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CARCINOGEN METABOLISM GENES, RED MEAT AND POULTRY INTAKE, AND COLORECTAL CANCER RISK

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Abstract

Diets high in red meat are established risk factors for colorectal cancer (CRC). Carcinogenic compounds generated during meat cooking have been implicated as causal agents. We conducted a family-based case-control study to investigate the association between polymorphisms in carcinogen metabolism genes (*CYP1A2* -154A>C, *CYP1B1* Leu432Val, *CYP2E1* -1054C>T, *GSTP1* Ile105Val, *PTGS2* 5UTR -765, *EPHX1* Tyr113His, *NAT2* Ile114Thr, *NAT2* Arg197Gln and *NAT2* Gly286Glu) and CRC risk. We tested for gene-environment interactions using case-only analyses (N = 577) and compared statistically significant results to those obtained using case-unaffected sibling comparisons (N = 307 sibships).

Our results suggested that *CYP1A2* -154A>C might modify the association between intake of red meat cooked using high temperature methods and well done on the inside and CRC risk (case-only interaction OR = 1.53; 95% CI = 1.19-1.97; p = 0.0008) and the association between intake of red meat heavily browned on the outside and rectal cancer risk (case-only interaction OR = 0.65; 95% CI = 0.48-0.86; p = 0.003). We also found that GSTP1 Ile105Val might modify the association between intake of poultry cooked with high temperature methods and CRC risk (p = 0.0035), a finding that was stronger among rectal cancer cases.

Our results support a role for heterocyclic amines that form in red meat as a potential explanation for the observed association between diets high in red meat and CRC. Our findings also suggest a possible role for diets high in poultry cooked at high temperatures in CRC risk.

Keywords

red meat; colorectal cancer; CYP1A2; GSTP1

Introduction

Colorectal (CRC) cancer is the third most common cancer and third leading cause of cancer death for both men and women in the United States[1]. Red meat consumption has been reported as a “convincing” risk factor for CRC in a large review conducted by the World

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Cancer Research Fund[2]. A meta-analysis of prospective studies published up to 2008 suggests that diets high in red meat or processed meat increase risk of CRC by about 20%[3]. In contrast, no overall association was found between diets high in poultry and CRC risk[3]. A few epidemiological studies, including our own, have taken into account cooking methods and doneness levels of red meat and poultry, and suggested positive associations between diets high in heavily browned red meat or red meat cooked using high temperature cooking methods and CRC[4-8].

Carcinogens that form during the cooking or processing of meats have been postulated as potential culprits for the association between red meats and CRC risk. These include: heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs) and N-nitroso compounds (NOCs)[9]. High cooking temperature or prolonged duration of cooking favors the formation of HCA[9, 10]. A few epidemiological studies have considered estimated levels of HCAs from diets high in well-done red meat and overall support a role for HCAs in CRC risk[4, 6, 8, 11]. PAHs are formed when meats are exposed to flames, such as when charbroiling and grilling[12], as well as during curing and processing of food with smoke[13]. Exposure to NOCs can occur from exogenous sources, such as cured meats with nitrites, or from endogenous formation due to nitrosating agents that react with amines derived from red meat[9, 14, 15]. The relative contribution of each of these three carcinogens to CRC is still uncertain.

Most absorbed dietary HCAs and PAHs are metabolized in the liver but are also transported back to the intestines via the bile acids and can be locally activated in the colon[16]. N-nitrosamines can be directly activated in human colon[17]. HCAs, PAHs, and NOCs require metabolic activation before they can react with macromolecules. These carcinogens, can also be detoxified and excreted, thus diminishing the amount of DNA damage induced by them. These metabolic reactions are carried out by specific combinations of Phase I and Phase II enzymes both in the liver and the colon. These enzymes vary in their metabolic activity in the human population; hence, it is biologically plausible to hypothesize that the inheritance of specific allelic variants of metabolizing genes may influence CRC risk. Whereas some epidemiological studies that focused on polymorphisms in Phase I and Phase II enzymes support this, overall results are inconclusive[18, 19]. However, studies on the role of key polymorphisms in some of these enzymes jointly with meat intake, considering cooking practices and/or level of doneness, find overall support for the hypothesis that variation in metabolic enzymes might modify the effect of diets high in red meat[11, 20-27]. However, few of these studies investigated potential interactions between these enzymes and diets high in poultry taking into account cooking methods[23, 24]. Furthermore, most of these studies have focused on only a few of the most relevant metabolic enzymes.

In this study, we investigated the role of polymorphisms in genes encoding seven enzymes that play key roles in the metabolism of the three main meat-induced carcinogens: CYP1A2 (HCA activation[16]), CYP1B1 (HCA[28] and PAH[29] activation), CYP2E1 (NOC activation[30]), GSTP1 (HCA, PAH, and NOC detoxification[31]), EPHX1 (PAH activation[29]), PTGS2 (also known as COX-2, HCA[1] and PAH[32] activation) and NAT2 (HCA activation[33]). We considered their overall association with CRC risk and their potential modifier role on the effect of diets high in red meat or poultry, taking into account cooking practices and doneness levels. All these SNPs were chosen based on their known impact on protein function and previous reports on their role on CRC risk.

Materials and Methods

Study Subjects

We conducted a family-based case-control association study with subjects recruited from the USC Consortium of the Colon Cancer Family Registry (Colon-CFR)[34]. Briefly, incident cases with CRC (probands) were recruited through the population-based registries affiliated with one of the component centers of the USC consortium[34]. Unaffected siblings and cousins in the family of the probands were enrolled, siblings were selected as controls. Preference was given to older and same-sex controls. Details on the ascertainment and eligibility criteria used by the USC Consortium have been published[34]. All subjects signed a written informed consent approved by the Institutional Review Board of each institution, donated a blood sample, and completed a risk factor questionnaire that provided demographic data, diet, physical activity and other life style factors. In our analyses we included affected probands (n=577) and unaffected siblings (n=362), for a total of 307 sibships, recruited between 1997-2002, as we previously described[7].

Exposure Assessment

Meat exposure was assessed using data from the baseline risk factor questionnaire used by all Colon CFR sites[7, 34]. Briefly, we included variables that captured servings per week of red meat (beef, steak, hamburger, prime rib, ribs, veal, lamb, bacon, pork, pork in sausages or venison) or poultry (chicken, turkey, fowl), and servings per week of red meat or poultry cooked by pan-frying, oven broiling, grilling or barbecuing (henceforth referred to as “high temperature methods”). Individuals were also asked about the level of doneness of red meat from inside (red, pink, brown) and the level of doneness of red meat or poultry from outside (lightly, medium or heavily browned) when red meat or poultry was cooked by pan-frying, oven broiling, grilling or barbecuing. All questions were referred to two years before cancer diagnosis of the proband.

