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Genetic polymorphisms of phase I metabolizing enzyme genes, their interaction with lifetime grilled and smoked meat intake, and breast cancer incidence

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Abstract

Purpose—To examine associations between 22 *CYP* single nucleotide polymorphisms (SNPs) and breast cancer incidence and their interactions with grilled–smoked meat intake, a source of polycyclic aromatic hydrocarbons.

Methods—White women with first primary *in situ* or invasive breast cancer (n = 988) and frequency-matched controls (n = 1021) from a population-based study were interviewed to assess lifetime grilled–smoked meat intake. SNPs with minor allele frequencies of greater than 0.05 were selected because of their links to carcinogenesis. We used multivariable unconditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs).

Results—Breast cancer was inversely associated with *CYP1A1* rs104C8943 AG + GG genotype (OR = 0.71, 95% CI = 0.50–0.99; vs. AA genotype) and positively associated with *CYP1B1* rs10175338 TT genotype (OR = 1.59, 95% CI = 1.12–2.26; vs. GG genotype) and the *CYP3A4* rs2242480 CT + TT genotype (OR = 1.25, 95% CI = 1.00–1.56; vs. CC genotype). The sum of the

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number of “at-risk” alleles for the *CYP* SNPs was positively associated with breast cancer incidence (4–6 “at-risk” alleles OR = 2.33, 95% CI = 1.37–3.99 vs. 0-1 alleles; $P_{Trend} < .01$). We observed multiplicative and additive interactions ($P < .05$) between grilled –smoked meat intake (low vs. high) with *CYP1A1* rs1048943 and *CYP1B1* rs10175338 SNPs.

Conclusions—Phase I metabolizing enzyme gene SNPs may play a role in breast cancer development and may modify the grilled–smoked meat intake–breast cancer association.

Keywords

Breast cancer; Cytochrome p450 enzymes; Polymorphism; Grilled and smoked meat; Gene–environment interactions

Introduction

Breast cancer is the most frequently diagnosed cancer among women in the United States with more than 231,000 new breast cancer cases estimated in 2016 [1]. Environmental exposures may play a role in breast carcinogenesis [2], particularly in concert with polymorphic low penetrance, but common genes [3]. Exposure to polycyclic aromatic hydrocarbons (PAHs), a group of more than 100 different chemicals that are formed during the incomplete combustion of coal, oil, and gas and other organic substances like tobacco and are in charbroiled and/or smoked meat [4], has been associated with elevated breast cancer incidence [5]. During grilling and barbecuing, specifically, PAHs are generated through pyrolysis of meat products when fat drips from the meat onto a heated surface and produces smoke that coats the food with the compounds [6]. Dietary PAH exposures from grilled–smoked meat, the primary source of PAH exposure among nonsmokers [7], have been associated with breast cancer incidence, with effect estimates ranging from 1.47 to 2.21 when comparing the highest to the lowest quantiles of intake of well-done meat [8–12].

Once ingested, PAHs induce expression of and are metabolized by phase I and phase II metabolizing enzymes. In phase I metabolism, PAH parent compounds are activated to dihydrodiol intermediates by cytochrome P450 (CYP) enzymes [13]. The dihydrodiols are further oxidized by CYPs into highly reactive diol epoxides, which are able to covalently bind to exocyclic amino groups of adenine and guanine, forming stable adducts on DNA [14,15]. The DNA adducts can cause mismatch in DNA replication and may alter promoter methylation or promoter binding, leading to inheritable DNA mutations or abnormal gene expression [16]. The main enzymes involved in phase I PAH metabolism include CYP1A1 (*CYP1A1* located on chromosome 15q24.1), CYP1A2 (*CYP1A2* located on chromosome 15q24.1), CYP1B1 (*CYP1B1* located on chromosome 2p22.2), and CYP3A4 (*CYP3A4* located on chromosome 7q21.1), four of approximately 60 CYP enzymes [13].

