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Red meat and poultry intake, polymorphisms in the nucleotide excision repair and mismatch repair pathways and colorectal cancer risk

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Diets high in red meat have been consistently associated with colorectal cancer (CRC) risk and may result in exposure to carcinogens that cause DNA damage [i.e polycyclic aromatic hydrocarbons, heterocyclic amines (HCAs) and *N*-nitroso compounds]. Using a family-based study, we investigated whether polymorphisms in the nucleotide excision repair (NER) (*ERCC1* 3′ untranslated region (UTR) G/T, *XPD* Asp312Asn and Lys751Gln, *XPC* intron 11 C/A, *XPA* 5′ UTR C/T, *XPF* Arg415Gln and *XPG* Asp1104His) and mismatch repair (*MLH1* Ile219Val and *MSH2* Gly322Asp) pathways modified the association with red meat and poultry intake. We tested for gene–environment interactions using case-only analyses ($n = 577$) and compared the results using case-unaffected sibling comparisons ($n = 307$ sibships). Increased risk of CRC was observed for intake of more than or equal to three servings per week of red meat [odds ratio (OR) = 1.8, 95% confidence interval (CI) = 1.3–2.5)] or high-temperature cooked red meat (OR = 1.6, 95% CI = 1.1–2.2). Intake of red meat heavily brown on the outside or inside increased CRC risk only among subjects who carried the *XPD* codon 751 Lys/Lys genotype (case-only interaction $P = 0.006$ and $P = 0.001$, respectively, for doneness outside or inside) or the XPD codon 312 Asp/Asp genotype (case-only interaction $P = 0.090$ and $P < 0.001$, respectively). These interactions were stronger for rectal cancer cases (heterogeneity test $P = 0.002$ for *XPD* Asp312Asn and $P = 0.03$ for *XPD* Lys751Gln) and remained statistically significant after accounting for multiple testing. Case-unaffected sibling analyses were generally supportive of the case-only results. These findings highlight the possible contribution of diets high in red meat to the formation of lesions that elicit the NER pathway, such as carcinogen-induced bulky adducts.

Introduction

In its recent report on diet, physical activity and cancer, the World Cancer Research Fund concluded that the available epidemiological evidence that red meat and processed meat increase the risk of colorectal cancer (CRC) is ‘convincing’ (1). Various mechanisms have been proposed to explain the link between red meat consumption and CRC. Chief among them is the exposure to three main families of carcinogens generated through cooking, processing or curing of meats: heterocyclic amines (HCAs) (2), primarily found in meat cooked at high temperatures (3); polycyclic aromatic hydrocarbons (PAHs) (2), formed by fat pyrolysis when meats are cooked above a direct flame, such as grilling and barbecuing (3) and *N*-nitroso compounds (NOCs) (4), formed in foods that have been preserved using nitrates or nitrites (e.g. cured meats/sausages) or processed by smoking or fire-drying. Interestingly, feeding studies confirm that NOCs can also be endogenously produced in the colon lumen by the reaction of amines and amides with dietary nitrites, a process facilitated by colonic flora (5–8). Among the epidemiological studies of CRC that have taken into account red meat and poultry cooking methods and doneness levels, some (9–11) reported a modest positive relationship between diets high in heavily brown red meats and CRC; however, others only found an association when the relevant bioactivation phenotypes were considered (12). A few epidemiological studies have considered estimated levels of HCAs (9,11,12) and overall support a role for HCAs in CRC risk, although results are not conclusive.

Results of animal studies support a role for HCAs, PAHs and NOCs in colorectal carcinogenesis (5,13,14), particularly by their ability to induce DNA damage. Specifically, HCA-induced DNA adducts can generate mutations in the colon, which is considered as the main extrahepatic target of HCA adduct formation and carcinogenicity (15). In addition, HCAs have been reported to induce frameshift mutations, microsatellite instability, strand breaks and oxidative base damage (15,16). There is also evidence that cells that have a deficiency in the mismatch repair (MMR) enzymes tend to accumulate more mutations after exposure to some HCAs (17). Similarly, PAH-induced bulky adducts can induce mutations that have been detected in the human colon (18,19) and its metabolism generates free radicals, which can induce base damages and strand breaks (20). NOCs can alkylate DNA bases (21), and aldehydes generated by NOC metabolism can also induce DNA strand breaks (22). These types of DNA damage are repaired by different pathways, which include the nucleotide excision repair (NER), base excision repair, MMR, homologous recombination repair, non-homologous end-joining repair and methylguanine-DNA methyltransferase pathways.

Given the potential role of carcinogens formed in cooked meats in colorectal carcinogenesis through the formation of mutations in the colorectal lumen, a role for DNA repair gene variants as susceptibility genes and effect modifiers is plausible. In the present study, we report results of our investigations on the role of single-nucleotide polymorphisms (SNPs) in genes that participate in the NER (*ERCC1* 3′ untranslated region (UTR) G/T, *XPD* Asp312Asn and Lys751Gln, *XPC* intron 11 C/A, *XPA* 5′ UTR C/T, *XPF* Arg415Gln and *XPG* Asp1104His) and MMR (*MLH1* Ile219Val and *MSH2* Gly322Asp) pathways. These two pathways are important for the repair of carcinogen-induced bulky adducts and mismatched bases, respectively. These SNPs were selected based on their putative impact on protein function (23–26) and/or previous evidence of cancer risk associations. We report here the role of these SNPs in CRC and their role as potential modifiers of the effect of red meat and poultry intake.

Materials and methods

Study subjects

This family-based case–control association study was conducted within the infrastructure of the Colon Cancer Family Registry (Colon-CFR) (27). Due to budget constraints, we conducted this study with subjects recruited from the University of Southern California (USC) Consortium, one of the six centers of the Colon-CFR, as a first step toward comprehensive analyses including the whole registry. Briefly, incident cases with CRC (probands) were recruited

Abbreviations: CI, confidence interval; Colon-CFR, Colon Cancer Family Registry; CRC, colorectal cancer; HCA, heterocyclic amine; IOR, interaction odds ratio; MMR, mismatch repair; NER, nucleotide excision repair; NOC, *N*-nitroso compound; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon; SNP, single-nucleotide polymorphism; USC, University of Southern California; UTR, untranslated region.

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through population-based registries in either of the component centers of the USC Consortium: Arizona Cancer Center, Dartmouth College, University of Colorado, University of Minnesota, University of North Carolina and University of Southern California. Unaffected siblings and cousins in the family of the probands 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 (27,28). All subjects signed a written informed consent approved by the Institutional Review Board of each institution, donated a blood sample and completed an in-person risk factor questionnaire.