Genotyping methods

Genomic DNA was extracted from peripheral blood lymphocytes and Taqman assays from Applied Biosystems (Foster City, CA) were used to determine the following SNPs: *CYP1A2* -154A>C (rs762551), *CYP1B1* Leu432Val (rs1056836), *CYP2E1* -1054C>T (rs2031920), *GSTP1* Ile105Val (rs1695, previously rs947894), *PTGS2* 5UTR -765 (rs20417), *EPHX1* Tyr113His (rs1051740), *NAT2* Ile114Thr (rs1801280), *NAT2* Arg197Gln (rs1799930) and *NAT2* Gly286Glu (rs1799931). Approximately 6% of the sample was randomly selected for repeated analysis. Call rates were >96% and we had 100% concordance between all duplicate samples.

Statistical analyses

Analyses of gene main effects—We found no statistically significant differences between the observed genotypic frequencies among Caucasian unaffected siblings (82.6% of all siblings) and those expected under HWE, compared using chi-square tests. Proband – unaffected sibling comparisons were conducted using 1: N matched conditional logistic regression. For gene main effect analyses we estimated odds ratios (ORs) and 95% confidence intervals (CI) for each genotype and per variant allele assuming a log-additive mode of action. Given that most of our matched siblings were older and the same gender of the probands, we did not further adjust age and gender in the final models.

The NAT2 predicted phenotype (slow/fast) was generated based on the three NAT2 SNPs-Ile114Thr (rs1801280), Arg197Gln (rs1799930) and Gly286Glu (rs1799931). These polymorphisms define different NAT2 alleles which have been characterized for their

impact on protein function[35, 36], and were inferred using haplotype probabilities estimated using the Expectation-Maximization algorithm[37]. In agreement with the existing classification[38], we classified carriers of two copies of the fast haplotype as '"fast" and carriers of all other haplotypes as "slow" phenotype.

Gene x Exposure analyses—Given that we had data and samples available for 577 probands, but only 307 of these had siblings available for case-unaffected sibling comparisons, to maximize statistical power we tested for gene-environment interactions using all 577 probands using case-only analyses. Proband-unaffected sibling analyses were done to corroborate results and are presented as supplementary analyses. Provided that the prevalence of the gene variants is independent of the exposure, ORs obtained from a case-only analysis can be used as estimates of interaction ORs (IOR)[39]. We tested this assumption of independence by testing the association between polymorphism frequencies and dietary exposures among the cousins of the probands, which are more representative of the underlying population than the unaffected siblings, and found no statistically significant associations. We created dichotomous exposure variables of meat intake using the median among cousins (N = 355), as we previously described[7, 34]. We tested for GxE interactions in proband-only analyses using unadjusted unconditional logistic regression models with the dichotomized exposure as the outcome variable and the 6 individual SNPs and NAT2 predicted phenotype as the independent variables. To confirm our significant case-only GxE ORs, we compared them to IORs computed using proband-unaffected siblings. We tested for interactions on a multiplicative scale using conditional logistic regression models that included an interaction term between dichotomous variables for each exposure and gene variables. In both probands-only and proband-sibling analysis, we assumed a log-additive mode of action.

For analyses of gene x meat interactions, we evaluated the potential confounding effect of relevant selected variables (age at interview, gender, history of Crohn's disease, ulcerative colitis, irritable bowel syndrome, diverticulitis, diabetes, high cholesterol, marital status, folate supplements, weight 2 years before interview and at age 20, height, years lived in the USA, BMI, aspirin/ibuprofen use, physical activity, fruits and vegetables per week, level of education, income and smoking status). Adjustment for these potential confounders did not change any of the ORs for the main exposure or gene variables by greater than 10%. Hence, they were not included in final gene-environment interaction models. For 87.5% of the subjects, we also had dietary data obtained with an FFQ[34] for total energy intake, total protein and total saturated fat intake. Among these subjects, we considered these variables as potential confounders of meat intake variables and found no evidence that they changed risk estimates by more than 10%; therefore, they were not included in our final models.

Analyses of heterogeneity by tumor sub-sites—For analyses of gene main effects and GxE interactions by tumor sub-sites we collapsed the site of 11 tumor ICD codes into two groups: colon cancer (ICD-O-2 C180-C188, n=351) and rectal cancer (ICD-O-2 C199, C209, n = 151), excluding from analyses cases with ICD code ICD-O-2 C189 (large intestines, not otherwise identified). We lacked tumor sub-site information for 75 probands. For proband-only analyses of G-E interactions, we tested for heterogeneity across tumor site by adding the tumor site variable and the product term between genotype and tumor site. Likelihood ratio tests from comparing nested models were used to assess statistical significance. For proband-sib analyses, we tested for heterogeneity of the gene main effects across anatomical sub- sites by assigning to each control the same code for tumor site as the associated proband. We test for heterogeneity in the effect of genotype or exposure by tumor sub-site by adding a product term between the gene or exposure and tumor site variable in the logistic regression model; thus allowing their log-OR to differ and testing the null hypothesis that the log-OR did not vary by tumor site. Furthermore, we examined 3-way

interaction by adding the product term of genotype, exposure and tumor site in the conditional logistic regression model.

To account for multiple testing we applied the Bonferroni correction. We present uncorrected ORs and CIs and indicate whether they were or not compatible with chance after Bonferroni correction. Tests of gene main effects are corrected for testing 7 variables (6 SNPs and one predicted phenotype), as are all GxE interaction tests for each exposure variable. All tests were two-sided and all analyses were done using the statistical software STATA version 11 (STATA Corporation, College Station, TX).

Results

Table 1 shows the demographic characteristics and meat consumption pattern of all individuals in our study. The mean age of probands and unaffected siblings were 60 and 59.3 respectively. Among probands, approximately 70% of the cancers were located in the colon and 30% were located in the rectum. Men were more likely to have rectal cancer.

