1.5)	1.5 (1.0–2.2)	0.76
CYP19027 (T,G)	0.8 (0.5–1.2)	1.0 (0.5–2.2)	0.35	0.6 (0.5–0.9)	0.8 (0.5–1.3)	0.64	1.3 (0.7–2.4)	1.1 (0.8–1.5)	0.84
CYP19034 (T,C)	0.9 (0.6–1.4)	1.1 (0.4–3.0)	0.59	0.8 (0.6–1.1)	0.7 (0.3–1.6)	0.77	1.0 (0.7–1.4)	1.2 (0.7–2.0)	0.84

[1] Results shown are the pooled odds ratio and 95% confidence intervals. Conditional (NHS/NHSII) and unconditional (NECC) logistic regression adjusted for age (NECC only), study center (NECC only), parity, oral contraceptive use, postmenopausal hormone use, tubal ligation, smoking history, and body mass index, family history of breast or ovarian cancer.

[2] Pooled *P* for interaction between dietary exposure (i.e. caffeine, coffee or tea) and genotype (i.e. wild type or Het + Hom).