In our analyses, we included subjects recruited during Phase I of the USC Consortium enrollment period (1997–2002). Briefly, of the 5684 subjects identified throughout the USC Consortium, 4734 (83%) were alive and available to be contacted. Of these, 3103 were eligible for screening, with 1055 of them being eligible for participation. A total of 633 subjects were alive and agreed to join the USC Consortium (60% of eligible subjects). From these subjects, a total of 650 siblings (controls) were contacted and a total of 389 agreed to participate. In this study, we only included subjects recruited from the population-based registries; therefore, 51 probands and 40 siblings from the Cleveland Clinic foundation, which maintains a clinic-based registry, were excluded. We had questionnaire risk factor with meat intake data and biospecimens available for genotyping for 577 probands, 362 siblings and 355 cousins. Among the 577 probands, 307 had at least one unaffected sibling who could serve as a control, for a total of 307 proband–sibling pairs (sibships). In addition, 87.5% subjects also completed a mailed food frequency questionnaire that was completed within the same year of administration of the risk factor questionnaire.

Exposure assessment

We used data collected in the baseline risk factor questionnaire, which was designed to be used by all Colon-CFR sites (27), and collected information regarding number of servings of red meat (beef, steak, hamburger, prime rib, ribs, veal, lamb, bacon, pork, pork in sausages or venison) per week, number of servings of red meat cooked by high-temperature methods (i.e. panfrying, oven broiling or grilling) per week, number of servings of poultry (chicken, turkey or fowl) per week and number of servings of poultry (chicken, turkey or fowl) cooked by panfrying, oven broiling or grilling. Furthermore, subjects were asked questions about the level of doneness of red meat from outside (lightly browned, medium browned and heavily browned), level of doneness from inside (red, pink and brown) and level of doneness of poultry from outside (lightly browned and medium browned), when these meats were cooked by the above-mentioned high-temperature methods. All questions were asked in reference to the 2 years before the cancer diagnosis. In our analyses, we defined ‘cooked’ red meat or poultry as those cooked with either panfrying, oven broiling or grilling/barbecuing, which serve as surrogates for ‘high-temperature’ methods, which are known sources of HCA and PAH formation. Level of doneness refers to the appearance of the outside or inside of the meat when cooked by these high-temperature methods.

Genotyping

DNA was extracted from peripheral blood lymphocytes by standard methods, resuspended in Tris–ethylenediaminetetraacetic acid buffer (10 mM Tris and 1 mM ethylenediaminetetraacetic acid) and frozen until use. We genotyped the following SNPs: *ERCC1* 3′ UTR G/T (rs3212986), *XPD* Asp312Asn (rs1799793) and Lys751Gln (rs13181), *XPC* intron 11 C/A (rs2279017), *XPA* 5′ UTR C/T (rs1800975), *XPF* Arg415Gln (rs1800067), *XPG* Asp1104His (rs17655), *MLH1* Ile219Val (rs1799977) and *MSH2* Gly322Asp (rs4987188). Genotype analyses of all SNPs were done using Taqman assays from Applied Biosystems (Foster City, CA). For quality control, ∼6% randomly selected samples were duplicated using a unique identification numbers and were blinded to laboratory personnel. In addition, there were 12 blank wells, serving as negative controls. We used an ABI 7900HT Sequence Detection and Scoring System for allele scoring. We observed 100% concordance between all duplicate samples. The calling rate for all assays ranged from 97.4 to 99.9%.

Data analysis

We checked among siblings for differences between the observed genotypic frequencies and those expected under Hardy–Weinberg equilibrium using chi-square tests. These tests were restricted to Caucasians who made up 82.6% of our siblings. Proband—unaffected sibling comparisons were done using a 1:n matched conditional logistic regression approach. For 42.3% of probands (n = 244), we had data collected on first-degree cousins (n = 355). We used data on these cousins to determine the median value for all exposure variables that we used to dichotomize all variables, as we assumed that the distribution of these variables among cousins would be more representative of the general population than among the siblings. For analyses of meat intake, we evaluated the potential confounding effect of the following variables available from the risk factor questionnaire: age at the time of interview (continuous), gender, past history of Crohn’s disease, ulcerative colitis, irritable bowel syndrome, diverticulitis, diabetes and high cholesterol, marital status, folate supplements, weight 2 years before interview, weight at the age of 20 years, height, number of years lived in the USA, body mass index, aspirin/ibuprofen use, physical activity, fruits per week, vegetables per week, level of education and income. Adjustment for these potential confounders did not change any of the odds ratios (ORs) for the main exposure or gene variables by >10%. Hence, they were not considered for further analysis of gene–environment interactions (29). For 87.5% of the subjects, we also had dietary data obtained with an food frequency questionnaire (27) for total energy intake, total protein and total saturated fat intake. Among these subjects (326 siblings and 265 probands), we considered these variables as potential confounders of meat intake variables and found no evidence that they changed risk estimates by >10%; therefore, they were not included in our final models.

For the gene main effect analyses, given the sample size of our study, we assumed a dominant mode of inheritance for all the SNPs and used conditional logistic regression models, to determine matched ORs. Age and gender did not confound the association between NER and MMR SNPs and CRC. Haplotype probabilities for SNPs in proximate chromosomal locations (*XPD* gene Asp312Asn, Lys751Gln and *ERCC1* 3′ UTR G/T) were calculated using expectation–maximization algorithm for multiheterozygous individuals (30). Among Caucasians, when considering the two *XPD* SNPs, we estimated that 98.1% of all double heterozygotes had the Asp-Lys and Asn-Gln alleles and the remaining 1.9% were estimated to be carriers of Asp-Gln and Asn-Lys alleles. D′ between the two loci was calculated to be 0.79 and the R^2 was 0.55, which were similar to corresponding values in the HapMap data. We found no evidence for strong linkage disequilibrium between the *ERCC1* 3′ UTR G/T SNP, which maps closely to *XPD*, and any of the two *XPD* SNPs. We performed a global test for association of haplotype alleles with CRC using likelihood ratio tests, assuming additive effects of haplotype alleles.