Carcinogen metabolism gene polymorphisms and colorectal cancer risk

We estimated per allele ORs assuming a log-additive mode of action and did not find any statistically significant associations for either of the 6 polymorphisms' variant alleles or the NAT2 predicted fast phenotype (Table 2). However, we observed a positive association between the *GSTP1* Ile/Val genotype and CRC risk (OR = 1.67; 95% CI = 1.05-2.66) and a similar but non-statistically significant association for the Val/Val genotype (OR = 1.59; 95% CI = 0.96-1.86). In light of these findings we estimated the association between one or two copies of the *GSTP1* Ile105Val Val allele which showed that carriers of the Ile/Val or Val/Val genotypes had approximately 70% increased CRC risk compared to individuals carrying Ile/Ile genotype (OR = 1.66, 95%CI = 1.05-2.63, p = 0.03). This association seemed slightly stronger for rectal cancer (OR = 2.42, 95%CI = 0.91-6.45) than colon cancer (OR = 1.63, 95%CI = 0.92-2.88), albeit the test of heterogeneity did not reach statistical significance (p = 0.099). We did not find evidence of heterogeneity by tumor sub-site for any of the other 5 SNPs and NAT2 predicted phenotype.

Carcinogen metabolism genes polymorphisms, red meat and colorectal cancer risk

Previously, we reported that intake of more than 3 servings of red meat per week was associated with an 80% increased risk of CRC (OR = 1.8; 95% CI = 1.3-2.5), and a similar intake of red meat cooked by pan-frying, oven-broiling or grilling was associated with a 60% increase of risk (OR = 1.6; 95% CI = 1.1-2.2) [7]. We examined possible gene-environment interactions between all 6 SNPs, along with the estimated NAT2 predicted phenotype, and the following meat intake variables: number of servings of red meat per week, number of servings of red meat cooked by high temperature (pan-fried, oven-broiled, barbecued or grilled) per week, level of doneness of red meat on the outside (light-medium brown/heavily browned-blackened), and level of doneness of red meat in the inside (rare-medium/well-done).

Using proband-only analyses we found evidence that the NAT2 predicted phenotype modified the effect of total red meat intake on CRC risk, as we observed that carriers of the fast NAT2 phenotype were less likely to have diets higher in red meat compared to carriers of the slow phenotype (proband-only interaction OR = 0.47; 95% CI = 0.26-0.85; p = 0.013). This finding did not differ when considering subsites determined by tumor location (colon versus rectum), and did not remain statistically significant after applying a Bonferroni correction. Similarly, when considering total red meat cooked by high temperature methods and all cases combined, we observed evidence that the NAT2 predicted phenotype modified

the association between intake of more than 3 servings per week and risk of CRC (proband-only interaction OR= 0.34; 95% CI = 0.17-0.68; p = 0.002)(Table 3). This finding remained statistically significant after Bonferroni correction. Again, we did not observe heterogeneity by tumor sub-type for this interaction. When considering colon and rectum cases separately, we observed opposite modifying effects of *CYP1A2* -154A>C on the association between high intake of pan-fried, oven-broiled or grilled red meat and rectal cancer (OR = 1.37, 95%CI = 0.83-2.25; p = 0.218) and colon cancer (OR = 0.65, 95%CI = 0.47-0.90; p = 0.01) (heterogeneity test for colon vs. rectal cancer = 0.014) (Table 3). However, this finding was compatible with chance after Bonferroni correction. A comparison of these interaction ORs with those obtained from proband-sibling analyses showed little support for these interactions (data not shown).

When considering red meat level of doneness, we found statistically significant evidence that the effect of doneness on the inside of red meat on risk of CRC was modified by *CYP1A2* -154A>C (proband-only interaction OR = 1.54; 95% CI = 1.19-1.98; p = 0.001) (Table 3). This finding remained statistically significant after Bonferroni correction. This interaction was slightly stronger among rectal cancer cases (OR = 1.81; 95% CI = 1.09-3.01; p = 0.023) than colon cancer cases (OR = 1.40; 95% CI = 1.02-1.91; p = 0.039); however this difference by tumor site did not reach statistical significance (heterogeneity test colon versus rectum p = 0.396). We found that this polymorphism also modified the effect of level of doneness on the outside of red meat, albeit only among rectal cancer cases (proband-only interaction OR = 2.27; 95% CI = 1.32-3.92; p = 0.003), but not colon cases (interaction OR = 0.76; 95% CI = 0.54-1.08; p = 0.121) (heterogeneity test for colon vs rectal cancer p = 0.0008)(Table 3). Again, this finding remained statistically significant after Bonferroni correction.

When we compared the results of red meat level of doneness and *CYP1A2* -154 A>C obtained from proband-only analyses to those obtained from proband-sibling analyses we found interaction ORs of similar magnitude, which provided additional support for the previously observed interactions, albeit statistical power was lower so estimates did not reach significance (supplementary Table 1). Specifically, for the interaction of *CYP1A2* -154A>C and red meat level of doneness on the inside among all CRC cases we observed an interaction OR of similar magnitude to the one observed among proband-only analyses (interaction OR = 1.35; 95% CI = 0.82-2.21; p = 0.237). Similarly, when considering level of doneness on the outside of the meat, among rectal cancer cases we observed an interaction OR of similar magnitude to the one observed among proband-only analyses (interaction OR = 3.16; 95% CI = 0.85-11.7; p = 0.086), and comparable estimates for the heterogeneity of colon vs. rectal cancer (p for heterogeneity = 0.154)(supplementary Table 1).

Carcinogen metabolism genes polymorphisms, poultry and CRC risk

We tested gene-environment interactions between the six SNPs and estimated NAT2 predicted phenotype and the following poultry variables: servings of pan-fried, oven-broiled, or grilled poultry per week and level of doneness of poultry on the outside (light-medium brown/heavily browned-blackened). When considering all tumors combined and using proband-only analyses, our results suggested *GSTP1* Ile105Val may modify the association between pan-fried, oven-broiled or grilled poultry intake and risk of CRC (interaction OR = 0.65, 95%CI = 0.49-0.87, interaction p = 0.0035) (Table 4). This finding remained statistically significant after Bonferroni correction. This interaction was slightly stronger among rectal cancer cases (test of heterogeneity colon versus rectum p = 0.043). Further examination of this gene-exposure interaction in proband-sibling analysis showed interaction ORs of similar magnitude as those observed with proband-only analyses

(interaction OR = 0.56; 95% CI = 0.31-1.01; p = 0.054)(supplementary Table 2). These analyses also supported a slightly stronger effect among rectal cancer cases (interaction OR = 0.36; 95% CI = 0.11-1.14; p = 0.082), although the heterogeneity test (colon versus rectum) was not statistically significant (p = 0.709) (supplementary Table 2). When considering poultry doneness level, we did not find evidence of effect modification for any either of the six SNPs or NAT2 predicted phenotype when considering all cancer sites combined, and no evidence of differential effects by tumor site.