CYP enzymes are primarily and abundantly expressed in the liver, kidneys, gastrointestinal tract, and lungs [17], but they are also expressed in normal breast tissue [18]. PAHs are activated and metabolized by breast epithelial cells [19], and overexpression of CYP enzymes and elevated levels of PAH-DNA adduct have been observed in breast tumor tissue [20]. Given the central role of CYP enzymes in the metabolism of xenobiotics, polymorphisms in *CYP* genes, especially those that may potentially alter the activity (e.g.,

rs1048943, rs2472299, and rs2567206 located in promoter regions) or inducibility (e.g., rs1056836, rs1800440, and rs10012 located in exonic regions) relative to the common alleles may influence breast cancer risk [21].

In the present study, we had two study goals. First, to examine the associations between 22 single nucleotide polymorphisms (SNPs) in four CYP enzyme genes (*CYP1A1*, *CYP1A2*, *CYP1B1*, and *CYP3A4*) and breast cancer incidence. Second, to examine whether the SNPs associated with breast cancer also modify the positive association between intake of grilled–smoked meat and breast cancer incidence previously reported by our group [8]. We hypothesized that polymorphisms that lead to increased enzymatic activity and therefore increased production of procarcinogens would be positively associated with breast cancer incidence, whereas those that lead to decreased enzymatic activity would be inversely associated with breast cancer. We also hypothesized that high intake of grilled–smoked meat and SNPs that confer increased enzymatic activity would act synergistically to increase the risk of incident breast cancer.

Materials and methods

This study used resources from the case–control component of the Long Island Breast Cancer Study Project (LIBCSP), a population-based study of adult women living in Nassau and Suffolk Counties on Long Island, New York [22].

Study population

Details of the LIBCSP case–control participants have been previously described [22]. In brief, women aged older than 20 years who were residents of Nassau and Suffolk Counties on Long Island, New York, and who were diagnosed with invasive or *in situ* breast cancer in 1996–1997 were identified using a rapid reporting system established for the LIBCSP. Diagnosed breast cancer cases were confirmed by each case's physician and by medical record review. Of the 2271 total women identified, consent was obtained for 1837, and of these, 1508 (82.1%) completed the main questionnaire. Control women were residents of the same two Long Island counties who were frequency matched to the expected age distribution of the case women on 5-year age group. Control women aged 65 years and older were identified by Health Care Finance Administration rosters and those aged younger than 65 years were identified by random digit dialing [23]. Of the 2714 women identified as potential controls, consent was obtained by 2481, and of these, 1556 (62.7%) completed the main questionnaire. Participants ranged in age from 20 to 95 years at diagnosis or date of identification for controls, and most were postmenopausal (66%) and self-identified as White (93%), which reflects the underlying racial distribution of these Long Island counties at the time of data collection.

Ethics

This study was approved by the Institutional Review Board of the University of North Carolina at Chapel Hill (approval no. 12-0131). Written informed consent was obtained from participants before data collection.

Assessment of dietary intake

On average, within 3 months of breast cancer diagnosis, LIBCSP participants completed an interviewer-administered main questionnaire and a self-administered food frequency questionnaire. The questionnaire elicited information on known and suspected risk factors for breast cancer, including reproductive, occupational, lifestyle, medical and environmental factors, as well as demographic characteristics. The food frequency questionnaire, which was completed by 98% of respondents, elicited information on approximately 100 food items that assessed usual diet in the previous year and was used to determine consumption of energy (kcal/day), fruit and vegetable intake (servings per day), and multivitamin supplement use (ever/never). The main questionnaire also included an assessment of intake of four categories of grilled–barbecued meat and smoked meat (grilled–barbecued beef, lamb, or pork; grilled–barbecued poultry or fish; smoked beef, lamb, or pork; and smoked poultry or fish) in each decade of life (<20 years, 20–29 years, 30–39 years, 40–49 years, 50–59 years, and ≥60 years), which was used to estimate the lifetime consumption of grilled, barbecued, and smoked meat [8,24]. We previously reported that total intake of grilled–smoked meat (lifetime servings) was the variable definition most strongly associated with breast cancer incidence [8], and thus it is the definition we use in the gene–environment study reported here.