We conducted analyses of gene–environment interactions for all nine SNPs and the exposure variables mentioned above using a case-only design, for which we had higher statistical power and next compared our statistically significant findings with those obtained using discordant sibships, paying special attention to the magnitude of the interaction odds ratios (IORs). Provided that genes and exposure are independent, ORs obtained from case-only analyses can be used as estimates of IORs (31). We tested this assumption of independence between the genes and exposures among the cousins of the probands, using a more liberal cutoff P-value of 0.15. We did not find any statistically significant association between any of the SNPs and the exposures, after correction for multiple comparisons. We tested for gene–exposure interactions on a multiplicative scale. 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 IOR. Further adjustment of these models by race, age at diagnosis and gender, did not change estimates by >10%. Therefore, these covariates were excluded from the final models (29). We also performed unconditional logistic regression using dichotomized exposure as outcome and the estimated number of haplotype alleles as independent variables to do a haplotype × exposure interaction test. Gene–exposure interaction models using proband–sibling pairs were done including in our regression model product terms between the gene and exposure variables in addition to the terms present in the main effect model and used likelihood ratio tests to compare these models with models that assumed no interaction. To do analyses by tumor anatomical subsite (colon versus rectum), we collapsed the site of tumors into two major groups: colon cancer [International classification of diseases for oncology (ICD-O-2) C180–C188, n = 351] and rectal cancer (ICD-O-2 C199, C209, n = 151), excluding cases with ICD code ICD-O-2 C189 (large intestines, NOS) (n = 74). One subject had tumor anatomical subsite information missing. Analyses of ‘CRC cases’ include all cases combined (n = 577). We tested for heterogeneity of the gene and exposures’ main effects across anatomical subsites by assigning to controls the same code for tumor site as probands and then adding a product term between the gene or exposure and tumor site variable in the conditional logistic regression model, thereby allowing their log OR to differ and testing the null hypothesis that the log OR did not vary by tumor site. For case-only analyses of gene–environment interactions, we tested for heterogeneity across tumor site by adding the tumor site variable and the product term between genotype and tumor site and comparing this with a model that only included the genotype and tumor site using likelihood ratio tests. We compared our gene main effect and G × E estimates analyses for multiple comparisons using the Benjamini–Hochberg method for controlling the false-discovery rate within each meat or poultry exposure considered across all polymorphisms analyzed (32). All tests were two sided and all analyses were done using the statistical software STATA version 8 (STATA Corporation, College Station, TX).

Results

The distributions of age, gender and meat intake variables for the probands and unaffected siblings are shown in Table I. No appreciable

Table I. Demographic characteristics of probands and unaffected siblings[a]

	Unaffected siblings (n = 362)	All probands (n = 577)	Colon cancer (n = 351)	Rectal cancer (n = 151)
Mean age at interview (SD)	59.3 (11.8)	60.0 (11.3)	60.2 (11.6)	59.4 (11.0)
Gender (%)				
Males	167 (46.1)	302 (52.3)	159 (45.3)	94 (62.3)
Females	195 (53.9)	275 (47.7)	192 (54.7)	57 (37.7)
Mean servings of red meat per week (SD)	4.4 (4.1)	5.5 (6.5)	5.2 (6.0)	6.0 (8.1)
Mean servings of cooked[b] RM per week (SD)	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[b] poultry per week (SD)	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)

[a]Percentages do not always add up to 100 because of rounding.
[b]Cooked by panfrying, grilling/barbecuing or oven broiling.

demographic differences were observed between the two groups with the exception of a slightly higher percentage of males in the probands compared with the sib controls.

Meat intake and CRC risk

The associations between the different meat intake variables and CRC risk are shown in Table II. Subjects who consumed more than three servings of red meat per week had a significantly higher risk of CRC compared with those consuming three or fewer servings (OR = 1.8, 95% CI = 1.3–2.5). Similarly, individuals who reported eating more than three servings of red meat cooked by panfrying, oven broiling or grilling had a significantly higher risk of CRC than those consuming three or fewer servings (OR = 1.6, 95% CI = 1.1–2.2). The association between total red meat intake and cancer did not vary by tumor site; however, that for cooked red meat appeared to be confined to risk of colon and not rectal cancer, although the test of heterogeneity did not reach statistical significance. We observed no association between the level of doneness of red meat and CRC risk. Similarly, no associations were observed for cooked poultry or its level of doneness and risk of CRC.

NER and MMR SNPs and CRC risk

The minor allele frequencies for each of the SNPs studied were similar to those reported in previous studies (33) (Table III). We found no statistically significant difference between the observed genotypic frequencies and those expected under the Hardy–Weinberg principle among siblings. Given the sample size of our study, for all SNPs we assumed a dominant mode of inheritance. We present in Table III ORs and 95% CI for all SNPs investigated comparing probands (cases) with unaffected siblings (controls). We observed no evidence of an association for any of the nine SNPs and CRC. We observed heterogeneity across tumor sites for the *XPG* Asp1104His SNP. The His allele was associated with a decreased risk of colon cancer (OR = 0.7, 95% CI = 0.4–1.3) but with an increased risk rectal cancer (OR = 2.1, 95% CI = 0.9–4.6). The test for heterogeneity *P*-value was 0.035. However, this *P*-value was no longer statistically significant after correcting for multiple comparisons. When considering *XPD* haplotypes, the global test for association between them and CRC was not statistically significant (P = 0.884).

NER and MMR SNPs, red meat intake and CRC risk

For G $\times$ E interactions, we considered total red meat intake, total red meat cooked by high-temperature methods such as by panfrying, oven broiling or grilling, level of doneness of red meat on the outside and level of doneness of red meat in the inside. Given 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 (34), we also considered potential heterogeneity of the G $\times$ E interactions by tumor subsite (colon versus rectum). As we describe in Materials and Methods, we first conducted case-only analyses, for which we had higher statistical power and compared our statistically significant findings with case–sib analyses.

Results of our case-only analyses for the interactions of the *XPD* SNPs and red meat intake showed evidence of effect modification for the presence of the *XPD* Lys751Gln SNP and higher level of doneness whether outside (P = 0.006) or inside the meat (P = 0.001) (Table IV). These findings were borderline (P = 0.054) and statistically significant (P = 0.009) after correcting for multiple comparisons, respectively. Similar findings, albeit with slightly less statistical significance, were observed for the *XPD* Asp312Asn, which is in linkage disequilibrium with Lys751Gln for level of doneness outside (P = 0.090) and inside (P = 0.001). Stratified analyses by anatomical location of the tumor were generally stronger for rectal compared with colon tumors, with statistically significant heterogeneity shown for outside but not inside doneness (Table IV). Haplotype analyses showed that cases consuming heavily brown meat on the outside or brown meat on the inside were less likely to have the Asn-Gln *XPD* haplotype as compared with the Asp-Lys haplotype (OR = 0.7, 95% CI = 0.5–0.9, Table IV). These findings were stronger for rectal cancer cases (OR = 0.4, 95% CI = 0.2–0.7, for doneness outside the meat; OR = 0.5, 95% CI = 0.3–0.8 for inside the meat, Table IV).