Discussion

We investigated the role of polymorphisms in seven metabolic enzymes that are relevant for the activation or detoxification of carcinogens formed in meats. These polymorphisms were selected due to their known impact on protein function, and due to the key roles these enzymes play in the metabolism of the main carcinogens formed in cooked red meats and poultry. Nevertheless, we cannot ignore that in our study we have conducted many different comparisons and some of these findings might be false positives due to chance. When taking into account Bonferroni corrections for multiple testing, and the comparison of IORs from proband-only analyses to those obtained from proband-sibling analyses, we found our strongest and most consistent findings were the modifier role of *CYP1A2* -154A>C on the effect of red meat level of doneness on the inside on CRC risk and on the outside of red meat on rectal cancer risk, and the modifier role of *GSTP1* Ile105Val on the effect of diets high in poultry cooked at high temperature on CRC risk. Overall, results were generally stronger for rectal than colon cancer.

The observed allelic frequencies of the SNPs we investigated were comparable to those previously reported[40]. We did not find strong evidence for an association between any of the six SNPs and the NAT2 predicted phenotype and CRC risk. However, our results suggest that the *CYP1A2* (-154A>C) SNP might modify the association between inside or outside level of doneness of red meat and CRC risk, with results suggesting an overall stronger effect for rectal cancer. Among individuals carrying the C allele, we found an approximately 30% increased risk associated with diets high in red meat well-done on the inside with no such association among individuals carrying A allele. Furthermore, our results suggested that among carriers of the C allele, diets high in red meat heavily browned on the outside might increase rectal cancer risk, but not colon cancer risk. CYP1A2 is an inducible phase I metabolizing enzyme and it plays a key role in the metabolism of HCAs[16]. The *CYP1A2* (-154A>C) polymorphism is common among Caucasians[41] and it may explain the reported variation in CYP1A2 inducibility[42]. The A allele is associated with higher enzymatic activity compared to the protein coded by the C allele[42]. Therefore, an effect modification of this SNP on the effect of HCAs on CRC risk is plausible. Our results suggest that the carcinogenic effects of diets high in red meat well done on the inside or outside would be greater in individuals carrying one or two copies of the C (slower) allele than individuals carrying two copies of the A (faster) allele. HCAs formation in red meat is a function of temperature and cooking time, and it is known to accumulate in meats cooked at high temperatures for longer periods of time, such as those heavily brown on the outside. Once absorbed in the colon, HCAs are rapidly transported to the liver where they can serve as substrates for N-oxidation by CYP1A2, or N-glucuronidation by UGT enzymes, or they can be converted to sulfamyl-HCAs. Sulfamyl-HCAs and HCA-N-glucuronides can be excreted back into the intestines via the bile acids, where they can be converted back into parent HCAs, which can undergo further activation into reactive species directly in the colon and rectum[43]. Therefore, it is possible that slower activation of HCAs in the liver by CYP1A2 might contribute to more or longer availability of HCAs in the colorectum, by the above mentioned mechanisms. This could explain our finding of stronger effects of red meat heavily browned among individuals who carry a slower *CYP1A2* allele. The finding of a

stronger, or exclusive effect modification on the rectum might be explained by the fact that distal parts of the large intestine are more likely to encounter higher concentrations of the carcinogenic exposures due to increased water absorption along the colon [44]. Recently, we have reported a similar finding for red meat level of doneness among carriers of polymorphisms in the *XPD* gene[7].

In our interpretation of the *CYP1A2* findings we cannot ignore that this enzyme is also able to locally metabolize NOCs in colon, even though CYP2E1 and CYP3A4 are considered the primary enzymes responsible for NOCs hydroxylation[30]. Hydroxylated forms of NOCs can react and induce DNA damage[45]. However, the existing evidence suggests that exposure to NOCs would occur via high intake of red meat, regardless of cooking method[14, 15]. In our results, we do not observe evidence of effect of modification of CYP1A2 on total red meat intake. The effect modification seems to be most relevant for red meat heavily browned on the outside. Therefore, our findings seem to implicate HCAs more strongly than NOCs. Red meat heavily browned on the outside could also accumulate PAHs, if the meat is grilled or barbecued with flames. The fact that *CYP1A2* plays a more central role in HCA than in PAH metabolism offers less support for a role of PAHs in the association between red meat heavily browned and rectal cancer risk.

In support of our findings, one previous study by Le Marchand and colleagues reported that the combination of the CYP1A2 and NAT2 predicted phenotypes, assessed using a caffeine-based test, exert interactions with well-done red meat, only among ever smokers[20]. To best compare our findings to those of Le Marchand *et al*, we also investigated a potential effect modification by smoking of the observed interaction between CYP1A2 and well-done meat on the inside, analyses we consider exploratory given the sample size of our study. Similarly to Le Marchand *et al*[20], we observed that the CYP1A2 x well-done meat interaction was restricted to ever smokers (interaction OR = 2.1; 95% CI = 1.42-3.06; p = <0.001) and absent among never smokers (interaction OR = 1.1; 95% CI = 0.76-1.66; p = 0.557)(case-only CYP1A2 x smoking status interaction p = 0.027). In contrast, two recently published studies investigating the *CYP1A2* -154A>C SNP did not find evidence that this SNP modified the relationship between red meat or doneness level of red meat and CRC[22, 46]. Possible explanations for the discrepancy between our study and these previous ones might include differences in meat variable definitions, and lack of stratification by tumor sub-site in these previous studies. In our study findings were stronger for rectal cancer.

We also found a statistically significant interaction between the *GSTP1* Ile105Val and diets high in pan-fried, oven-broiled or grilled poultry. Altogether, our results suggest that diets high in poultry cooked using high temperature methods associated with increased CRC risk only among carriers of the Ile allele. This effect modification seemed stronger for rectal cancer cases. HCAs are known to accumulate in poultry cooked at high temperature[47]. GSTs are a supergene family of Phase II metabolism genes, that catalyze the binding of a large number of electrophiles to the sulfhydryl group of glutathione[48, 49]. Carcinogens formed in meats cooked at high temperatures, such as HCAs and PAHs, become electrophilic after activation; therefore, GSTs become crucial in their detoxification process. Experimental studies suggest that proteins coded by the Ile allele have reduced enzyme activity compared to those coded by the Val allele[50], which has been reported to have approximately up to 3-fold activity towards PAH bay-region diol epoxides [51]. Therefore, our findings are plausible as they indicate that among subjects who carry the less efficient GSTP1 enzyme, diets high in poultry cooked at high temperatures might have a more detrimental effect on CRC risk due to deficient excretion of activated carcinogens. To our knowledge, this is the first study to report a modifier role of *GSTP1* in the relationship between cooked poultry intake and CRC risk.