SNP selection and genotyping

We selected 24 polymorphisms representing four genes of phase I metabolizing CYP enzymes: *CYP1A1* (rs1531163, rs2472299, rs2606345, rs4646903, rs1048943, and rs1800031), *CYP1A2* (rs2069522, rs2470890, rs2472304, and rs762551), *CYP1B1* (rs10175338, rs10175368, rs1056836, rs10916, rs162330, rs162555, rs162556, rs162557, rs162562, and rs1800440), and *CYP3A4* (rs11773597, rs12333983, rs2242480, and rs2740574). The 24 polymorphisms were selected because of the documented role of the CYP enzymes in the activation of PAHs [13], the reported link of the SNPs to carcinogenesis [25], and previously reported minor allele frequencies of at least 0.05 in Caucasian women [25].

At the time of the interview, approximately 73% of participants provided a nonfasting blood sample. As previously reported [22], participants were more likely to donate blood if they were aged younger than 65 years, used oral contraceptives–hormone replacement, drank alcohol, and had ever received a mammogram, but were nonsmokers. Genomic DNA was extracted from the mononuclear cells in whole blood separated by Ficoll (Sigma Chemical Co., St. Louis, MO). *CYP1A1* SNPs rs4646903, rs1048943, and rs1800031 were genotyped by BioServe Biotechnologies (Laurel, MD) using Sequenom's (San Diego, CA) high-throughput matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [26]. The remainder of the SNPs were genotyped at the University of North Carolina at Chapel Hill using the Illumina GoldenGate assay (Illumina, Inc., San Diego, CA). For the latter, assay intensity data and genotype cluster images for all SNPs were reviewed individually using GenomeStudio software, v. 2011.1. Blind duplicates of 56 samples were genotyped to verify the reproducibility of genotype calls. Concordance between duplicates was greater than 99.4% for all pairs.

Statistical analysis

This study is restricted to the 2009 women (*n* cases = 988 and *n* controls = 1021) who self-identified as White and for whom genotyping data were available because of the small sample sizes in other race–ethnicity groups. We tested for Hardy–Weinberg equilibrium among the control women using Proc Allele on SAS/Genetics version 9.3 (Cary, NC) at an alpha level of 0.05. Two SNPs (rs4646903 and rs11773597) exhibited significant departure from Hardy–Weinberg equilibrium and were not considered further. We also examined all SNPs for linkage disequilibrium (LD) using the SNAP database based on HapMap [27]. The following pairwise SNPs were in LD: *CYP1A2* rs2470890 and rs2472304; *CYP1B1* rs10175338 and rs10175368; and *CYP1B1* rs10916 and rs162562. For the three pairs of SNPs in LD, only the results for one SNP (rs2470890, rs10175338, rs10916) are presented.

For the statistical analysis, we addressed our two study goals. First, to examine whether 22 SNPs in four CYP genes are associated with breast cancer risk. And, second, to examine whether interactions between these SNPs and intake of grilled–smoked meat were associated with breast cancer. To address the first goal, odds ratios (ORs) and 95% confidence intervals (CIs) of the associations between 22 *CYP* genotypes and breast cancer incidence were estimated using unconditional logistic regression [28] in SAS version 9.4 (Cary, NC). In the text, we present results of dominant models; results of additive models are presented in Supplemental Table 1. All models were adjusted for age to account for frequency matching. Common homozygote genotypes defined among the control women were the referent group for all analyses. We created a summary SNP index for each participant by summing the number of “at-risk” alleles present for each SNP that was found to be associated with breast cancer (total of three SNPs). Based on the associations presented in Table 1, the “at-risk” allele was defined as the less common (variant) allele (*CYP1B1* rs10175338: T allele; and *CYP3A4* rs2242480: T allele), with the exception of the *CYP1A1* rs1048943 SNP where the more common allele (A allele) was associated with increased risk in this population. SNPs that were significantly associated with breast cancer incidence were further considered as interactions with lifetime grilled–smoked meat intake, dichotomized at the median (high = 4297 to 51,652 vs. low = 0 to 4296) among controls.

