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# Nucleotide excision repair gene polymorphisms, meat intake and colon cancer risk

Susan E. Steck[a,*], Lesley M. Butler[b], Temitope Keku[c], Samuel Antwi[a], Joseph Galanko[d], Robert S. Sandler[d], Jennifer J. Hu[e]

[a] Department of Epidemiology and Biostatistics, Cancer Prevention and Control Program, Arnold School of Public Health, University of South Carolina, 915 Greene Street, RM 236, Columbia, SC 29208, USA
[b] Cancer Control and Population Sciences, University of Pittsburgh Cancer Institute, UPMC Cancer Pavilion, Suite 4C-466, 5150 Centre Avenue, Pittsburgh, PA 15232, USA
[c] Division of Gastroenterology and Hepatology, School of Medicine, University of North Carolina, 103 Mason Farm Road, 7340-C Medical Biomolecular Research Building, CB#7032, Chapel Hill, NC 27599-7032, USA
[d] Cancer Epidemiology Gastrointestinal Biology and Disease, School of Medicine, University of North Carolina at Chapel Hill, CB#7555, 4157 Bioinformatics Building, Chapel Hill, NC 27599-7555, USA
[e] Sylvester Comprehensive Cancer Center and Department of Public Health Sciences, University of Miami Miller School of Medicine, Miami, FL, 33136, USA

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ABSTRACT

*Purpose:* Much of the DNA damage from colon cancer-related carcinogens, including heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH) from red meat cooked at high temperature, are repaired by the nucleotide excision repair (NER) pathway. Thus, we examined whether NER non-synonymous single nucleotide polymorphisms (nsSNPs) modified the association between red meat intake and colon cancer risk.
*Methods:* The study consists of 244 African-American and 311 white colon cancer cases and population-based controls (331 African Americans and 544 whites) recruited from 33 counties in North Carolina from 1996 to 2000. Information collected by food frequency questionnaire on meat intake and preparation methods were used to estimate HCA and benzo(a)pyrene (BaP, a PAH) intake. We tested 7 nsSNPs in 5 NER genes: *XPC A499V* and *K939Q, XPD D312N* and *K751Q, XPF R415Q, XPG D1104H*, and *RAD23B A249V*. Adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated using unconditional logistic regression.
*Results:* Among African Americans, we observed a statistically significant positive association between colon cancer risk and *XPC 499 AV+VV* genotype (OR = 1.7, 95% CI: 1.1, 2.7, *AA* as referent), and an inverse association with *XPC 939 QQ* (OR = 0.3, 95%CI: 0.2, 0.8, *KK* as referent). These associations were not observed among whites. For both races combined, there was interaction between the *XPC 939* genotype, well-done red meat intake and colon cancer risk (OR = 1.5, 95% CI = 1.0, 2.2 for high well-done red meat and *KK* genotype as compared to low well-done red meat and *KK* genotype, $p_{interaction} = 0.05$).
*Conclusions:* Our data suggest that NER nsSNPs are associated with colon cancer risk and may modify the association between well-done red meat intake and colon cancer risk.


*Abbreviations:* BaP, benzo(a)pyrene; BPDE, benzo(a)pyrene diol epoxide; CI, confidence interval; DiMeIQX, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; HCA, heterocyclic amine; HWE, Hardy Weinberg Equilibrium; LRT, likelihood ratio test; MeIQx, 2-amino-3,8-dimethylimidazo[4.5-f]quinoxaline; NCCCS, North Carolina Colon Cancer Study; NER, nucleotide excision repair; nsSNP, non-synonymous single nucleotide polymorphism; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon; PhIP, 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine; RR, relative risk; XPX, eroderma Pigmentosa.

* Corresponding author. Tel.: +1 803 576 5638; fax: +1 803 576 5624.
*E-mail addresses:* ssteck@sc.edu (S.E. Steck), BUTLERL3@upmc.edu (L.M. Butler), tokeku@med.unc.edu (T. Keku), galanko@med.unc.edu (J. Galanko), rsandler@med.unc.edu (R.S. Sandler), JHu@med.miami.edu (J.J. Hu).

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## 1. Introduction

African Americans have higher colorectal cancer incidence and mortality compared to all other racial/ethnic groups in the U.S. [1]. Differences in socioeconomic status, access to health care, environmental and dietary exposures, and genetic susceptibility may explain the disparities, though the exact contribution of each factor is unknown [2,3]. Red meat intake has been well-studied as a risk factor for colorectal cancer [4]. High red meat intake was associated with a modest increased risk of colorectal cancer in three meta-analyses [5–8].

The hypothesized etiologically relevant components in red meat include heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs), saturated fat, and nitrosamines [9–13]. Heme iron has also been implicated in colon carcinogenesis, though a cohort study found no association between heme iron intake and risk of colorectal cancer in Canadian women [14]. We previously reported statistically significant associations with well-done and pan-fried red meat, as well as the HCA, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx), for colon cancer in the North Carolina Colon Cancer Study (NCCCS), a population-based, case-control study, of African Americans and whites [15].

