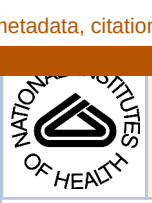

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UGT1A1 and *UGT1A9* functional variants, meat intake, and colon cancer, among Caucasians and African Americans

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Abstract

Glucuronidation by the UDP-glucuronosyltransferase enzymes (UGTs) is one of the primary detoxification pathways of dietary heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs). In a population-based case-control study of 537 cases and 866 controls, we investigated whether colon cancer was associated with genetic variations in *UGT1A1* and *UGT1A9* genes and we determined if those variations modify the association between colon cancer and dietary HCA and PAH exposure. We measured functional *UGT1A1* polymorphisms at positions −53 (*28; A(TA)6TAA to A(TA)7TAA), −3156 (G>A), −3279 (T>G) and the *UGT1A9*-275(T>A) polymorphism, and found no association with colon cancer overall. However, when stratified by race, the *UGT1A1*-3279 GG/TG intermediate/low activity genotypes were associated with an increased risk of colon cancer (odds ratio (OR) = 1.5, 95% confidence interval (CI)=1.1–2.0) in Caucasians. This finding is also supported by haplotype analyses where the UGT1A1-3279G-allele-bearing haplotype is overrepresented in case group. Overall, *UGT1A1*-53 and -3156 genotypes modified the association between dietary benzo(a)pyrene (BaP) and colon cancer (P for interaction=0.02 and 0.03, respectively). The strongest association was observed for those with <7.7 ng/day BaP exposure and the low activity genotypes, for both UGT1A1*28/*28 (OR=1.8, 95% CI=1.1–2.9) and −3156AA (OR=1.7, 95% CI=1.0–3.0), compared to ≥7.7 ng/day and combined high/intermediate genotypes. These data support a hypothesis that UGTs modify the association between meat-derived PAH exposure and colon cancer by their role in the elimination of dietary carcinogens.

Keywords

glucuronidation; amines; colon cancer; meat; polymorphism; genetic

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1. Introduction

Consumption of red meat is associated with an increased risk of colorectal cancer (CRC) [1, 2]. Cooking of red meat using high temperature cooking methods produces several mutagens, including heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) [3,4]. As demonstrated by the Ames test, those molecules are potent mutagens and have carcinogenic properties in animal models (reviewed by Turesky [5]). While such environmental factors could have a great influence on cancer susceptibility, approximately 35% of the risk of colon cancer is attributable to heritable factors [6]. Thus, variation in genes involved in the metabolism of HCAs and PAHs could influence risk of cancer via the exposure to these carcinogenic molecules.

Once consumed, HCAs are bioactivated by cytochrome P450s into N-hydroxy-HCAs and then esterified by N-acetyltransferases (NATs) or sulfotransferases (SULTs) thus potentializing their carcinogenic properties through the formation of DNA-adducts by the esterified HCAs [7,8]. However, the genotoxic potential of N-hydroxy molecules is also influenced by phase II enzymes, namely UDP-glucuronosyltransferases (UGTs) [9].

Several case-control studies investigated the role of UGT polymorphisms on susceptibility to CRC. The most common finding showed that the presence of variant in *UGT1A7*, particularly the *UGT1A7*3* allele, is associated with an increased risk of colorectal cancer or adenoma [10–12]. In addition, the influence of the *UGT1A1*28* allele was also investigated in two of these studies but did not revealed any significant association with CRC [11,12], while carriers of the mutant allele for *UGT1A1* G71R polymorphism, frequently found in the Asian population, have an increase risk of CRC [11]. Two case-control studies showed no association between CRC and UGT1A6 variants T181A and R184S [13,14]. Finally, using a food frequency questionnaire developed by Sinha et al. [15,16], we have previously reported modification by NAT1 and UGT1A7 genotypes on the association with meat and meat-derived HCA exposure in a population-based case-control study of colon cancer [17,18].

Other individual UGT enzymes may have a more important role in detoxification of food-borne carcinogens. For instance, UGT1A1 is the main hepatic enzyme responsible for the *in vitro* glucuronidation of *N*-OH-PhIP, the most abundant HCA found in cooked meat [19,20]. Moreover, the formation of *N*-OH-PHIP-N^2-G and N^3-G in human liver microsomes is strongly correlated with UGT1A1 expression. The promoter polymorphisms at positions -3279(T>G), -3156(G>A) and -53 (presence of 7 TA repeats in the *UGT1A1* promoter; *UGT1A1*28*) reducing UGT1A1 protein expression, have been correlated with lower levels of formation of N^2-G and N^3-G metabolites in human liver microsomes [19]. Additionally, UGT1A9 is one of the most active UGT towards the hydroxy metabolites of BaP, namely 3-OH-BaP, 7-OH-BaP and 9-OH-BaP [21,22] and it also has the capacity to conjugate *N*-OH-PhIP particularly at the N^3-position [19,23]. This suggests that any genetic alterations reducing UGT1A9 activity or expression could influence the elimination of HCAs or PAHs. For instance, previous studies showed that the *UGT1A9*-275 AT genotype is associated *in vitro* with a higher level of UGT1A9 expression and this is translated *in vivo* by a reduced exposure to the immunosuppressive drug MPA [24,25].