To address our second study goal, the interaction of *CYP* genotypes with intake of grilled–smoked meat was evaluated by including cross-product terms in the logistic regression models. Multiplicative interactions were examined by comparing the models with the interaction term against a reduced model that included only terms for the main effects and conducting a likelihood ratio test; the results are presented by stratifying the grilled–smoked meat intake–breast cancer association by *CYP* genotype. For additive interaction, we explored the joint effects of lifetime grilled–smoked meat intake and *CYP* genotype (heterozygous + variant allele vs. common allele); we created three indicator variables for each SNP and included all three variables in a single logistic regression model. The following covariates were considered as potential confounders of the association between grilled–smoked meat intake and incident breast cancer: age, menopausal status, education, parity, use of hormone replacement therapy, smoking status, physical activity, body mass index, alcohol intake, energy intake, intake of fruits and vegetables, and single and multiple vitamin use [8]. Of these, the following covariates were found to change the effect estimate

for the association between grilled–smoked meat intake and breast cancer incidence by more than 10% and were included in the final multivariable models: age at diagnosis (5-year age groups), energy intake (continuous), fruit and vegetable intake (categorized as quintiles of intake in the past 12 months), and multivitamin supplement use (yes vs. no).

Results

Results corresponding to our two study goals (to determine whether breast cancer risk was associated with 22 SNPs in four CYP genes and the interaction between CYP SNPs and intake of grilled–smoked foods) are presented in the following.

CYP SNPs and breast cancer risk

The *CYP1A1* rs1048943 AG + GG genotype was inversely associated with breast cancer incidence (OR = 0.71, 95% CI = 0.50–0.99) relative to the AA genotype, as shown in Table 1. We observed increased ORs for the associations between breast cancer incidence and *CYP1B1* rs10175338 TT ($OR_{TT\ vs.\ GG}$ = 1.59, 95% CI = 1.12–2.26), which was in LD with rs10175368, and *CYP3A4* rs2242480 CT + TT ($OR_{CT+TT\ vs.\ CC}$ = 1.25, 95% CI = 1.00–1.56). The sum of the number of "at-risk" alleles for these three *CYP* SNPs (rs10175338, rs2242480, and rs1048943) was positively associated with breast cancer incidence (P_{Trend} < .01); the odds of breast cancer incidence among women with 4–6 "at-risk" alleles was 2.33 (95% CI = 1.37–3.99) times the odds among women with 0 or 1 "at-risk" alleles.

CYP SNP interactions with grilled–smoked meat intake and breast cancer risk

We observed significant effect modification on a multiplicative scale of the association between total grilled–smoked meat intake over the life-course and breast cancer incidence by *CYP1A1* rs1048943 ($P_{Interaction}$ = .03) and *CYP1B1* rs10175338 ($P_{Interaction}$ < .01) SNPs (Table 2). Among women with the *CYP1B1* rs10175338 GG genotype, the OR for the association with high grilled–smoked meat intake, compared with low intake, was increased approximately 60% (OR = 1.59, 95% CI = 1.15–2.20); but among women with the GT + TT genotypes and high grilled–smoked meat intake, the OR was decreased approximately 30% (OR = 0.71, 95% CI = 0.50–1.02). There was some suggestion of effect modification by the *CYP3A4* rs2242480 genotype ($P_{Interaction}$ = .07); the OR for the grilled–smoked meat–breast cancer association was elevated 59% (OR = 1.59, 95% CI = 0.91–2.77) among women with the CT + TT genotype, but not among women with the CC genotype (OR = 1.01, 95% CI = 0.78–1.30). In addition, as shown in Table 3, we observed effect modification on the additive scale for *CYP1A1* rs1048943 (interaction contrast ratio = −0.82, 95% CI = −1.62 to −0.01) and *CYP1B1* rs10175338 (interaction contrast ratio = −0.97, 95% CI = −1.72 to −0.23).