We did not find any evidence of effect modification related to red meat or poultry intake for the polymorphisms we investigated in *EPHX1*, *CYP1B1*, *CYP2E1*, and *PTGS2* enzymes. In contrast, one study suggested that *CYP1B1* variants significantly interacts with red meat doneness intake[22] and another study reported a modifier role for a *CYP2E1* insertion variant on the association between processed meats and rectal cancer[21].

The use of proband-only analysis allowed us to maximize statistical power by using data from all available probands regardless of the availability of siblings. Analyses of proband-sibling pairs allowed us to internally validate our results using a family-based design that eliminates the need for gene-exposure independence in addition to confounding by population admixture. However, our study has a few limitations. First, our sample size was not large enough for detecting gene-environment interactions of small effects, which we may have missed. In particular, sample size was smaller for analyses by tumor sub-site. Secondly, we did not consider direct measures of carcinogens but instead we considered information from the questionnaire with respect to the frequency of meat intake and the meat-cooking methods, which indirectly captures the formation of the carcinogens. Lastly, we only considered SNPs presumed to impact protein function based on prior knowledge, rather than a comprehensive tag SNP-based approach that would capture most of the genetic variation in each gene. Therefore, we cannot discard a role for the genes for which we report no associations with overall CRC risk.

In conclusion, our findings suggest that diets high in red meat well-done on the inside or outside may increase CRC risk, particularly rectal cancer, presumably through the formation of HCAs. Furthermore, our results indicate that diets high in poultry cooked at high temperature might also be detrimental for CRC risk, perhaps through the formation of PAHs or HCAs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Cancer facts and figures. American Cancer Society; 2010.
2. World Cancer Research Fund. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. American Institute for Cancer Research; 2007.
3. Huxley RR, Ansary-Moghaddam A, Clifton P, Czernichow S, Parr CL, Woodward M. The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. International journal of cancer. 2009; 125:171–80.
4. Butler LM, Sinha R, Millikan RC, Martin CF, Newman B, Gammon MD, Ammerman AS, Sandler RS. Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. American journal of epidemiology. 2003; 157:434–45. [PubMed: 12615608]
5. Navarro A, Munoz SE, Lantieri MJ, del Pilar Diaz M, Cristaldo PE, de Fabro SP, Eynard AR. Meat cooking habits and risk of colorectal cancer in Cordoba, Argentina. Nutrition (Burbank, Los Angeles County, Calif). 2004; 20:873–7.
6. Nowell S, Coles B, Sinha R, MacLeod S, Luke Ratnasinghe D, Stotts C, Kadlubar FF, Ambrosone CB, Lang NP. Analysis of total meat intake and exposure to individual heterocyclic amines in a

case-control study of colorectal cancer: contribution of metabolic variation to risk. Mutation research. 2002; 506-507:175–85. [PubMed: 12351157]

7. Joshi AD, Corral R, Siegmund KD, Haile RW, Le Marchand L, Martinez ME, Ahnen DJ, Sandler RS, Lance P, Stern MC. Red meat and poultry intake, polymorphisms in the nucleotide excision repair and mismatch repair pathways, and colorectal cancer risk. Carcinogenesis. 2009; 30:472–9. [PubMed: 19029193]
8. Cross AJ, Ferrucci LM, Risch A, Graubard BI, Ward MH, Park Y, Hollenbeck AR, Schatzkin A, Sinha R. A large prospective study of meat consumption and colorectal cancer risk: an investigation of potential mechanisms underlying this association. Cancer Res. 2010; 70:2406–14. [PubMed: 20215514]
9. Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. Environ Mol Mutagen. 2004; 44:44–55. [PubMed: 15199546]
10. Sugimura T. Carcinogenicity of mutagenic heterocyclic amines formed during the cooking process. Mutat Res. 1985; 150:33–41. [PubMed: 3889618]
11. Le Marchand L, Hankin JH, Pierce LM, Sinha R, Nerurkar PV, Franke AA, Wilkens LR, Kolonel LN, Donlon T, Seifried A, Custer LJ, Lum-Jones A, et al. Well-done red meat, metabolic phenotypes and colorectal cancer in Hawaii. Mutation research. 2002; 506-507:205–14. [PubMed: 12351160]
12. van Maanen JM, Moonen EJ, Maas LM, Kleinjans JC, van Schooten FJ. Formation of aromatic DNA adducts in white blood cells in relation to urinary excretion of 1-hydroxypyrene during consumption of grilled meat. Carcinogenesis. 1994; 15:2263–8. [PubMed: 7955064]
13. Phillips DH. Polycyclic aromatic hydrocarbons in the diet. Mutat Res. 1999; 443:139–47. [PubMed: 10415437]
14. Hughes R, Cross AJ, Pollock JR, Bingham S. Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. Carcinogenesis. 2001; 22:199–202. [PubMed: 11159760]
15. Bingham SA, Pignatelli B, Pollock JR, Ellul A, Malaveille C, Gross G, Runswick S, Cummings JH, O'Neill IK. Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis. 1996; 17:515–23. [PubMed: 8631138]
16. Boobis AR, Lynch AM, Murray S, de la Torre R, Solans A, Farre M, Segura J, Gooderham NJ, Davies DS. CYP1A2-catalyzed conversion of dietary heterocyclic amines to their proximate carcinogens is their major route of metabolism in humans. Cancer Res. 1994; 54:89–94. [PubMed: 8261468]
17. Autrup H, Harris CC, Trump BF. Metabolism of acyclic and cyclic N-nitrosamines by cultured human colon. Proc Soc Exp Biol Med. 1978; 159:111–5. [PubMed: 714925]
18. Kiyohara C. Genetic polymorphism of enzymes involved in xenobiotic metabolism and the risk of colorectal cancer. J Epidemiol. 2000; 10:349–60. [PubMed: 11059519]
19. Dalhoff K, Buus Jensen K, Enghusen Poulsen H. Cancer and molecular biomarkers of phase 2. Methods in enzymology. 2005; 400:618–27. [PubMed: 16399374]
20. Le Marchand L, Hankin JH, Wilkens LR, Pierce LM, Franke A, Kolonel LN, Seifried A, Custer LJ, Chang W, Lum-Jones A, Donlon T. Combined effects of well-done red meat, smoking, and rapid N-acetyltransferase 2 and CYP1A2 phenotypes in increasing colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 2001; 10:1259–66. [PubMed: 11751443]
21. Le Marchand L, Donlon T, Seifried A, Wilkens LR. Red meat intake, CYP2E1 genetic polymorphisms, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 2002; 11:1019–24. [PubMed: 12376502]
22. Cotterchio M, Boucher BA, Manno M, Gallinger S, Okey AB, Harper PA. Red meat intake, doneness, polymorphisms in genes that encode carcinogen-metabolizing enzymes, and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 2008; 17:3098–107. [PubMed: 18990750]
23. Murtaugh MA, Ma KN, Sweeney C, Caan BJ, Slattery ML. Meat consumption patterns and preparation, genetic variants of metabolic enzymes, and their association with rectal cancer in men and women. J Nutr. 2004; 134:776–84. [PubMed: 15051825]