These data suggest that genetic alterations in the *UGT1* gene could modify the metabolism of certain carcinogenic compounds and may partially explain the interindividual variation observed in HCA and PAH metabolism. In this study, we investigated whether colon cancer was associated with genetic variations in the *UGT1A1* and *UGT1A9* genes and we determined if those variations modify the association between colon cancer and red meat consumption (by type, cooking methods, and doneness preferences) or dietary carcinogen exposure (HCA and PAH).

2. Materials and Methods

2.1 Study Population

Cases and controls of the North Carolina Colon Cancer Study were selected from 33 counties in North Carolina and frequency matched by race, age, and sex [26]. Study design and sample characteristics have been previously described [27]. In brief, cases were selected through a rapid ascertainment system [28] established in conjunction with the North Carolina Central Cancer Registry. Cases were eligible if they were between 40 and 84 years of age at first primary diagnosis of invasive adenocarcinoma of the colon and diagnosed between 10/01/96 and 09/30/00. The age range was chosen to include cases less likely to be associated with familial disease. Controls were randomly selected from North Carolina Division of Motor Vehicle lists if they were under 65 years of age, or from the Center for Medicare and Medicaid Services list if they were 65 years or older. Of those who were eligible, 84% of cases and 62% of controls were interviewed. For the analysis of *UGT1A1* and *UGT1A9* genotypes, 537 cases and 866 controls were included. The controls comprised 325 African Americans and 541 Caucasians, and the cases, 227 African-Americans and 310 Caucasians. The study was approved by the Institutional Review Boards at the University of North Carolina School of Medicine, CHUL Research Center, Laval University, and by equivalent committees at the collaborating hospitals.

In this study population, African American controls were younger, less educated, and although more likely to be never smokers, were also more likely to be long-term current smokers, compared to white controls. Statistically significant case-control differences were not observed for demographic or smoking characteristics, regardless of race, with the exception of age among African Americans [27]. Mean daily energy, total fat, total meat, red meat and the HCAs, DiMeIQx, MeIQx, and 2-amino-1-methyl-6-phenyl-imidazo[4,5-*b*]pyridine (PhIP) were greater among cases, compared to controls, regardless of race [27]. Dietary fiber was inversely associated with colorectal cancer [29], while calcium was only inversely associated among whites [30]. Although folate intake was not associated with colorectal cancer in this population [30], an approximate two-fold increase in risk was reported for those with low folate and the wild-type methylenetetrahydrofolate reductase genotype, regardless of race [31].

2.2 Exposure assessment

Questionnaires were administered in person in the participants' homes by specially trained registered nurses. The questionnaire collected information on lifestyle factors, such as physical activity and tobacco use; medical, family, and work histories; and use of over-the-counter medications. A 150-item food frequency questionnaire was used to measure usual dietary intake over the year preceding diagnosis for cases, and over the year before date of selection for controls [32]. The questionnaire was modified to assess individual exposure to dietary carcinogens based on a meat cooking and doneness module developed by Sinha et al. [16]. Meat intake frequency data, cooking method, and level of doneness were used to estimate values of three HCAs (MeIQx, PhIP, and DiMeIQx) and BaP, using Sinha's exposure index (described in [16,33]). Details regarding the collection of dietary history and the estimated HCA and PAH exposure have been previously documented [17,27].

2.3 *UGT1A1* and *UGT1A9* genotyping

Of the individuals with completed questionnaire data, 88% (93% of cases and 85% of controls) also agreed to provide a blood sample for DNA analyses. In order to assess the influence of UGT polymorphisms on colon cancer risk, we first genotyped functional polymorphisms of the *UGT1A1* and *UGT1A9* genes in 537 colon cancer cases and 866 control subjects by direct sequencing or by the GeneScan method. The polymorphisms included in the analysis were selected because they had previously been associated with a significant alteration of the protein

level or activity [19,24]. *UGT1A1* genotyping at position -53 (5, 6, 7 (UGT1A1*28), 8 or 9 TA repeats) was performed by the previously described GeneScan analysis [34]. The PCR amplifications were performed with different sets of primers; the forward primers were tagged with fluorescent markers VIC F39-5'-GTCACGTGACACAGTCAAAC-3' or FAM F35-5'-GAGGTTCTGGAAGTACTTTGC-3' while the reverse primers were as followed R107-5'-GTTTCTTTTTGCTCCTGCCAGAGGTT-3' and R108-5'-GTTTCTTCCGCTCGAGCGGCCATGGCGCCTTTGCTCC-3'. The conditions were: 10 cycles of 94°C for 15 sec, 58°C for 15 sec, 72°C for 30 sec, followed by 25 cycles at 89°C for 15 sec, 58°C for 15 sec and 72°C for 15 sec. An initial denaturation step at 95°C for 3 min and a final extension at 72°C for 15 min were performed. The three amplification products of different lengths (268 bp, 290 bp and 123 bp) were diluted and mixed with the GeneScan™ 500 LIZ™ Size Standard molecular weight maker in 20 μl. Finally, 1 μl of the mix was separated on a fragment analysis gel on the ABI Prism 3775 DNA Sequencer and analyzed by GeneScan 2.1 Analysis software (PE Applied Biosystems). The accuracy of the genotyping method was verified by sequencing of randomly selected PCR products.