We next compared the results of the statistically significant findings from our case-only analyses with analyses using the case–unaffected sibling design. Case–sib analyses showed stronger support for the case-only findings that considered level of doneness of red meat on the outside and *XPD* SNPs, with stronger effects observed for rectal compared with colon cancer (Table V). Findings were stronger for the *XPD* Lys751Gln SNP (IOR for CRC = 0.6, P = 0.128; IOR for colon cancer = 1.1, P = 0.817 and IOR for rectal cancer = 0.2, P = 0.03). Our results indicated that intake of red meat heavily browned on the outside was only associated with CRC among carriers of the *XPD* codon 751 Lys/Lys genotype. Furthermore, in support to our case-only findings, this interaction was stronger among rectal cases, among whom intake of heavily brown red meat on the outside had an OR = 3.8 (95% CI = 1.1–13) among *XPD* codon 751 Lys/Lys subjects and OR = 0.7 (95% CI = 0.2–1.8) among carriers of

Table II. Red meat and poultry intake and CRC risk

Exposure variables	CRC				Colon cancer				Rectal cancer			
	Co/Ca	OR[a]	95% CI	*P*-value	Co/Ca	OR[a]	95% CI	*P*-value	Co/Ca	OR[a]	95% CI	*P*-value
Servings of red meat												
≤3/week	191/131	1.0[Ref]			113/79	1.0[Ref]			49/40	1.0[Ref]		
>3/week	170/177	1.8	1.3–2.5	0.001	102/106	1.8	1.1–2.8	0.019	49/44	1.3	0.6–2.5	0.517
Heterogeneity colon versus rectum *P*-value				0.419								
Servings of cooked[b] red meat												
≤3/week	233/172	1.0[Ref]			142/104	1.0[Ref]			58/51	1.0[Ref]		
>3/week	128/134	1.6	1.1–2.2	0.009	73/81	1.7	1.1–2.6	0.022	40/32	1.0	0.5–1.8	0.885
Heterogeneity colon versus rectum *P*-value				0.150								
Doneness of red met from outside												
Light or medium browned	256/214	1.0[Ref]			147/124	1.0[Ref]			80/64	1.0[Ref]		
Heavily browned	104/94	1.1	0.8–1.6	0.559	67/61	1.1	0.7–1.8	0.674	18/20	1.4	0.7–2.9	0.387
Heterogeneity colon versus rectum *P*-value				0.613								
Doneness of red met from inside												
Red/pink	191/153	1.0[Ref]			114/91	1.0[Ref]			53/48	1.0[Ref]		
Brown	170/155	1.2	0.8–1.6	0.362	101/94	1.2	0.8–1.9	0.379	45/36	0.8	0.4–1.6	0.600
Heterogeneity colon versus rectum *P*-value				0.351								
Servings of cooked[b] poultry												
≤2/week	257/227	1.0[Ref]			157/138	1.0[Ref]			68/57	1.0[Ref]		
>2/week	104/80	0.9	0.6–1.2	0.440	58/47	0.9	0.6–1.5	0.747	30/27	1.1	0.6–2.2	0.674
Heterogeneity colon vs. rectum *P*-value				0.596								
Doneness of poultry from outside												
Light or medium browned	274/238	1.0[Ref]			161/142	1.0[Ref]			76/64	1.0[Ref]		
Heavily browned	87/69	0.9	0.6–1.4	0.765	54/43	0.9	0.6–1.5	0.790	22/19	1.0	0.5–2.2	0.929
Heterogeneity colon versus rectum *P*-value				0.826								

[a]Unadjusted.
[b]Panfried, oven-broiled or grilled.

Table III. NER SNPs and CRC risk

Gene	CRC					Colon cancer				Rectal cancer				Site diff *P*-value[b]
	Minor allele frequency	Co/Ca	OR[a]	95% CI	*P*-value	Co/Ca	OR[a]	95% CI	*P*-value	Co/Ca	OR[a]	95% CI	*P*-value	
MLH1/Ile219Val														
Ile/Ile	0.29	194/161	1.0[Ref]			80/69	1.0[Ref]			111/92	1.0[Ref]			
Ile/Val + Val/Val		160/140	1.1	0.7–1.6	0.678	67/59	1.0	0.6–1.9	0.915	93/81	1.1	0.7–1.9	0.65	0.83
MSH2 Gly322Asp														
Gly/Gly	0.02	348/291	1.0[Ref]			144/124	1.0[Ref]			201/167	1.0[Ref]			
Gly/Asp + Asp/Asp		13/16	3.6	0.7–18	0.113	5/6	2.0	0.2–22	0.571	8/10	5.3	0.6–46	0.134	0.559
ERCC1 3′ UTR														
G/G	0.27	206/162	1.0[Ref]			82/69	1.0[Ref]			124/93	1.0[Ref]			
G/T + T/T		153/142	1.3	0.8–1.9	0.255	66/59	1.2	0.6–2.2	0.622	84/83	1.4	0.8–2.4	0.275	0.704
XPD Asp312Asn														
Asp/Asp	0.33	161/142	1.0[Ref]			73/65	1.0[Ref]			87/77	1.0[Ref]			
Asp/Asn + Asn/Asn		200/165	1.0	0.6–1.4	0.813	76/65	1.0	0.5–1.9	0.911	122/100	0.9	0.6–1.6	0.831	0.962
XPD Lys751Gln														
Lys/Lys	0.34	153/124	1.0[Ref]			62/56	1.0[Ref]			90/68	1.0[Ref]			
Lys/Gln + Gln/Gln		209/184	1.2	0.8–1.8	0.447	88/74	0.9	0.5–1.8	0.778	119/110	1.4	0.8–2.4	0.229	0.325
XPC intron 11														
C/C	0.39	139/117	1.0[Ref]			64/52	1.0[Ref]			74/65	1.0[Ref]			
C/A + A/A		222/191	1.0	0.7–1.5	0.862	85/78	1.1	0.6–2.1	0.748	135/113	1.0	0.6–1.7	0.968	0.784
XPA 5′ UTR														
C/C	0.33	149/136	1.0[Ref]			52/61	1.0[Ref]			94/75	1.0[Ref]			
C/T + T/T		200/166	0.8	0.5–1.2	0.211	91/66	0.4	0.2–0.8	0.011	109/100	1.1	0.7–2.0	0.641	0.016
XPF Arg415Gln														
Arg/Arg	0.10	313/265	1.0[Ref]			129/112	1.0[Ref]			181/153	1.0[Ref]			
Arg/Gln + Gln/Gln		47/40	1.0	0.5–1.9	0.978	19/17	0.9	0.3–2.7	0.851	28/23	1.0	0.5–2.3	0.919	0.832
XPG Asp1104His														
Asp/Asp	0.22	213/183	1.0[Ref]			85/75	1.0[Ref]			126/108	1.0[Ref]			
Asp/His + His/His		148/125	1.0	0.7–1.6	0.950	65/55	0.9	0.5–1.9	0.835	82/70	1.1	0.6–1.9	0.807	0.753

[a]Unadjusted.
[b]*P*-value from colon versus rectum heterogeneity test.