Discussion

In our population-based case-control study of 22 SNPs of four CYP genes, the variant allele of *CYP1A1* rs1048943 was associated with a reduced OR for breast cancer, whereas the variant alleles of *CYP1B1* rs10175338 and *CYP3A4* rs2242480 were associated with an increased OR for breast cancer incidence. The association with breast cancer was further elevated in a dose-response manner when we considered the sum of the risk alleles of the

three SNPs. When considering effect measure modification, we observed multiplicative interactions between lifetime grilled–smoked meat intake with *CYP1A1* rs1048943 and *CYP1B1* rs10175338 and additive interactions for *CYP1A1* rs1048943 and *CYP1B1* rs10175338.

The *CYP1A1* rs1048943, an A >G transition in exon 7, leads to the substitution of isoleucine (*Ile*) for valine (*Val*), resulting in increased enzymatic activity [29]. This suggests more efficient generation of reactive PAH intermediates, and thus increased breast cancer risk. However, CYP1A1 also catalyzes the conversion of estradiol into noncarcinogenic 2-hydroxyestradiol in extra-hepatic tissues, which may explain the inverse association we observed in this study between the *CYP1A1* rs1048943 *Ile/Val*-genotype and breast cancer. Our findings are consistent with at least one other case–control study [30] of Japanese women in which the *Ile*/*Val*-genotype was found to be associated with reduced breast cancer incidence compared with the *Ile/Ile* genotype; however, a meta-analysis did not report reduced breast cancer incidence for the *Ile/Val* or *Val/Val* genotypes [31]. Prior studies also have not supported an association between breast cancer risk and *CYP3A4* rs2242480 [32] and *CYP1B1* rs10175368 [33,34] variants. In contrast, in this study, we report a positive association with the variant alleles of *CYP1B1* rs10175338 and *CYP3A4* rs2242480. These SNPS occur within the noncoding regions of *CYP1B1* and *CYP3A4*, and the associations may be explained by linkage of these SNPS with other polymorphisms that have an effect on enzymatic function. For example, in addition to being in LD with each other, *CYP1B1* rs10175338 and rs10175368 are also in LD with *CYP1B1* rs1056827 [27], located in the second exon of chromosome 2p21.22 that results in an amino acid change from alanine to serine [35]. However, it is possible that these SNPS may have functional effects as well. Polymorphisms in noncoding sequences may influence gene function by altering the level, location, stability, or timing of gene expression [36]. rs2242480, a C > T substitution in intron 10 of *CYP3A4*, significantly increases its transcription [37].

No previous studies have examined the joint effects on breast cancer risk of *CYP* genotype and grilled–smoked meat intake, an important source of PAH exposure [7]. However, the interaction between the *CYP1A1* variants and environmental exposures has been studied in relation to cigarette smoking, polychlorinated biphenyls, and alcohol intake with some positive results [31]. For example, Ambrosone et al. found that carriers of the *CYP1A1 Ile/Val* genotype versus the *Ile/Ile* genotype who were nonsmokers, light smokers, and heavy smokers had relative risks of breast cancer of 1.30 (95% CI = 0.62–2.70), 5.22 (95% CI = 1.16–23.56), and 0.86 (95% CI = 0.24–3.09), respectively [38]. Consistent with the findings of an elevated risk of breast cancer among nonsmokers and light smokers with *CYP* variant alleles, we observed elevated ORs among women with the rs10175338 variant allele and 4–6 “at-risk” alleles and low intake of grilled–smoked meats (Table 3). As is hypothesized in the smoking and lung cancer literature [39], this may suggest that subtle changes in enzyme function based on genotype are masked among those who have high levels of exposure. Our results reported here show that the *CYP* genes may act antagonistically and synergistically to influence breast cancer development in the presence of dietary exposures. These results further contribute to the growing evidence for a role of PAHs in the etiology of breast cancer [40].