The *UGT1A1* PBREM (phenobarbital responsive enhancer module) region was amplified with primers F652-5'-CTGGGGATAAACATGGGATG-3' and R653-5'-CACCACCACTTCTGGAACCT-3'. The PCR conditions were: 35 cycles at 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec, with an initial denaturation step for 3 min at 95°C and a final extension at 72°C for 7 min. The UGT1A1 promoter variations in the PBREM region at positions -3156 and -3279 (relative to the ATG) were genotyped by automated sequencing using primer F652.

The *UGT1A9-275* variation was genotyped by sequencing as described previously [24]. Briefly, PCR amplification was performed with primers F248-5'-TTGAGACAGAGTCGTGCTGTTT-3' and R608-5'-GCAAAGCCACAGGTCAGC-3' and PCR products were submitted to automated sequencing with primer F516-5'-GCATTGCAGAGACACAGG-3'. For quality control purposes, 5% of the samples were randomly selected for both UGT1A1 and UGT1A9 genotyping. In addition positive and negative controls were included in each experiment. Only three samples failed the amplification process and thus were not included in the analysis.

2.4 Haplotypes and Linkage Analyses

Haplotypes for UGT1A1 and UGT1A9 were determined using Phase v2.1 program, and analysis was performed with either Caucasian (n=851) or African-American subjects (n=552). Haplotypes of 2 or 3 successive markers were estimated with the expectation-maximization (EM) algorithm [35] implanted in the cocaphase module of UNPHASE version 2.40 [36]. Considering that EM algorithm has limited precision to estimate haplotype frequencies <1%, such haplotypes were excluded using the –droprare option. Global and individual (-individual option) likelihood-ratio p-values were estimated for each analysis. The linkage between different polymorphisms was determined with the linkage disequilibrium (LD) plotter tool program found at https://innateimmunity.net/.

2.5 Statistical analysis

UGT1A1 and *UGT1A9* genotype and allelic frequencies were calculated among African-American and Caucasian subjects. Differences in genotype and allelic frequencies between cases and controls among individual race/ethnic groups were assessed by a chi-square test or Fisher exact test when the number of subjects was lower than five in one of the groups. *UGT1A1* and *UGT1A9* genotypes were categorized in three groups based on the predicted UGT expression and activity [19,24,34]. *UGT1A1*-53 genotypes was categorized as follows, based on the number of TA repeats: predicted high (56 and 66), intermediate (57, 58, 67, 68 and 69)

and low activity (77, 78 and 77). *UGT1A1*-3156 and -3279 genotypes were classified as predicted high (GG and TT), intermediate (GA and TG) and low activity (AA and GG). Finally, *UGT1A9* -275 genotype TT was classified as predicted high activity, TA as intermediate and AA as low. Departure from Hardy-Weinberg disequilibrium was measured among African-American and Caucasian controls for each polymorphism with a degree of freedom equal to the number of alleles - 1.

Meat consumption and dietary HCA exposure data were derived from an adapted food frequency questionnaire, as previously reported [17,27]. All meat (by cooking methods and doneness preferences), HCAs (MeIQx, DiMeIQx, and PhIP), and BaP exposure assessments were dichotomized based on the median values of the control group. Joint effect variables were created with a common reference group based on the control's median meat intake or exposure to carcinogens and on the predicted activity associated with *UGT1A1* or *UGT1A9* genotypes. For joint effect variables with UGT1A1 genotypes, high and intermediate genotypes were combined *for UGT1A1*-53, -3156, while intermediate and low genotypes were combined for *UGT1A1*-3279. The pooling strategy was based on observed associations between UGT1A1 genotypes and colon cancer. We also grouped *UGT1A9* intermediate and high activity genotypes because of the low frequency of *UGT1A9*-275 AA high activity genotypes and classified the AA and AT genotypes as high and intermediate based on Girard et al [24].

Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for colon cancer were calculated from unconditional logistic regression models [37]. PROC LOGISTIC of the software package SAS (version 9.1; SAS Institute, Cary, NC) was used with the option in the MODEL statement to incorporate offsets, which takes into account the selection probabilities by age, race, and sex [26]. Multivariable gene effect models included the following variables to adjust for potential confounding factors: race (African Americans and Caucasians), 5-year age groups (≤ 45, 46–50, […], ≥ 76 years), and sex. Multivariable joint effect models were adjusted for race, age, and sex, in addition to dietary fiber, energy adjusted fat intake and total energy intake. These potential confounding variables were determined previously [17].

As described above, indicator variables were created to estimate the joint effect of dietary exposure and *UGT1A1* or *UGT1A9* polymorphisms. Individuals with the lowest hypothesized associations, less than the median daily intake, and predicted low risk genotypes comprised the common reference group (OR_{00}). The following ORs were used to assess the expected joint effect for multiplicative interaction (OR_{10} X $OR_{01} > OR_{11}$), where OR_{10} was for high intake and an expected low risk genotype, OR_{01} was for low intake and an expected high-risk genotype, and OR_{11} was for their combined effects. The multiplicative interactions between the meat intake and the genotypes were evaluated using a likelihood ratio (LR) test where $p<0.05$ was considered statistically significant.