Table IV. Case-only analyses of interactions of XPD polymorphisms with red meat level of doneness

	CRC cases				Colon cancer cases				Rectal cancer cases				Heterogeneity P-value[c]
		OR[a,b]	95% CI	P-value		OR[a,b]	95% CI	P-value		OR[a,b]	95% CI	P-value	
	Level of doneness of red meat from outside (light or medium brown versus heavily brown)												
	Light or medium/heavy[d]				Light or medium/heavy[d]				Light or medium/heavy[d]				
XPD Asp312Asn[e]													
Asp/Asp	181/95	1.0[Ref]			117/51	1.0[Ref]			40/29	1.0[Ref]			
Asp/Asn + Asn/Asn	215/83	0.7	0.5–1.0	0.090	122/58	1.1	0.7–1.7	0.708	68/14	0.3	0.1–0.6	0.001	0.002
XPD Lys751Gln[e]													
Lys/Lys	148/89	1.0[Ref]			96/50	1.0[Ref]			37/27	1.0[Ref]			
Lys/Gln + Gln/Gln	247/90	0.6	0.4–0.9	0.006	142/60	0.8	0.5–1.3	0.369	71/16	0.3	0.1–0.6	0.002	0.027
XPD haplotype[f]													
Asp-Lys	463/232	1.0[Ref]			285/138	1.0[Ref]			121/61	1.0[Ref]			
Asp-Gln	60/28	0.9	0.6–1.5	0.768	38/14	0.7	0.4–1.4	0.329	10/7	1.8	0.6–5.4	0.301	
Asn-Lys	23/12	1.1	0.5–2.3	0.869	14/7	0.9	0.3—2.6	0.909	6/3	1.3	0.3–6.2	0.716	
Asn-Gln	244/84	0.7	0.5–0.9	0.016	139/59	0.9	0.6–1.3	0.502	79/15	0.4	0.2–0.7	0.004	
Global test P-value				0.102				0.743				0.008	
	Level of doneness of red meat from inside (red or pink versus brown)												
	Red or pink/brown[g]				Red or pink/brown[g]				Red or pink/brown[g]				
XPD Asp312Asn[e]													
Asp/Asp	123/153	1.0[Ref]			79/89	1.0[Ref]			30/39	1.0[Ref]			
Asp/Asn + Asn/Asn	174/124	0.6	0.4–0.8	0.001	99/81	0.7	0.5–1.1	0.137	56/26	0.4	0.2–0.7	0.002	0.076
XPD Lys751Gln[e]													
Lys/Lys	102/135	1.0[Ref]			64/82	1.0[Ref]			29/35	1.0[Ref]			
Lys/Gln + Gln/Gln	194/143	0.6	0.4–0.8	0.001	113/89	0.6	0.4–0.9	0.026	57/30	0.4	0.2–0.8	0.014	0.392
XPD haplotype[f]													
Asp-Lys	339/356	1.0[Ref]			206/217	1.0[Ref]			95/87	1.0[Ref]			
Asp-Gln	46/42	0.9	0.5–1.4	0.594	30/22	0.7	0.3–1.2	0.181	8/9	1.6	0.5–4.7	0.401	
Asn-Lys	16/19	1.2	0.6–2.5	0.589	11/10	0.8	0.3–2.1	0.652	4/5	2.0	0.5–9.1	0.357	
Asn-Gln	191/137	0.7	0.5–0.9	0.006	107/91	0.8	0.6–1.1	0.230	65/29	0.5	0.3–0.8	0.009	
Global test P-value				0.040				0.423				0.021	

[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 IOR.
[b]Unadjusted.
[c]Colon versus rectum heterogeneity test.
[d]Light or medium as referent group.
[e]Number of individuals carrying the genotype.
[f]Number of haplotype alleles.
[g]Red or pink as referent group.

one or two copies of the Gln allele (IOR = 0.2, P = 0.03) (Table V). Analyses considering haplotyes determined by the two *XPD* SNPs indicated that their effect modification on intake of red meat heavily brown on the outside was restricted to rectal cancer cases (IOR = 0.2, P = 0.034, global test of interaction P = 0.088) (supplementary Table I is available at *Carcinogenesis* Online).

Case–sib comparisons that considered level of doneness of red meat in the inside and *XPD* SNPs showed a similar trend as that observed in case-only analyses, although effects were modest and non-statistically significant (supplementary Table II is available at *Carcinogenesis* Online). Overall, these data lend weak support for an effect of internal brown red meat on rectal cancer among carriers of the *XPD* codon 751 Lys/Lys genotypes.

We did not find evidence that any of the nine SNPs modified the association between total red meat intake or total red meat cooked by panfrying, oven broiling or grilling and CRC risk or colon cancer or rectal cancer risk separately (data not shown).

NER and MMR SNPs, poultry intake and CRC risk

Using case-only analysis, we observed that CRC cases who consumed more than two servings of poultry cooked by panfrying, oven broiling or grilling were less likely to be carriers of at least one copy of the 'A' allele in *XPC* intron 11 (CRC IOR = 0.7, P-value = 0.040) (Table VI). Furthermore, we observed that cases who consumed heavily browned poultry were less likely to have the *XPD* codon 751 Gln allele (CRC IOR = 0.6, P = 0.016) or the A allele at the *XPC* intron 11 C/A locus (CRC IOR = 0.7, P-value = 0.031) (Table VI). Furthermore, the *XPD* Lys751Gln × poultry level of doneness interaction seemed stronger among rectal cancer cases (IOR = 0.4, P-value = 0.005) (Table VI). These interactions did not remain statistically significant after correcting for multiple comparisons. Nonetheless, a comparison of these case-only statistically significant findings to case–sib analyses showed IORs of similar magnitude for *XPC* intron 11 and intake of cooked poultry (CRC IOR = 0.5; P = 0.054), *XPD* Lys751Gln and level of doneness of poultry outside (rectal cancer IOR = 0.2, P-value = 0.04), *XPC* intron 11 and level of doneness of poultry outside (CRC IOR = 0.5, P-value = 0.08) (supplementary Table III is available at *Carcinogenesis* Online).