HCAs and PAHs are carcinogens that can cause bulky DNA lesions. These DNA adducts can be repaired by the nucleotide excision repair pathway (NER). The NER pathway involves multiple factors in damage recognition and damage repair [16–18]. The Xeroderma Pigmentosa Group C (XPC)-RAD23B complex recognizes DNA damage, which is followed by unwinding of the DNA helix around the damaged site by the transcription factor IIH (TFIIH) complex of proteins. The TFIIH complex includes ERCC2/XPD and ERCC3/XPB [19–21]. Next, ERCC5/XPG and ERCC1/XPF make 3′ and 5′ incisions to the lesion [22–24], and the gap is filled by repair synthesis and sealed by DNA ligase [25,26]. Decreased NER capacity in the presence of accumulating DNA damage has been associated with sporadic colorectal cancer [18].

Multiple polymorphisms within genes involved in the NER pathway have been identified [27], many of which have functional significance [17,28,29]. Several of these NER gene nsSNPs have been examined in relation to colorectal cancer risk, with equivocal results thus far [30]. However, few data are available for joint effects of meat intake and NER gene polymorphisms on colon cancer risk [31–34], particularly among African Americans, an underserved population with higher incidence of colon cancer than other racial/ethnic groups [35]. The presence of joint effects would provide support for an underlying mechanism for our previously reported HCA-colon cancer associations that would involve the NER pathway. In the present study, we examined joint effects of meat intake and seven nsSNPS in five genes involved in NER on colon cancer risk. These nsSNPS were selected based on their putative functional impact and previous evidence of colon cancer risk associations. We hypothesize that nsSNPs in genes in the NER pathway modify associations between meat and meat-derived carcinogen (HCAs and PAHs) intake and colon cancer risk, and the extent of the effect modification varies by race.

## 2. Materials and methods

The NCCCS has been described in detail previously [36]. In brief, cases were selected through a rapid ascertainment system [37] established in conjunction with the North Carolina Central Cancer Registry and enrolled within one year of diagnosis. 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 10/01/1996 and 09/30/2000. 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. A total of 643 colon cancer cases (294 African Americans and 349 whites) and 1048 population-based controls (437 African Americans and 611 whites) were enrolled in the study. Cases and controls were frequency matched by race, sex and 5-year age group through a variation of randomized recruitment as previously described [38]. The study was approved by the School of Medicine Institutional Review Board at the University of North Carolina and by equivalent committees at the collaborating hospitals.

A 150-item food frequency questionnaire was used to measure usual dietary intake over the year prior to diagnosis for cases, or year prior to date of interview for controls [39]. The questionnaire was modified to assess individual exposure to dietary carcinogens based on a meat-cooking and doneness module developed by Sinha and Rothman [40]. Details regarding the collection of dietary history and specifically HCA and PAH exposure have been previously described [15]. In brief, meat intake frequency data, cooking method, and level of doneness were used to estimate values of three HCAs [DiMeIQx, 2-amino-3,8-dimethylimidazo[4.5-f]quinoxaline (MeIQx), and 2-amino-1-methyl-6-phenyl-imidazo[4,5-b]pyridine (PhIP)], and one PAH (BaP) using an exposure-index that has been previously described in detail [40,41].

Blood samples were obtained from 86% of cases and 83% of controls. Cases and controls who provided blood samples were more likely to be male, white, and never-smokers ($p < 0.01$), as previously reported [36]. No other differences between those who provided blood samples and those who did not were noted for variables such as age, education level, income, family history of colorectal cancer, or total meat intake. Genomic DNA was extracted using the PureGene® DNA isolation kit (Gentra Systems, Inc., Minneapolis, MN). Seven SNPs in five genes involved in DNA repair were genotyped. The MassARRAY system (Sequenom, Inc., San Diego, CA) was used to determine genotypes. Genotyping was first completed on a panel of 90 DNA samples from Coriell Institute for Medical Research (Camden, NJ) and compared to the reported genotype data on 2 websites: http://www.ncbi.nlm.nih.gov and http://egp.gs.washington.edu. As part of the quality control protocol, four control samples were genotyped with 92 patient samples on each 96-well plate, and study cases and controls were loaded on each plate to minimize systematic bias. The average call rate was >95% for the genotype assay. The concordance rate for the quality control samples was 100% and the concordance rate for the Coriell samples ranged from 91 to 100%. For each genotype, there was a 100% concordance rate for the 4 internal control samples on each plate. Hardy Weinberg Equilibrium (HWE) [42] was examined among controls for each SNP stratified by race using a goodness of fit $\chi^2$ test to compare the observed genotype frequencies with expected genotype frequencies calculated on the basis of the observed allele frequencies.

Unconditional logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) using SAS statistical software version 9.1. All tests were two-sided. Models presented here are adjusted for offset terms to account for randomized recruitment sampling method, used to maintain somewhat equal numbers by age, race and gender categories [43]. Additional adjustment of total energy intake, energy-adjusted fat intake, dietary fiber intake, and total meat intake were made for the main effect of diet and the joint effects. Based on previous findings for smoking history and colon cancer risk in this dataset [38], smoking status was evaluated as a potential confounder but it was ultimately not retained in the final adjusted model because its inclusion did not change effect estimates. Meat intake and meat-derived carcinogen variables were categorized into “low” versus “high” intake, based on median cutpoints within the control population. We examined the