24. Murtaugh MA, Sweeney C, Ma KN, Caan BJ, Slattery ML. The CYP1A1 genotype may alter the association of meat consumption patterns and preparation with the risk of colorectal cancer in men and women. J Nutr. 2005; 135:179–86. [PubMed: 15671210]
25. Roberts-Thomson IC, Butler WJ, Ryan P. Meat, metabolic genotypes and risk for colorectal cancer. Eur J Cancer Prev. 1999; 8:207–11. [PubMed: 10443949]
26. Barrett JH, Smith G, Waxman R, Gooderham N, Lightfoot T, Garner RC, Augustsson K, Wolf CR, Bishop DT, Forman D. Investigation of interaction between N-acetyltransferase 2 and heterocyclic amines as potential risk factors for colorectal cancer. Carcinogenesis. 2003; 24:275–82. [PubMed: 12584178]
27. Reszka E, Wasowicz W, Gromadzinska J. Genetic polymorphism of xenobiotic metabolising enzymes, diet and cancer susceptibility. The British journal of nutrition. 2006; 96:609–19. [PubMed: 17010218]
28. Turesky RJ. Heterocyclic aromatic amine metabolism, DNA adduct formation, mutagenesis, and carcinogenesis. Drug Metab Rev. 2002; 34:625–50. [PubMed: 12214671]
29. Shimada T, Fujii-Kuriyama Y. Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. Cancer Sci. 2004; 95:1–6. [PubMed: 14720319]
30. Yamazaki H, Inui Y, Yun CH, Guengerich FP, Shimada T. Cytochrome P450 2E1 and 2A6 enzymes as major catalysts for metabolic activation of N-nitrosodialkylamines and tobacco-related nitrosamines in human liver microsomes. Carcinogenesis. 1992; 13:1789–94. [PubMed: 1423839]
31. Strange RC, Fryer AA. The glutathione S-transferases: influence of polymorphism on cancer susceptibility. IARC Sci Publ. 1999; 148:231–49. [PubMed: 10493261]
32. Wiese FW, Thompson PA, Kadlubar FF. Carcinogen substrate specificity of human COX-1 and COX-2. Carcinogenesis. 2001; 22:5–10. [PubMed: 11159734]
33. Kato R, Yamazoe Y. Metabolic activation and covalent binding to nucleic acids of carcinogenic heterocyclic amines from cooked foods and amino acid pyrolysates. Jpn J Cancer Res. 1987; 78:297–311. [PubMed: 3108208]
34. Newcomb PA, Baron J, Cotterchio M, Gallinger S, Grove J, Haile R, Hall D, Hopper JL, Jass J, Le Marchand L, Limburg P, Lindor N, et al. Colon Cancer Family Registry: An International Resource for Studies of the Genetic Epidemiology of Colon Cancer. Cancer Epidemiol Biomarkers Prev. 2007; 16:2331–43. [PubMed: 17982118]
35. Chan AT, Tranah GJ, Giovannucci EL, Willett WC, Hunter DJ, Fuchs CS. Prospective study of N-acetyltransferase-2 genotypes, meat intake, smoking and risk of colorectal cancer. International journal of cancer. 2005; 115:648–52.
36. Hein DW, Grant DM, Sim E. Update on consensus arylamine N-acetyltransferase gene nomenclature. Pharmacogenetics. 2000; 10:291–2. [PubMed: 10862519]
37. Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol. 1995; 12:921–7. [PubMed: 7476138]
38. Gertig DM, Hankinson SE, Hough H, Spiegelman D, Colditz GA, Willett WC, Kelsey KT, Hunter DJ. N-acetyl transferase 2 genotypes, meat intake and breast cancer risk. International journal of cancer. 1999; 80:13–7.
39. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! American journal of epidemiology. 1996; 144:207–13. [PubMed: 8686689]
40. NCI. SNP500 Project. 2011. http://variantgps.nci.nih.gov/cgfseq/pages/snp500.do
41. Sachse C, Bhambra U, Smith G, Lightfoot TJ, Barrett JH, Scollay J, Garner RC, Boobis AR, Wolf CR, Gooderham NJ. Polymorphisms in the cytochrome P450 CYP1A2 gene (CYP1A2) in colorectal cancer patients and controls: allele frequencies, linkage disequilibrium and influence on caffeine metabolism. Br J Clin Pharmacol. 2003; 55:68–76. [PubMed: 12534642]
42. Sachse C, Brockmoller J, Bauer S, Roots I. Functional significance of a C-->A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. Br J Clin Pharmacol. 1999; 47:445–9. [PubMed: 10233211]
43. Kinoshita N, Gelboin HV. beta-Glucuronidase catalyzed hydrolysis of benzo(a)pyrene-3-glucuronide and binding to DNA. Science. 1978; 199:307–9. [PubMed: 619459]