3. Results

The allele and genotype frequencies for functional polymorphisms of the *UGT1A1* and *UGT1A9* genes are presented in the Table 1. Genotype and allelic frequencies for UGT1A1-3279 were not significantly different ($p>0.05$) between Caucasians and African Americans. In addition, the *UGT1A9*-275A allele frequency is twice as high in African-American sample (0.18 vs. 0.07 in Caucasians). None of the typed polymorphisms deviated from Hardy-Weinberg equilibrium in control group. The most common allele for the *UGT1A1* polymorphism at position -53 (TATA box region of the promoter), was the six TA repeats (*UGT1A1*1*). We also observed polymorphic alleles with five (*UGT1A1*36*), seven (*UGT1A1*28*), or eight (*UGT1A1*37*) TA repeats. A novel allele including six and nine TA repeats was observed in one African-American control. There were no significant differences in allelic or genotype frequencies between cases and controls for any polymorphisms

investigated (Table 1). Strong linkage disequilibrium (LD) was observed between *UGT1A1*-53 and *UGT1A1*-3156 in both populations (r2 = 0.86 in Caucasians and 0.64 in African-Americans) while a low degree of LD was observed between *UGT1A9* and *UGT1A1* polymorphisms (r2 < 0.10).

Haplotype analyses for *UGT1A1–UGT1A9* were performed within either Caucasians, African-Americans or combined subjects. A larger variability was observed in haplotype in the African-Americans population compared to Caucasians (14 different haplotypes generating 48 diplotypes versus 11 haplotypes generating 23 diplotypes). When all subjects were combined 14 different haplotypes and 51 diplotypes were found. We observed an ethnic-specific effect in haplotype frequency, most notably with frequent haplotypes. Indeed, the most common haplotype in African-Americans is *UGT1A9*-275T, *UGT1A1*-3279G, *UGT1A1*-3156A and *UGT1A1*-53$(TA)_7$ (TGA7; n=293) followed by TTG6 (n=211), whereas the TTG6 haplotype was the most frequent (n=879) in Caucasians followed by TGA7 (n=486).

SNP-based colorectal cancer study when sample was stratified by race demonstrated a trend toward an allelic association for the SNP UGT1A1-3279 (p=0.07) in Caucasians (table 1). In addition, when we performed genotypic analysis under a dominant model, we showed a significant association (OR=1.5, 95% CI, 1.1–2.0; table 2). More interestingly, this finding is supported by 2-SNPs and 3-SNPs haplotype analyses where the UGT1A1-3279G-allele-bearing haplotype is overrepresented in case group (likelihood-ratio p-value=0.0082 G-G and 0.023 G-G-6; table 3). This effect was not observed within African-Americans (cases= 227, controls= 325), likely because the striking difference in *UGT1A1*-3279 allele frequency (table 1).

In Table 4, joint effects for UGT1A1-53, UGT1A1-3156, meat intake, and meat-derived compound exposure on risk of colon cancer. Both genotypes modified the association between colon cancer risk and BaP exposure (P for interaction = 0.02 and 0.03, respectively). The strongest associations were observed for those with less than the median intake of BaP and with low activity genotypes (Table 4). The association between BaP and colon cancer was not modified by either the *UGT1A1*-3279 (OR=1.0, 95% CI, 0.7–1.7, P for interaction = 0.2) or UGT1A9 (OR=0.9, 95% CI, 0.6–1.3, P for interaction=0.2) genotypes. However, we did observe modification by UGT1A9-275 genotype for pan-fried red meat consumption and colon cancer (P for interaction=0.04). The strongest association was observed for 9.0 g/day pan-fried red meat and the high/intermediate genotype, compared to 9.0 g/day pan-fried red meat (OR=1.7, 95% CI, 1.1–2.4). We did not observe statistically significant departure from the multiplicative scale for the remaining UGT1A1-3279 or UGT1A9 genotype-meat or meat compound joint effects. When Caucasians and African Americans were investigated separately for interaction, we observed odds ratios with similar magnitude, compared to odds ratios among the entire study, but they were very imprecise, due to small numbers in each cell (data not shown). Thus, we only presented results combining race/ethnic groups.

4. Discussion

Using a population-based case-control study, we showed that *UGT1A1*-3279 TG/GG intermediate/low genotypes were associated with an increased risk of colon cancer, compared to high genotypes (*UGT1A1*-3279 TT). We report statistically significant modification by *UGT1A* genotypes for dietary carcinogen and colon cancer associations. Specifically, we report that individuals with *UGT1A1*-53 (*28/*28) and -3156 (AA) low genotypes and less than median BaP exposure were at 1.8- and 1.7-fold greater risk of colon cancer, respectively, compared to those with high/intermediate genotypes with the same BaP exposure. In addition, carriers of *UGT1A9-275* (AA/AT) high/intermediate genotypes and greater than median intake

of pan-fried red meat were at a 1.7-fold greater risk of colon cancer, compared to those with less than median intake with the same genotypes.