Our study used a large population-based case–control design to examine the genetic variants of the four main CYP enzymes involved in the metabolism of PAHs; however, several limitations should be noted. First, the large number of statistical tests could have resulted in spurious results. However, the associations examined here are biologically plausible as polymorphisms in genes encoding the CYP metabolizing enzymes are known to have the potential to alter enzyme activity and inducibility. In this report, we were interested understanding the main effects of the *CYP* genetic polymorphisms as well as their interactions with intake of grilled–smoked meat. We therefore do not present Bonferroni-corrected results, which can be overly conservative. Second, it is possible that the associations observed here are confounded by other SNPs not measured in our study. This may be especially true for the SNPs that occur in noncoding regions. However, the SNPs examined in this study have been shown to impact CYP enzyme activity. Third, approximately, 73% of case participants and 73% of control participants provided blood samples for analyses resulting in a sample size of 2009 for the present study. Donation of biological samples varied with age with lower proportions of older women, and in particular older control women, donating blood [22]. It is possible that this selection could bias our results; however, the proportion of eligible subjects who donated blood is comparable with other population-based studies that collected blood [41]. Thus, LIBCSP study results are likely to be as representative of the general population as those from other major population-based studies of breast cancer. Fourth, we used a self-reported measure of lifetime intake of grilled–smoked meat rather than an estimate of PAH intake specifically or a PAH biomarker. Grilling and smoking foods is well-known to increase the level of carcinogenic PAHs [7]. Importantly, measurement of PAH exposure by use of biomarkers reflects short-term exposure as adducts are excised, and DNA is repaired. Therefore, self-reported measurements of long-term exposure are our best alternative. Although the validity of recall of lifetime intake of grilled–smoked meat has not been directly examined, prior studies have reported modest correlations between dietary assessment at one point in time and recall of the same diet up to 20 years later [42,43]. Misclassification of the intake of grilled–smoked meat could result in bias toward or away from the null. In addition, while our categorization of lifetime intake of grilled–smoked meat intake at the median maximized our power to examine interactions and allowed us to more easily examine both multiplicative and additive interactions, this categorization scheme limits our ability to make clinical or public health recommendations regarding specific quantities in intake of grilled–smoked meat as related to risk of breast cancer. In a previous report where we describe our results of examining the intake of grilled–smoked foods in association with the risk of developing breast cancer [8], we considered intake at various ages and lag times. However, the strongest associations with breast cancer risk were with cumulative lifetime intake. Thus, for the interaction analysis, we *a priori* decided to consider lifetime intake only. Furthermore, we also decided against including a lag period since the main carcinogens in grilled–smoked meat, PAHs, could act as both initiators and promoters—several PAHs including chrysene and fluoranthene show estrogenic activity *in vitro* [44]— and so including a lag may underestimate the associations. Finally, it is possible that there could be differential recall of grilled– smoked meat intake by case–control status; however, previous studies of long-term recall of diet have not found differential recall between cancer cases and controls [45].

In summary, results from our population-based study indicate that genetic variants of four phase I metabolizing enzyme genes may play a role in breast cancer development and may modify the positive association between grilled–smoked meat intake and breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for cytochrome P450 genotypes in breast cancer cases and controls in the Long Island Breast Cancer Study Project (n = 2009)

Gene (rs id)	Cases/controls	OR (95% CI)
CYP1A1 (rs1531163)		
AA	891/912	Ref.
AG	93/106	0.87 (0.65–1.17)
GG	1/2	0.60 (0.05–6.52)
AG + GG	94/108	0.87 (0.64–1.16)
CYP1A1 (rs2472299)		
GG	483/515	Ref.
GA	422/420	0.97 (0.69–1.36)
AA	78/83	1.07 (0.89–1.29)
GA + AA	500/503	1.06 (0.88–1.26)
CYP1A1 (rs2606345)		
AA	407/423	Ref.
AC	437/448	1.02 (0.84–1.24)
CC	138/149	0.98 (0.75–1.29)
AC + CC	575/597	1.01 (0.85–1.21)
CYP1A1 (rs1048943)		
AA	873/890	Ref.
AG	60/92	0.69 (0.49–0.97)
GG	4/3	1.19 (0.26–5.38)
AG + GG	64/95	0.71 (0.50–0.99)
CYP1A1 (rs1800031)		
TT	903/954	Ref.
TC	9/5	1.97 (0.65–5.95)
CC	1/0	—
TC + CC	10/5	2.19 (0.74–6.49)
CYP1A2 (rs2069522)		
TT	931/963	Ref.
TC	53/57	1.00 (0.67–1.47)
CC	1/0	—
TC + CC	54/57	1.02 (0.69–1.50)
CYP1A2 (rs2470890) *		
TT	371/394	Ref.
TC	447/471	1.01 (0.84–1.23)
CC	162/151	1.12 (0.86–1.46)
TC + CC	609/622	1.04 (0.87–1.25)
CYP1A2 (rs762551)		
AA	471/510	Ref.
AC	422/417	1.10 (0.91–1.32)