We did not find evidence of effect modification of high intake of cooked poultry or its level of doneness by any of the other SNPs. Overall, results did not differ by tumor site (colon versus rectum).

Discussion

In this study, we observed that consumption of more than three servings of red meat per week was associated with an increased risk of CRC. Similar findings were shown for red meat cooked by panfrying, oven broiling or grilling. When we modeled servings of red meat per week continuously, we observed an OR of 1.4 for an increase in 75 g/day of red meat consumption (~1.6 for every 100 g/day increase). The

Table V. Interaction of *XPD* polymorphisms with level of doneness of red meat on the outside: case–sib comparisons

	Light or medium brown				Heavily browned				Genotype-specific OR[a]		
	Co/Ca	OR[b]	95% CI	*P*-value	Co/Ca	OR[a]	95% CI	*P*-value	OR[a]	95% CI	*P*-value
XPD Asp312Asn											
CRC											
Asp/Asp	114/98	1.0[Ref]			47/44	1.1	0.6–1.9	0.717	1.1	0.6–1.9	0.717
Asp/Asn and Asn/Asn	142/116	1.0	0.6–1.5	0.882	56/49	1.1	0.6–2.0	0.727	1.1	0.7–1.9	0.579
IOR		1.0	0.5–2.1	0.922							
Colon cancer											
Asp/Asp	65/59	1.0[Ref]			28/23	0.9	0.4–1.9	0.812	0.9	0.4–1.9	0.812
Asp/Asn and Asn/Asn	82/65	1.0	0.6–1.9	0.933	38/37	1.3	0.6–2.0	0.468	1.3	0.7–2.3	0.409
IOR		1.4	0.6–3.6	0.475							
Rectal cancer											
Asp/Asp	33/24	1.0[Ref]			7/13	2.3	0.7–7.0	0.147	2.3	0.7–7.0	0.147
Asp/Asn and Asn/Asn	47/40	1.0	0.4–2.4	0.982	11/7	0.8	0.3–2.7	0.753	0.8	0.3–2.5	0.730
IOR		0.4	0.1–1.8	0.209							
XPD Lys751Gln											
CRC											
Lys/Lys	108/77	1.0[Ref]			45/47	1.6	0.9–2.9	0.114	1.6	0.9–2.9	0.114
Lys/Gln and Gln/Gln	148/137	1.5	0.9–2.4	0.127	59/47	1.3	0.7–2.4	0.357	0.9	0.6–1.4	0.674
IOR		0.6	0.3–1.2	0.128							
Colon cancer											
Lys/Lys	54/46	1.0[Ref]			31/27	1.0	0.5–2.2	0.900	1.0	0.5–2.2	0.900
Lys/Gln and Gln/Gln	93/78	1.1	0.6–2.1	0.740	36/34	1.3	0.6–2.8	0.497	1.2	0.6–2.1	0.603
IOR		1.1	0.4–2.8	0.817							
Rectal cancer											
Lys/Lys	39/22	1.0[Ref]			7/13	3.8	1.1–13	0.037	3.8	1.1–13	0.037
Lys/Gln and Gln/Gln	41/42	1.8	0.7–4.6	0.184	11/7	1.2	0.4–3.9	0.752	0.7	0.2–1.8	0.418
IOR		0.2	0.03–0.9	0.030							

Co/Ca, controls/cases.
[a]Genotype-specific OR is the OR for heavily browned red meat intake within each genotype subgroup.
[b]Unadjusted.

magnitude of this OR (1.6) is higher than the pooled summary statistic of 1.3 for every 100 g/day consumption of red meat in a pooled analysis of prospective studies (35). We did not observe an association between consumption of heavily browned red meat (inside or outside) or between frequency of consumption of cooked poultry or heavily brown poultry and CRC. Further adjustment of our models by other dietary factors known to reduce CRC risk (e.g. fruits and vegetable intake) did not statistically significantly change our estimates.

In contrast to our findings, some studies (9–11) reported a positive association between diets high in well-done red meat and CRC, whereas other studies reported an association only among carriers of specific metabolic phenotypes (12). Studies done on colorectal adenomas, precursors of CRC, are also inconclusive, with one prospective study reporting an association between doneness of red meat and risk of adenomas (36), whereas two other case–control studies failed to find an association (37,38). Similarly, association studies that took into account different cooking methods and colorectal adenoma/cancer risk were inconclusive (9,10,37,39). Overall, our findings suggest that components present in total red meat, and also in red meats cooked by broiling, grilling or barbecuing, may contribute to CRC risk. Our definition of total red meat included some processed meat items (e.g. sausages and bacon); therefore, our findings may suggest that NOCs present in cured meats or NOCs formed endogenously due to high red meat intake (6–8), might be stronger candidates to explain the red meat and CRC association. In addition, PAHs and HCAs that formed in grilled and barbecued red meat may also explain this association. Nonetheless, we cannot discard other red meat components, such as heme-iron, that have also been suggested as cancer risk factors and that we did not evaluate in this study.

The main objective of our study was to identify potential effect modifiers among SNPs in the NER and MMR pathways. The results of our study based on case-only analyses suggest that the two SNPs we studied in the NER gene *XPD* may modify the effects of level of doneness inside and outside of red meat (beef, pork, lamb and sausage), especially for rectal cancer. Our analyses using sibships generally supported these findings. In particular, our main finding was that subjects who frequently ate heavily browned red meat were at a higher risk of developing rectal cancer if they were carriers of two copies of the *XPD* codon 312 Asp or *XPD* 751 Lys alleles but not if they carried at least one copy of the Asn^{321} or Gln^{751} alleles. Furthermore, our analyses considering poultry intake suggested that among carriers of the *XPD* 751 Lys allele, intake of poultry heavily browned on the outside might also increase risk of rectal cancer. To our knowledge, few previous studies have assessed interactions between NER genes and intake of meat or poultry in CRC risk or colorectal adenoma risk. In a Danish prospective study, Hansen *et al.* (40) reported no interactions between intake of red meat, processed meat and white meat (fish and poultry) and genetic variants in the NER pathway on CRC risk. Berndt *et al.* (41) did not find any statistically significant interaction between consumption of red meat and NER variants in the causation of CRC. However, both of these studies did not consider level of doneness or cooking methods, so we cannot fully compare with our results. Our finding of an *XPD* by level of doneness interaction restricted to rectal cancer cases is in agreement with a study in Hawaii that reported an association between estimated levels of HCAs and rectal cancer but not with colon cancer among men (12). It has been suggested previously that if the route of exposure to the colorectal mucosa is via the lumen, distal parts of the large intestine are more likely to encounter higher concentrations of the exposures due to the increase in concentration of lumen components as water absorption increases along the colon (34). Therefore, our findings are consistent with this hypothesis.