We report similar *UGT1A1* genotype frequencies to what has been observed in other populations [38,39]. The largest difference we observed for *UGT1A1*-3279 between Caucasians and African Americans was for the TT genotype (0.30 and 0.02, respectively). Similar genotype frequencies were reported by Innocenti et al [38]. In their study, the *UGT1A1*-3279 TT genotype frequencies in Caucasians (n=55) was 0.28, and in African Americans (n=37) it was 0.03. The *UGT1A9*-275A frequency of 0.07 was within the range previously reported (0.04 to 0.09) in Caucasians [39,40].

All three *UGT1A1* polymorphisms were in strong linkage disequilibrium, where the strongest LD was observed between *UGT1A1*-3156 and *UGT1A1*-53. There was no significant LD between *UGT1A1* and *UGT1A9*. This is consistent with the results of Innocenti et al., who reported an r^2 value between 0.7 and 0.9 for *UGT1A1*-53, -3156 and -3279, and $r^2<0.2$ for *UGT1A9*-275 and *UGT1A1* polymorphisms [40].

Without assuming the food intake effect, the *UGT1A1*-3279G-allele, under a dominant model, demonstrated an increase risk to colorectal cancer in Caucasians, and this is supported by haplotypic analysis. We did not observe any significant association between CRC and UGT1A1-3279 in African-Americans. However, the *UGT1A1*-3279G-allele in this subset sample is more common (0.82), and our sample is not enough powered to detect such a difference in allele frequency (0.05 in Caucasians). Moreover, the difference in *UGT1A1*-3279 allele frequency between TT case and control groups would argue for an overrepresentation of T-allele in cases, contrasting with results from Caucasian subset. Consequently, we may not assume a role for *UGT1A1*-3279 in CRC risk, but we hypothesize that it might genetically link with other most likely functional polymorphisms in the UGT1 locus.

Dietary BaP exposure was positively associated with colon cancer among carriers of *UGT1A1*-53 (*28/*28) and -3156 (AA) low activity genotypes, compared to those with combined high/intermediate genotypes. Interestingly, this modification by UGT1A1 genotypes resulted in a stronger association for those who had less than median BaP exposure, compared to the median or greater exposure. Previously, Fang et al., demonstrated that carriers of the *UGT1A1*-53 (*28/*28) low activity genotype had a significant reduction of BPD(−) glucuronidation when UGT1A9 was inhibited in assays with human liver microsomes [41]. Thus, the exact contribution of UGT1A1 to *in-vivo* glucuronidation of BaP remains to be elucidated, but our result might indicated that intake of even small amounts of carcinogens influences the risk of colon cancer and that consequently the median daily intake value might not constitute the most appropriate classification method for certain meats or carcinogens.

Another possible explanation for a stronger association with less than median BaP exposure is the idea of “saturation” of the enzyme at higher levels of exposure. For example, it has been hypothesized that the metabolic genetic effects, such as those with the UGTs, are most relevant at low to middle level exposures to carcinogenic compounds, such as BaP, rather than at high levels where the exposure is likely to saturate the enzyme activity and diminish the differences between UGT1A1 “high/intermediate” and “low” activity [42,43]. We have previously reported the same antagonism effect for *UGT1A7* low-activity genotypes and dietary BaP on risk of colon cancer in this population [17]. Thus, our findings appear to support a low-dose joint effects model between UGT1A1, UGT1A7, and dietary BaP on the association with colon cancer.

We previously found that *UGT1A1* polymorphisms were strongly associated with the *in-vitro* hepatic glucuronidation of the *N*-OH-PHIP [19]. However, modification by UGT1A1 genotypes for PhIP and colon cancer was not observed in our data. This result could be

explained by the interindividual variability observed in the CYP1A2 activity, the enzyme responsible for the conversion of PhIP to *N*-OH-PhIP [44]. Because of this variability, it is possible that the estimation of the PhIP exposure is not a precise measure of the *N*-OH-PhIP exposure.

We showed for the first time the impact of *UGT1A9*-275 polymorphism on colon cancer risk, the joint effect is observed for pan-fried meat and -275 high/intermediate (AA/AT) activity genotype (P for interaction=0.04). Meat that is cooked above a heat source, by methods such as barbecuing, contain the highest levels of PAHs [33], because the meat is exposed to smoke formed from the pyrolysis of fatty juices that drip down onto the heat source [45]. In contrast, the optimal conditions for HCA formation include high-temperature cooking such as pan-frying [46].

The influence of UGT1A9 on cancer risk through pan-fried red meat deserves further exploration. Pan-fried red meat was associated with a two-fold increase in risk of colon cancer in this population [27]. We observed the strongest association among the high/intermediate UGT1A9 genotype, suggesting either poor genotype-phenotype correlation, or that there is something in pan-fried red meat other than HCAs that are driving the association.