44. Chao A, Thun MJ, Connell CJ, McCullough ML, Jacobs EJ, Flanders WD, Rodriguez C, Sinha R, Calle EE. Meat consumption and risk of colorectal cancer. Jama. 2005; 293:172–82. [PubMed: 15644544]
45. Verna L, Whysner J, Williams GM. N-nitrosodiethylamine mechanistic data and risk assessment: bioactivation, DNA-adduct formation, mutagenicity, and tumor initiation. Pharmacol Ther. 1996; 71:57–81. [PubMed: 8910949]
46. Saebo M, Skjelbred CF, Brekke Li K, Bowitz Lothe IM, Hagen PC, Johnsen E, Tveit KM, Kure EH. CYP1A2 164 A-->C polymorphism, cigarette smoking, consumption of well-done red meat and risk of developing colorectal adenomas and carcinomas. Anticancer research. 2008; 28:2289–95. [PubMed: 18751408]
47. Knize MG, Felton JS. Formation and human risk of carcinogenic heterocyclic amines formed from natural precursors in meat. Nutr Rev. 2005; 63:158–65. [PubMed: 15971410]
48. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Critical reviews in biochemistry and molecular biology. 1995; 30:445–600. [PubMed: 8770536]
49. Beckett GJ, Hayes JD. Glutathione S-transferases: biomedical applications. Advances in clinical chemistry. 1993; 30:281–380. [PubMed: 8237562]
50. Zimniak P, Nanduri B, Pikula S, Bandorowicz-Pikula J, Singhal SS, Srivastava SK, Awasthi S, Awasthi YC. Naturally occurring human glutathione S-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. Eur J Biochem. 1994; 224:893–9. [PubMed: 7925413]
51. Sundberg K, Johansson AS, Stenberg G, Widersten M, Seidel A, Mannervik B, Jernstrom B. Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-1 towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons. Carcinogenesis. 1998; 19:433–6. [PubMed: 9525277]

Abbreviations

HCA	heterocyclic amines
PAH	polycyclic aromatic hydrocarbons
NOC	N-nitroso compounds
CRC	colorectal cancer
OR	odds ratio
CI	confidence interval
IOR	interaction odds ratio
FFQ	food frequency questionnaire
CYP1A2	cytochrome P450 1A2
CYP1B1	cytochrome P450 1B1
CYP2E1	cytochrome P450 2E1
GSTP1	glutathione-S-transferase P1
EPHX1	epoxy hydrolase 1
PTGS	prostaglandin endoperoxide synthase 1
NAT1	N-acetyltransferase 2

Novelty Statement

This study examines polymorphisms in seven enzymes that participate in the metabolism of three carcinogens associated with red meat and poultry intake and reports on their association with colorectal cancer risk and possible gene-environment interactions. Few studies have investigated the role of poultry on colorectal cancer risk taking into account cooking practices and relevant metabolic enzyme polymorphisms.

Impact Statement

Chemical carcinogens that form in red meat and poultry cooked using high temperature methods may be relevant contributors to colorectal carcinogenesis. Their effect may be modified by genetic variants in carcinogen metabolism genes, which would thus modulate the colorectal cancer risk associated with diets high in red meat and poultry intake.

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Table 1

Demographic characteristics of probands and unaffected siblings

Characteristics	Sibling N=362	Proband N=577	Colon Cancer N=351	Rectal Cancer N=151
Age	59.3 (0.6)	60.0 (0.5)	60.2 (11.6)	59.4 (11.0)
Gender				
Male	168(46.3)	302(52.3)	159 (45.3)	94 (62.3)
Female	195(53.7)	275(47.7)	192 (54.7)	57 (37.7)
Race				
Caucasian	299 (82.6)	425(73.7)	266 (75.8)	108 (71.5)
African American	19 (5.3)	54(9.4)	32 (9.1)	11 (7.3)
Hispanic	27 (7.4)	53(9.2)	30 (8.6)	15 (9.9)
Asian	7 (1.9)	19(3.3)	9 (2.6)	8 (5.3)
Others	10 (2.8)	26(4.5)	14 (4.0)	9 (6.0)
Mean servings of red meat per week	4.4 (4.1)	5.5 (6.5)	5.2 (6.0)	6.0 (8.1)
Mean servings of cooked red meat per week	3.6 (3.7)	4.5 (6.2)	4.3 (5.7)	5.0 (7.7)
Doneness of red meat on the outside				
Lightly browned	69 (19.2)	98 (17.0)	52 (14.9)	34 (22.5)
Medium browned	187 (51.9)	298 (51.8)	187 (53.6)	74 (49.0)
Heavily browned	104 (28.9)	179 (31.1)	110 (31.5)	43 (28.5)
Doneness of red meat on the inside				
Red (rare)	52 (14.4)	66 (11.5)	34 (9.7)	24 (15.9)
Pink (medium)	139 (38.5)	231 (40.2)	144 (41.3)	62 (41.1)
Brown (well-done)	170 (47.1)	278 (48.3)	171 (49.0)	65 (43.0)
Mean servings of cooked poultry per week	2.1 (3.1)	2.0 (3.1)	2.0 (3.0)	2.3 (3.6)
Doneness of poultry on the outside				
Lightly browned	109 (30.2)	176 (30.7)	123 (35.1)	34 (22.8)
Medium browned	165 (45.7)	256 (44.6)	148 (42.3)	72 (48.3)
Heavily browned	87 (24.1)	142 (24.7)	79 (22.6)	43 (28.9)

Note: for continuous variable, mean (SD) is given; for categorical variables, N (%) is given

Table 2

Carcinogen metabolism SNPs, NAT2 phenotype and CRC risk

Gene	Probands	Siblings	OR (95%CI)	P
CYP1A2 -154A>C				
AA	164	184	1[ref]	
AC	117	144	0.90 (0.58-1.39)	0.624
CC	24	29	0.86 (0.37-1.97)	0.717
per allele C OR*			0.91 (0.63-1.31)	0.621
allelic frequency among Caucasian C allele = 27%				
CYP1B1 Leu432Val				
Leu/Leu	86	118	1[ref]	
Leu/Val	139	151	1.43 (0.91-2.26)	0.121
Val/Val	75	81	1.51 (0.81-2.84)	0.196
per allele Val OR*			1.25 (0.92-1.71)	0.158
allelic frequency among Caucasian Val allele = 46%				
CYP2E1 -1054C>T				
CC	277	329	1[ref]	
CT	26	26	1.30 (0.62-2.72)	0.492
TT	0	0	-	-
per allele T OR*			1.30 (0.62-2.72)	0.492
allelic frequency among Caucasian T allele = 2.6%				
GSTP1 Ile105Val				
Ile/Ile	127	171	1[ref]	
Ile/Val	137	144	1.67 (1.05-2.66)	0.029
Val/Val	38	43	1.59 (0.80-3.16)	0.183
per allele Val OR*			1.34 (0.96-1.86)	0.087
allelic frequency among Caucasian Val allele = 31%				
EPHX1 Tyr113His				
Tyr/Tyr	167	188	1[ref]	
Tyr/His	108	141	0.92 (0.58-1.48)	0.745
His/His	28	29	1.30 (0.61-2.78)	0.497
per allele His OR*			1.07 (0.75-1.51)	0.711
allelic frequency among Caucasian His allele = 28%				
PTGS 2 -765G>C				
GG	207	238	1[ref]	
GC	87	111	0.78 (0.49-1.24)	0.295
CC	11	10	1.21 (0.39-3.74)	0.735
per allele C OR*			0.88 (0.59-1.33)	0.556
allelic frequency among Caucasian C allele = 18%				
NAT2 phenotype				
Slow	281	320	1[ref]	