It is biologically plausible that *XPD* SNPs could modify the effect of well-done meats and CRC. Both carcinogens generated with charred meats (PAHs) and well-done meats (HCAs) can induce bulky adducts, which elicit NER. The XPD protein functions in NER as an adenosine triphosphate-dependent $5'$–$3'$ DNA helicase. Its C-terminal domain (amino acids 478–759 that include codon 751) interacts with the p53 protein (24). It has been reported that the *XPD* polymorphism at codon 312, but not the one at codon 751, is

Table VI. Case-only analyses of interactions of XPD and XPC polymorphisms with poultry frequency and level of doneness

	CRC cases				Colon cancer cases				Rectal cancer cases				Heterogeneity *P*-value[c]
		OR[a,b]	95% CI	*P*-value		OR[a,b]	95% CI	*P*-value		OR[a,b]	95% CI	*P*-value	
	Frequency of cooked poultry consumption per week												
	≤2 Servings/>2 servings[d]				≤2 Servings/>2 servings[d]				≤2 Servings/>2 servings[d]				
XPC intron 11													
C/C	148/66	1.0[Ref]			96/36	1.0[Ref]			33/20	1.0[Ref]			
C/A + A/A	277/83	0.7	0.5–1.0	0.040	171/47	0.7	0.4–1.2	0.224	69/28	0.7	0.3–1.4	0.267	0.838
	Level of doneness of poultry from outside												
	Light or medium brown/heavily browned[e]				Light or medium brown/heavily browned[e]				Light or medium brown/heavily browned[e]				
XPD Lys751Gln													
Lys/Lys	166/71	1.0[Ref]			110/37	1.0[Ref]			37/26	1.0[Ref]			
Lys/Gln + Gln/Gln	265/71	0.6	0.4–0.9	0.016	160/42	0.8	0.5–1.3	0.335	69/17	0.4	0.2–0.8	0.005	0.075
XPC intron 11													
C/C	151/64	1.0[Ref]			94/38	1.0[Ref]			35/18	1.0[Ref]			
C/A + A/A	281/78	0.7	0.4–1.0	0.031	177/41	0.6	0.3–1.0	0.031	71/25	0.7	0.3–1.4	0.308	0.694

[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 IOR.
[b]Unadjusted.
[c]Colon versus rectum heterogeneity test.
[d]Less than or equal to two servings as referent group.
[e]Light or medium brown as referent group.

associated with a 2.5-fold increase in ultraviolet-induced apoptosis among lymphoblastoid cell lines (24). Interaction with p53 is known to reduce the helicase activity of the XPD gene, and it was hypothesized that inhibition of helicase activity may allow a stable formation of the complex of the damaged DNA and the NER machinery, resulting in a more efficient repair (42). If either *XPD* variant alleles we studied here (Asn^{312} and Gln^{751}), or another SNP in linkage disequilibrium with either of them, reduced p53 binding, this could explain why diets high in well-done or heavily brown red meat may have a detrimental effect among carriers of the more common Asp^{312} or Lys^{751} alleles, as these subjects would be less efficient in removing the damage induced by PAHs and HCAs. The literature for genotype–phenotype association studies for *XPD* SNPs have been inconsistent, therefore no final interpretations can be made regarding our findings (43). Further studies on the functional impact of genetic variants in the *XPD* gene will help better understand our results.

The inherent advantage in this family-based study design is that it reduces the likelihood of confounding by population stratification. The population-based nature of this study is another advantage. Lastly, given that the USC Consortium is one of six members of the Colon-CFR, we will be able in the future to extend these studies to other centers in this large Consortium to validate and expand on our findings. Our study had three main limitations. First, 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, based on our findings, we cannot discard a potential role in CRC risk, or effect modifier role, of those NER genes for which we did not find associations. Furthermore, for the same reason, we are also unable to comment on which steps of the NER pathway might be most important for CRC risk. Second, we did not utilize summary measures of HCAs, PAHs and NOCs to determine which one might explain the association between consumption of cooked meat and CRC. Instead, we used data on frequency of intake of red meat or poultry cooked by panfrying, oven broiling and grilling, which serves as a surrogate measure for the formation of either HCAs or PAHs. The two variables that assessed level of doneness on the inside or outside of the meat may also serve as surrogates for the accumulation of carcinogens. Third, we were unable to investigate potential racial disparities in the role of meat intake, cooking practices and CRC risk, as most of the subjects in our study were white (75% probands). However, we did not find evidence that our main findings differed when restricting analyses to whites or non-whites (non-whites composition: 36% African-American, 34% Hispanics, 12% Asians, 17.5% other racial groups or unknown). Future larger studies using the entire Colon-CFR Consortium will allow us to better address any potential disparities.

In summary, our findings confirm a role for diets high in red meat as CRC risk factor and support the hypothesis that carcinogens that form in red meat heavily brown on the outside might play a role in this association, particularly for rectal cancer. Additional studies using a more comprehensive genetic approach in a larger study population as well as data from prospective studies are needed to confirm our results.

Supplementary material

Supplementary Tables I–III can be found at http://carcin.oxfordjournals.org/

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References

1. WCRF. (2007) *Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective*. World Cancer Research Fund & American Institute for Cancer Research, Washington, DC.
2. Wakabayashi,K. *et al*. (1992) Food-derived mutagens and carcinogens. *Cancer Res.*, **52**, 2092s–2098s.
3. Knize,M.G. *et al*. (1999) Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. *Adv. Exp. Med. Biol.*, **459**, 179–193.