The predicted activity of *UGT1A9* genotypes was based on our previous results where we demonstrated a higher level of UGT1A9 protein in human liver microsomes in subjects carrying the -275A allele [24]. UGT1A9 is the most efficient enzyme in the formation of N-OH-PhIP-N^3-G [12]. Higher formation of *N*-OH-PhIP-N^3-G associated with the -275A allele has the potential to increase the exposure of the colon to N^3-G, which can be further hydrolyzed to *N*-OH-PhIP by bacterial β-glucuronidases and converted locally to reactive metabolites [47]. However, the -275 polymorphism could have a different impact in other tissues and still its functional impact on gene transcription in various tissues has not been resolved yet. It could also be influenced by UGT inducers found in the diet [48,49] and thus UGT1A9 results should be analyzed cautiously in regard of the classification of the predicted activity. On the other hand, polymorphisms in the *UGT1A1* promoter are well known to reduce UGT1A1 protein expression [19,34,50], bilirubin [51] and SN-38 glucuronidation [52,53], and consequently misclassification is less probable.

In this study, we stratified the data by both *UGT1* genotype and meat-related dietary factors to determine their joint effects on the association for colon cancer. Although these statistical comparisons were based on a priori hypotheses driven by experimental and epidemiologic evidence, we cannot exclude the possibility that our statistically significant main finding for BaP was due to change.

Overall, the results of the present study and of Butler et al. [17] support the hypothesis that UGTs may play a role in carcinogens elimination and, as a result, influence colon cancer risk. An investigation of UGT expression in normal and malignant tissues revealed that, in normal large bowel mucosa, UGT proteins are expressed at high levels whereas there is a considerable down-regulation in low-grade adenomas and no expression in high-grade adenomas and colon cancer [54]. In addition, UGT proteins are essentially expressed in the luminal cells with which the carcinogens from the diet come into direct contact. Based on this expression profile, Giuliani et al. concluded that UGT proteins may participate in the early phase of colon malignant transformation and could play a role of prevention against carcinogenesis [54]. The data obtained in the present study are in agreement with this hypothesis; subjects with high expression of UGT1A1 would eliminate HCAs or PAHs more rapidly and thus could be less at risk of colon cancer through benzo(a)pyrene exposure. In conclusion, our data point toward a potential influence of *UGT1A1* and *UGT1A9* polymorphisms on colon cancer risk through

meat consumption and PAH exposure and suggest that UGT enzymes have an important role of elimination for food-borne carcinogens.

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Table 1

UGT1A1 and UGT1A9 allele and genotype frequencies

	African-Americans (n = 552)		Caucasians (n = 851)	
	Cases (n = 227)	**Controls (n = 325)**	**Cases (n = 310)**	**Controls (n = 541)**
UGT1A1				
−53	% (n)	% (n)	% (n)	% (n)
Alleles				
5	0.06 (27)	0.08 (50)	0.001 (1)	0 (0)
6	0.48 (220)	0.49 (318)	0.68 (420)	0.68 (738)
7	0.40 (183)	0.39 (256)	0.32 (198)	0.32 (342)
8	0.05 (24)	0.04 (25)	0.002 (1)	0.002 (2)
9	0 (0)	0.001 (1)	0 (0)	0 (0)
Fisher exact test p		0.54		0.72
Genotypes				
55	0 (0)	0.009 (3)	0 (0)	0 (0)
56	0.05 (11)	0.06 (19)	0.0032 (1)	0 (0)
57	0.05 (12)	0.06 (20)	0 (0)	0 (0)
58	0.02 (4)	0.02 (5)	0 (0)	0 (0)
66	0.25 (57)	0.24 (79)	0.4548 (141)	0.47 (252)
67	0.37 (84)	0.38 (125)	0.4387 (136)	0.43 (233)
68	0.05 (11)	0.05 (15)	0.0032 (1)	0.002 (1)
69	0 (0)	0.003 (1)	0 (0)	0 (0)
77	0.18 (40)	0.17 (54)	0.10 (31)	0.10 (54)
78	0.03 (7)	0.009 (3)	0 (0)	0.002 (1)
88	0.004 (1)	0.003 (1)	0 (0)	0 (0)
Fisher exact test *P*		0.73		0.85
Hwe *P* for controls		0.17		0.94
−3156				
G	0.68 (309)	0.70(456)	0.72 (444)	0.71 (770)
A	0.32 (145)	0.30 (194)	0.28 (176)	0.29 (312)
χ^2 test *P*		0.46		0.84
AA	0.10 (23)	0.10 (30)	0.08 (24)	0.08 (45)
AG	0.44 (99)	0.41 (134)	0.41 (128)	0.41 (222)
GG	0.46 (105)	0.50 (161)	0.51 (158)	0.51 (274)
χ^2 test *P*		0.74		0.96
Hwe controls		0.77		0.91
−3279				
T	0.21 (94)	0.18 (119)	0.50 (310)	0.55 (590)
G	0.79 (360)	0.82 (531)	0.50 (310)	0.45 (492)
χ^2 test *P*		0.32		0.07
GG	0.64 (145)	0.66 (213)	0.23 (71)	0.21 (111)
GT	0.31 (70)	0.32 (105)	0.54 (168)	0.50 (270)
TT	0.05 (12)	0.02 (7)	0.23 (71)	0.30 (160)
χ^2 test *P*		0.14		0.11
Hwe controls		0.14		0.73
UGT1A9				
−275				
T	0.82 (374)	0.823 (532)	0.91 (563)	0.93 (1002)
A	0.18 (80)	0.18 (118)	0.09 (57)	0.07 (80)
χ^2 test *P*		0.82		0.19
AA	0.04 (6)	0.02 (8)	0.01 (3)	(0)
AT	0.30 (68)	0.31 (102)	0.16 (51)	0.15 (80)
TT	0.67 (153)	0.66 (215)	0.83 (256)	0.85 (461)
χ^2 or Fisher exact test *P*		0.93[1]		0.06[2]

	African-Americans (n = 552)		Caucasians (n = 851)	
	Cases (n = 227)	**Controls (n = 325)**	**Cases (n = 310)**	**Controls (n = 541)**
Hwe controls		0.30		0.05

For allele frequencies, N = total number of chromosomes, and for genotype frequencies, N=total number of individuals.