Gene (rs id)	Cases/controls	OR (95% CI)
CC	82/90	0.97 (0.70–1.34)
AC + CC	504/507	1.07 (0.90–1.28)
CYP1B1 (rs10175338)*		
GG	525/564	Ref.
GT	369/388	1.03 (0.85–1.24)
TT	90/64	1.59 (1.12–2.26)
GT + TT	459/452	1.11 (0.93–1.32)
CYP1B1 (rs1056836)		
CC	331/337	Ref.
CG	469/480	0.97 (0.79–1.18)
GG	179/201	0.88 (0.68–1.13)
CG + GG	648/681	0.94 (0.78–1.14)
CYP1B1 (rs10916)*		
TT	629/666	Ref.
TG	307/304	1.07 (0.88–1.30)
GG	47/47	1.06 (0.69–1.61)
TG + GG	354/351	1.07 (0.89–1.29)
CYP1B1 (rs162330)		
TT	290/305	Ref.
TG	488/478	1.05 (0.85–1.29)
GG	206/236	0.89 (0.69–1.14)
TG + GG	694/714	1.00 (0.82–1.21)
CYP1B1 (rs162555)		
AA	618/614	Ref.
AG	321/346	0.89 (0.73–1.07)
GG	43/56	0.74 (0.49–1.13)
AG + GG	364/402	0.87 (0.72–1.04)
CYP1B1 (rs162556)		
TT	297/302	Ref.
TC	472/490	0.98 (0.80–1.21)
CC	215/224	0.98 (0.77–1.26)
TC + CC	687/714	0.98 (0.81–1.19)
CYP1B1 (rs162557)		
CC	610/647	Ref.
CT	327/319	1.09 (0.90–1.33)
TT	48/55	0.93 (0.62–1.39)
CT + TT	375/374	1.07 (0.90–1.28)
CYP1B1 (rs1800440)		
AA	653/637	Ref.
AG	294/338	0.84 (0.70–1.02)
GG	36/40	0.89 (0.56–1.42)
AG + GG	330/378	0.85 (0.71–1.02)

Gene (rs id)	Cases/controls	OR (95% CI)
CYP3A4 (rs12333983)		
TT	749/798	Ref.
TA	217/204	1.15 (0.92–1.43)
AA	17/17	1.08 (0.54–2.15)
TA + AA	234/221	1.14 (0.92–1.41)
CYP3A4 (rs2242480)		
CC	771/831	Ref.
CT	199/171	1.26 (1.00–1.59)
TT	16/15	1.13 (0.55–2.32)
CT + TT	215/186	1.25 (1.00–1.56)
CYP3A4 (rs2740574)		
AA	894/942	Ref.
AG	84/75	1.18 (0.85–1.63)
GG	4/2	2.43 (0.44–13.42)
AG + GG	88/77	1.21 (0.87–1.67)
Number of “at-risk” alleles[†]		
0–1	27/47	Ref.
2–3	743/809	1.58 (0.96–2.57)
4–6	161/121	2.33 (1.37–3.99)

Ref. = reference.

[*] SNPs in linkage disequilibrium rs2470890 and rs2472304; rs10175338 and rs10175368; rs10916 and rs162562.

[†] The “at-risk” allele was defined as follows: rs1048943: A allele; rs10175338: T allele; rs2242480: T allele.