Gene	Probands	Siblings	OR (95%CI)	P
Fast	20	35	0.51 (0.24-1.10)	0.079

* per allele OR assuming a log-additive model

Table 3

Proband-only analysis of interactions between *CYP1A2* -154A>C, NAT2 phenotype and red meat intake

	Colorectal cancer				Colon cancer				Rectal cancer				Heterog. p-value[b]
	N	OR[a]	95% CI	p-value	N	OR[a]	95% CI	p-value	N	OR[a]	95% CI	p-value	
***CYP1A2* -154 A>C**													
Number of servings of cooked[c] red meat per week													
	≤ 3/>3				***≤ 3/>3***				***≤ 3/>3***				
A/A	168/128	1[ref]			97/83	1[ref]			52/31	1[ref]			
A/C	121/96	1.04	0.73-1.48	0.822	48/52	0.78	0.49-1.23	0.285	27/27	1.68	0.84-3.36	0.144	
C/C	40/17	0.56	0.30-1.03	0.062	29/8	0.32	0.14-0.74	0.008	7/6	1.44	0.44-4.67	0.546	
per C allele		*0.85*	*0.66-1.09*	*0.206*		*0.65*	*0.47-0.90*	*0.010*		*1.37*	*0.83-2.25*	*0.218*	*0.014*
Level of doneness of meat in the inside (rare-medium = RM or well-done = WD)[d]													
	RM/WD				***RM/WD***				***RM/WD***				
A/A	176/121	1[ref]			102/78	1[ref]			56/27	1[ref]			
A/C	95/123	1.88	1.32-2.68	<0.0001	59/71	1.57	1.0-2.48	0.050	23/32	2.89	1.42-5.84	0.003	
C/C	25/32	1.86	1.05-3.29	0.033	16/21	1.72	0.84-3.51	0.138	7/6	1.78	0.54-5.80	0.341	
per C allele		*1.54*	*1.19-1.98*	*0.001*		*1.39*	*1.02-1.91*	*0.039*		*1.81*	*1.09-3.01*	*0.023*	*0.396*
Level of doneness of red meat from outside (light-medium brown = LMB or heavily browned = HB)[e]													
	LMB/HB				***LMB/HB***				***LMB/HB***				
A/A	208/89	1[ref]			119/61	1[ref]			67/16	1[ref]			
A/C	145/73	1.18	0.81-1.71	0.396	89/41	0.90	0.56-1.45	0.664	35/20	2.39	1.10-5.19	0.027	
C/C	41/16	0.91	0.49-1.71	0.774	30/7	0.46	0.19-1.10	0.079	6/7	4.88	1.44-16.5	0.011	
per C allele		*1.03*	*0.79-1.34*	*0.819*		*0.76*	*0.54-1.08*	*0.121*		*2.27*	*1.32-3.92*	*0.003*	*0.0008*
NAT2 predicted phenotype													
Number of servings of total red meat per week													
	≤ 3/>3				***≤ 3/>3***				***≤ 3/>3***				
Slow	222/291	1[ref]			141/177	1[ref]			54/77	1[ref]			
Fast	32/20	0.47	0.26-0.85	0.013	15/11	0.58	0.26-1.31	0.193	11/7	0.44	0.16-1.22	0.117	0.683
Number of servings of cooked[c] red meat per week													
	≤ 3/>3				***≤ 3/>3***				***≤ 3/>3***				

	Colorectal cancer				Colon cancer				Rectal cancer				Heterog. p-value[b]
	N	OR[a]	95% CI	p-value	N	OR[a]	95% CI	p-value	N	OR[a]	95% CI	p-value	
Slow	283/228	1[ref]			180/137	1[ref]			71/59	1[ref]			
Fast	40/11	0.34	0.17-0.68	0.002	20/6	0.39	0.15-0.10	0.0519	14/4	0.34	0.1-1.1	0.072	0.858

[a] Obtained from case-only analyses done using unconditional logistic regression models using the dichotomized exposure as the outcome variable and individual SNPs as the independent variables to obtain ORs that would be equivalent to interaction OR (IOR).

[b] colon vs rectum heterogeneity test;

[c] pan-fried, oven-broiled, or grilled;

[d] rare-medium as referent group;

[e] Light or medium browned as referent group.

Table 4

Proband-only analysis of interaction between GSTP1 Ile105Val and number of sevings of cooked[c] poultry per week

	Colorectal cancer				Colon cancer				Rectal cancer				Heterog. p-value[b]
	N	OR[a]	95% CI	p-value	N	OR[a]	95% CI	p-value	N	OR[a]	95% CI	p-value	
	≤ 3/>3				***≤ 3/>3***				***≤ 3/>3***				
Ile/Ile	164/80	1[ref]			107/42	1[ref]			33/29	1[ref]			
Ile/Val	195/55	0.58	0.39-0.86	0.007	126/32	0.65	0.38-1.09	0.106	50.16	0.36	0.17-0.77	0.008	
Val/Val	59/14	0.49	0.26-0.92	0.028	29/9	0.79	0.34-1.81	0.578	19/3	0.18	0.05-0.67	0.011	
per Val allele		*0.65*	*0.49-0.87*	*0.004*		*0.79*	*0.54-1.16*	*0.232*		*0.40*	*0.22-0.70*	*0.001*	*0.043*

[a] Case-only analyses were done using unadjusted unconditional logistic regression models using the dichotomized exposure as the outcome variable, using individual SNPs as the independent variables to obtain ORs that would be equivalent to interaction OR (IOR).

[b] colon vs rectum heterogeneity test;

[c] pan-fried, oven-broiled, or grilled