[1] χ^2 test

[2] Fisher exact test

Table 2

Odds ratios (ORs) for UGT1A1 and UGT1A9 genotypes and colon cancer among Caucasians and African-Americans

UGT	Overall		Overall Caucasians		African Americans	
	Cases/ Controls	OR (95% CI)*	Cases/ Controls	OR (95% CI)†	Cases/ Controls	OR (95% CI)†
	537/866		310/541		227/325	
UGT1A1-53						
High (55, 56, 66)	210/353	1.0 (Ref)	142/252	1.0 (Ref)	68/101	1.0 (Ref)
Int (57, 58, 67, 68, 69)	248/400	1.0 (0.8–1.3)	137/234	1.1 (0.8–1.4)	111/166	1.0 (0.7–1.5)
Low (77, 78, 88)	79/113	1.2 (0.8–1.6)	31/55	1.0 (0.6–1.7)	48/58	1.3 (0.8–2.1)
Low versus high/int as reference group		1.2 (0.8–1.6)		1.0 (0.6–1.6)		1.3 (0.8–2.0)
Low/int versus high as reference group		1.1 (0.8 – 1.3)		1.1 (0.8 – 1.4)		1.1 (0.7 – 1.5)
UGT1A1-3156						
High (GG)	263/435	1.0 (Ref)	158/274	1.0 (Ref)	23/30	1.0 (Ref)
Int (GA)	227/156	1.1 (0.8–1.3)	128/222	1.0 (0.8–1.4)	105/161	1.2 (0.8–1.7)
Low (AA)	47/75	1.0 (0.7–1.5)	24/45	0.9 (0.5–1.6)	99/134	1.2 (0.6–2.2)
Low versus high/int as reference group		1.0 (0.7 – 1.5)		0.9 (0.6 – 1.6)		1.1 (0.6 – 2.0)
Low/int versus high as reference group		1.1 (0.9 – 1.3)		1.0 (0.8 – 1.3)		1.2 (0.8 –1.7)
UGT1A1-3279						
High (TT)	83/167	1.0 (Ref)	71/160	1.0 (Ref)	12/7	1.0 (Ref)
Int (TG)	238/375	1.3 (0.9 –1.7)	168/270	1.5 (1.0 – 2.1)	70/105	0.4 (0.1 –1.0)
Low (GG)	216/324	1.3 (0.9–1.8)	71/111	1.5 (0.9–2.2)	145/213	0.4 (0.1–1.0)
Low versus high/int as reference group		1.0 (0.8–1.3)		1.1 (0.8–1.6)		0.9 (0.6–1.3)
Low/int versus high as reference group		1.3 (0.9 –1.7)		1.5 (1.1–2.0)		0.4 (0.1 –1.0)
UGT1A9-275						
Low (TT)	409/676	1.0 (Ref)	256/461	1.0 (Ref)	153/215	1.0 (Ref)
Int (AT)	119/182	1.0 (0.8 – 1.3)	51/80	1.1 (0.7–1.6)	68/102	0.9 (0.6 –1.3)
High (AA)	9/8	1.8 (0.7 – 4.7)	3/0	-	6/8	1.1 (0.4 –3.3)
Low versus high/int as reference group		1.0 (0.7–1.3)		0.8 (0.6–1.3)		1.1 (0.8–1.6)
Low/int versus high as reference group		0.6 (0.2 –1.5)		-		0.9 (0.3 – 2.7)

*OR are adjusted for age, race, sex and offsets.

†OR are adjusted for age, sex and offsets.

Table 3

Haplotypes association studies in colon cancer case/control samples from a Caucasian population

SNP-Haplotype	Allele	Frequency		χ^2 test (p-value)	LRS* (global p-value)
		Case	Control		
UGT1A1-3279/-3156	G-A	0.28	0.29	0.7048	0.0277
	G-G	0.22	0.16	0.0082	
	T-G	0.50	0.55	0.0858	
UGT1A1-3279/-3156/-53	G-A-7	0.28	0.29	0.7048	0.0557
	G-G-6	0.18	0.14	0.0230	
	G-G-7	0.03	0.03	0.3442	
	G-G-8	0.001	0.001	0.6945	
	T-G-6	0.50	0.55	0.0925	

* LRS : Likelihood ratio statistic

Table 4

Odds ratios (ORs) for meat and meat-derived compound exposure and colon cancer by UGT1A1-53 and −3156 genotypes