4. Sen,N.P. *et al*. (1976) Nitrosamines in cured meat products. *IARC Sci. Publ.*, 333–342.
5. Forman,D. (1987) Dietary exposure to *N*-nitroso compounds and the risk of human cancer. *Cancer Surv.*, **6**, 719–738.
6. Bingham,S.A. *et al*. (1996) Does increased endogenous formation of *N*-nitroso compounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis*, **17**, 515–523.
7. Bingham,S.A. *et al*. (2002) Effect of white versus red meat on endogenous *N*-nitrosation in the human colon and further evidence of a dose response. *J. Nutr.*, **132**, 3522S–3525S.
8. Lewin,M.H. *et al*. (2006) Red meat enhances the colonic formation of the DNA adduct O6-carboxymethyl guanine: implications for colorectal cancer risk. *Cancer Res.*, **66**, 1859–1865.
9. Butler,L.M. *et al*. (2003) Heterocyclic amines, meat intake, and association with colon cancer in a population-based study. *Am. J. Epidemiol.*, **157**, 434–445.
10. Navarro,A. *et al*. (2004) Meat cooking habits and risk of colorectal cancer in Cordoba, Argentina. *Nutrition*, **20**, 873–877.
11. Nowell,S. *et al*. (2002) 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. *Mutat. Res.*, **506–507**, 175–185.
12. Le Marchand,L. *et al*. (2002) Well-done red meat, metabolic phenotypes and colorectal cancer in Hawaii. *Mutat. Res.*, **506–507**, 205–214.
13. Ito,N. *et al*. (1997) Carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the rat. *Mutat. Res.*, **376**, 107–114.
14. Herbst,U. *et al*. (2006) Malignant transformation of human colon epithelial cells by benzo[*c*]phenanthrene dihydrodiolepoxides as well as 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine. *Toxicol. Appl. Pharmacol.*, **212**, 136–145.
15. Turesky,R.J. (2002) Heterocyclic aromatic amine metabolism, DNA adduct formation, mutagenesis, and carcinogenesis. *Drug Metab. Rev.*, **34**, 625–650.
16. Pfau,W. *et al*. (1999) Heterocyclic aromatic amines induce DNA strand breaks and cell transformation. *Carcinogenesis*, **20**, 545–551.
17. Leong-Morgenthaler,P.M. *et al*. (2001) Comparison of the mutagenic responses of mismatch repair-proficient (TK6) and mismatch repair-deficient (MT1) human lymphoblast cells to the food-borne carcinogen PhIP. *Environ. Mol. Mutagen.*, **38**, 323–328.
18. Miller,K.P. *et al*. (2001) Impact of cellular metabolism on the biological effects of benzo[*a*]pyrene and related hydrocarbons. *Drug Metab. Rev.*, **33**, 1–35.
19. Alexandrov,K. *et al*. (1996) Evidence of anti-benzo[*a*]pyrene diolepoxide-DNA adduct formation in human colon mucosa. *Carcinogenesis*, **17**, 2081–2083.
20. Ramesh,A. *et al*. (2004) Bioavailability and risk assessment of orally ingested polycyclic aromatic hydrocarbons. *Int. J. Toxicol.*, **23**, 301–333.
21. Kyrtopoulos,S.A. (1998) DNA adducts in humans after exposure to methylating agents. *Mutat. Res.*, **405**, 135–143.
22. Sierra,L.M. *et al*. (2001) The importance of distinct metabolites of *N*-nitrosodiethylamine for its *in vivo* mutagenic specificity. *Mutat. Res.*, **483**, 95–104.
23. Khan,S.G. *et al*. (2002) The human XPC DNA repair gene: arrangement, splice site information content and influence of a single nucleotide polymorphism in a splice acceptor site on alternative splicing and function. *Nucleic Acids Res.*, **30**, 3624–3631.
24. Seker,H. *et al*. (2001) Functional significance of XPD polymorphic variants: attenuated apoptosis in human lymphoblastoid cells with the XPD 312 Asp/Asp genotype. *Cancer Res.*, **61**, 7430–7434.
25. Ellison,A.R. *et al*. (2001) Functional analysis of human MLH1 and MSH2 missense variants and hybrid human-yeast MLH1 proteins in *Saccharomyces cerevisiae*. *Hum. Mol. Genet.*, **10**, 1889–1900.
26. Chen,P. *et al*. (2000) Association of an ERCC1 polymorphism with adult-onset glioma. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 843–847.
27. Newcomb,P.A. *et al*. (2007) Colon cancer family registry: an international resource for studies of the genetic epidemiology of colon cancer. *Cancer Epidemiol. Biomarkers Prev.*, **16**, 2331–2343.
28. Haile,R.W. *et al*. (1999) Study-design issues in the development of the University of Southern California Consortium's Colorectal Cancer Family Registry. *J. Natl Cancer Inst. Monogr.*, 89–93.
29. Mickey,R.M. *et al*. (1989) The impact of confounder selection criteria on effect estimation. *Am. J. Epidemiol.*, **129**, 125–137.
30. Excoffier,L. *et al*. (1995) Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol. Biol. Evol.*, **12**, 921–927.
31. Khoury,M.J. *et al*. (1996) Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls!. *Am. J. Epidemiol.*, **144**, 207–213.
32. Benjamini,Y. *et al*. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc.*, **57**, 289–300.
33. Naccarati,A. *et al*. (2007) Sporadic colorectal cancer and individual susceptibility: a review of the association studies investigating the role of DNA repair genetic polymorphisms. *Mutat. Res.*, **635**, 118–145.
34. Chao,A. *et al*. (2005) Meat consumption and risk of colorectal cancer. *JAMA*, **293**, 172–182.
35. Sandhu,M.S. *et al*. (2001) Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. *Cancer Epidemiol. Biomarkers Prev.*, **10**, 439–446.
36. Sinha,R. *et al*. (2005) Meat, meat cooking methods and preservation, and risk for colorectal adenoma. *Cancer Res.*, **65**, 8034–8041.
37. Gunter,M.J. *et al*. (2005) Meat intake, cooking-related mutagens and risk of colorectal adenoma in a sigmoidoscopy-based case-control study. *Carcinogenesis*, **26**, 637–642.
38. Tiemersma,E.W. *et al*. (2004) Risk of colorectal adenomas in relation to meat consumption, meat preparation, and genetic susceptibility in a Dutch population. *Cancer Causes Control*, **15**, 225–236.
39. Murtaugh,M.A. *et al*. (2004) Meat consumption patterns and preparation, genetic variants of metabolic enzymes, and their association with rectal cancer in men and women. *J. Nutr.*, **134**, 776–784.
40. Hansen,R.D. *et al*. (2007) XPA A23G, XPC Lys939Gln, XPD Lys751Gln and XPD Asp312Asn polymorphisms, interactions with smoking, alcohol and dietary factors, and risk of colorectal cancer. *Mutat. Res.*, **619**, 68–80.
41. Berndt,S.I. *et al*. (2006) Genetic variation in the nucleotide excision repair pathway and colorectal cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **15**, 2263–2269.
42. Wang,X.W. *et al*. (1995) p53 modulation of TFIIH-associated nucleotide excision repair activity. *Nat. Genet.*, **10**, 188–195.
43. Benhamou,S. *et al*. (2005) ERCC2/XPD gene polymorphisms and lung cancer: a HuGE review. *Am. J. Epidemiol.*, **161**, 1–14.

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