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Table 2

Multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for total grilled–smoked meat intake (lifetime servings) over the life course, stratified by cytochrome P450 genotype, Long Island Breast Cancer Study Project (*n* = 2009)

Gene (rs id)*	Genotype	Lifetime servings grilled–smoked meat	Cases/controls, *n*	OR† (95% CI)	*P* value, ‡ indicating multiplicative interaction
CYP1A1 (rs1048943)					.03
	AA	Low	359/425	Ref.	
		High	473/417	1.21 (0.94–1.55)	
	AG + GG	Low	33/44	Ref.	
		High	30/47	0.64 (0.23–1.80)	
CYP1B1 (rs10175338) §					<.01
	GG	Low	195/281	Ref.	
		High	309/258	1.59 (1.15–2.20)	
	GT + TT	Low	225/201	Ref.	
		High	213/220	0.71 (0.50–1.02)	
CYP3A4 (rs2242480)					.07
	CC	Low	328/373	Ref.	
		High	410/414	1.01 (0.78–1.30)	
	CT + TT	Low	91/108	Ref.	
		High	115/66	1.59 (0.91–2.77)	
Number of “at-risk” alleles‖					.44
	0–3 alleles	Low	313/398	Ref.	
		High	422/412	1.20 (0.92–1.55)	
	4–6 alleles	Low	76/69	Ref.	
		High	78/46	0.97 (0.48–1.97)	

Ref. = reference.

*Low = 0–4296 times consumed, High = 4297–51,652 times consumed.

†Adjusted for age at diagnosis, energy intake, fruit and vegetable intake, and multivitamin supplement use.

‡*P* value for likelihood ratio test indicating interaction on a multiplicative scale.

§rs10175338 is in linkage disequilibrium with rs10175368; only the results for rs10175338 are presented.

[//] The "at-risk" allele was defined as follows: *CYP1A1* (rs1048943): A allele; *CYP1B1* (rs10175338): T allele; *CYP3A4* (rs2242480): T allele.

Table 3

Multivariable-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for the joint effects of total grilled–smoked meat intake (lifetime servings) and cytochrome P450 genotype, Long Island Breast Cancer Study Project (n = 2009)

Gene (rs id)	Lifetime servings grilled–smoked meat*	OR† (95% CI)	ICR‡ (95% CI), indicating additive interaction
CYP1A1 (rs1048943)			
AA	Low	Ref.	—
AA	High	1.22 (0.96–1.57)	—
AG + GG	Low	1.15 (0.64–2.05)	—
AG + GG	High	0.55 (0.30–1.03)	−0.82 (−1.62 to −0.01)
CYP1B1 (rs10175338) §			
GG	Low	Ref.	—
GG	High	1.54 (1.12–2.11)	—
GT + TT	Low	1.67 (1.20–2.33)	—
GT + TT	High	1.24 (0.88–1.73)	−0.97 (−1.72 to −0.23)
CYP3A4 (rs2242480)			
CC	Low	Ref.	—
CC	High	1.00 (0.78–1.30)	—
CT + TT	Low	1.07 (0.72–1.60)	—
CT + TT	High	1.83 (1.20–2.80)	0.75 (−0.06 to 1.57)
Number of “at-risk” alleles‖			
0–3 alleles	Low	Ref.	—
0–3 alleles	High	1.20 (0.93–1.55)	—
4–6 alleles	Low	1.61 (1.03–2.53)	—
4–6 alleles	High	1.49 (0.91–2.45)	−0.32 (−1.33 to 0.69)

Ref. = reference.

*Low = 0–4296 times consumed, High = 4297–51,652 times consumed.

†Adjusted for age at diagnosis, energy intake, fruit and vegetable intake, and multivitamin supplement use.

‡Interaction contrast ratio (ICR) indicating interaction on the additive scale. In the absence of interaction, ICR = 0.

§rs10175338 is in linkage disequilibrium with rs10175368; only the results for rs10175338 are presented.

‖The “at-risk” allele was defined as follows: *CYP1A1* (rs1048943): A allele; *CYP1B1* (rs10175338): T allele; *CYP3A4* (rs2242480): T allele.