	All participants		UGT1A1-53				UGT1A1-3156			
			High/Intermediate		Low		High/Intermediate		Low	
	Cases /Controls	OR (95% CI)*	Cases /Controls	OR (95% CI)*	Cases /Controls	OR (95% CI)*	Cases /Controls	OR (95% CI)*	Cases /Controls	OR (95% CI)*
n	537/866		458/753		79/113		490/791		47/75	
Red meat (g/d)										
<28.5	213/433	1.0 (Ref)	184/374	1.0 (Ref)	29/59	1.0 (0.6–1.7)	194/393	1.0 (Ref)	19/40	1.0 (0.5–1.7)
≥28.5	324/433	1.3 (1.0–1.8)	274/379	1.3 (1.0–1.7)	50/54	1.8 (1.1–2.9)	296/398	1.3 (1.0–1.8)	28/35	1.5 (0.9–2.7)
p-value†						0.37				0.65
Well/Very-well done red meat (g/d)										
<21.5	213/433	1.0 (Ref)	183/374	1.0 (Ref)	30/59	1.1 (0.7–1.8)	193/392	1.0 (Ref)	20/41	1.0 (0.6–1.8)
≥21.5	324/433	1.3 (1.1–1.7)	275/379	1.3 (1.0–1.7)	49/54	1.7 (1.1–2.8)	297/399	1.3 (1.0–1.7)	27/34	1.5 (0.8–2.6)
p-value†						0.55				0.84
Pan-fried red meat (g/d)										
<9.0	202/433	1.0 (Ref)	172/370	1.0 (Ref)	30/63	1.1 (0.7–1.9)	182/391	1.0 (Ref)	20/42	1.1 (0.6–2.0)
≥9.0	335/433	1.5 (1.1–1.9)	286/383	1.4 (1.1–1.9)	49/50	1.9 (1.2–3.0)	308/400	1.5 (1.1–1.9)	27/33	1.5 (0.8–2.6)
p-value†						0.62				0.83
Grilled/BBQ red meat (g/d)										
<3.7	267/435	1.0 (Ref)	224/382	1.0 (Ref)	43/53	1.6 (1.0–2.4)	242/402	1.0 (Ref)	25/33	1.4 (0.8–2.5)
≥3.7	270/431	1.0 (0.7–1.2)	234/371	1.0 (0.8–1.3)	36/60	1.0 (0.6–1.6)	248/389	1.0 (0.8–1.3)	22/42	0.8 (0.5–1.4)
p-value†						0.17				0.15
DiMeIQx (ng/d)										
<2.4	206/433	1.0 (Ref)	178/378	1.0 (Ref)	28/55	1.1 (0.7–1.9)	189/394	1.0 (Ref)	17/39	1.0 (0.5–1.8)
≥2.4	331/433	1.2 (0.9–1.7)	280/375	1.2 (0.9–1.7)	51/58	1.6 (1.0–2.6)	301/397	1.3 (0.9–1.7)	30/36	1.4 (0.8–2.5)
p-value†						0.71				0.72
MeIQx (ng/d)										
<37.3	198/433	1.0 (Ref)	164/373	1.0 (Ref)	34/60	1.3 (0.8–2.2)	175/390	1.0 (Ref)	23/43	1.2 (0.7–2.2)
≥37.3	339/433	1.2 (0.9–1.7)	294/380	1.2 (0.9–1.8)	45/53	1.4 (0.8–2.4)	315/401	1.2 (0.9–1.7)	24/32	1.1 (0.6–2.2)
p-value†						0.27				0.47
PhIP (ng/d)										
<45.9	228/433	1.0 (Ref)	188/377	1.0 (Ref)	40/56	1.5 (0.9–2.4)	202/394	1.0 (Ref)	26/39	1.3 (0.8–2.3)
≥45.9	309/433	1.0 (0.7–1.3)	270/376	1.0 (0.8–1.3)	39/57	1.0 (0.6–1.7)	288/397	1.0 (0.8–1.3)	21/36	0.9 (0.5–1.6)
p-value†						0.24				0.30
BaP (ng/d)										
<7.7	240/433	1.0 (Ref)	199/383	1.0 (Ref)	41/50	1.8 (1.1–2.9)	215/400	1.0 (Ref)	25/33	1.7 (1.0–3.0)
≥7.7	297/433	1.1 (0.9–1.4)	259/370	1.2 (0.9–1.5)	38/63	1.0 (0.7–1.6)	275/391	1.2 (0.9–1.5)	22/42	0.8 (0.5–1.4)
p-value†						0.02				0.03

Abbreviations: BaP, benzo(a)pyrene; BBQ, barbecued.

* The ORs calculated for UGT1A and meat groups were adjusted for age, race, sex, total meat, energy-adjusted fat intake, dietary fiber intake, total energy, and offsets. The ORs calculated for UGT1A and heterocyclic amines (DiMeIQx , MeIQx and PhIP) were adjusted for all heterocyclic amines as well as age, race, sex, energy-adjusted fat intake, dietary fiber intake, total energy, and offsets. The ORs calculated for BaP were adjusted for age, race, sex, energy-adjusted fat intake, dietary fiber intake, total energy, and offsets. Variable cut points are median values based on the distribution among controls.

† Likelihood ratio test p-value to assess mutiplicative interaction