

Meat-derived carcinogens, genetic susceptibility and colorectal adenoma risk

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Abstract Exposure to heterocyclic aromatic amines (HAAs), carcinogens produced when meat is cooked at high temperatures, is an emerging risk factor for colorectal cancer (CRC). In a cross-sectional study of 342 patients undergoing a screening colonoscopy, the role of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx), the three most abundant HAAs found in cooked meats, and total mutagenic activity in cooked meats were examined in relation to colorectal adenoma risk. Given that genetic differences in the

ability to biotransform HAAs and repair DNA are postulated to modify the HAA–CRC relationship, gene–diet interactions were also examined. Among the total study population, no relationships were observed between dietary HAAs or meat mutagenicity, and colorectal adenoma risk; however, in males, positive associations between dietary HAAs/meat mutagenicity exposures and adenoma risk were suggestive of a relationship. In a separate analysis, polymorphisms in *CYP1B1* were found to be associated with colorectal adenoma risk. Additionally, gene–diet interactions were observed for dietary PhIP and polymorphisms in *CYP1B1* and *XPB*, dietary DiMeIQx and *XPB* polymorphisms, and meat mutagenicity exposure and *CYP1B1* polymorphisms. Overall, increased colorectal adenoma risk was observed with higher HAA/meat mutagenicity exposures among those with polymorphisms which confer greater activity to biotransform HAAs and/or lower ability to repair DNA. This research supports the link between dietary HAAs and genetic susceptibility in colorectal adenoma etiology. The vast majority of CRCs arise from colorectal adenomas; thus, the results of this study suggest that changes in meat preparation practices limiting the production of HAAs may be beneficial for CRC prevention.

Keywords Heterocyclic aromatic amines · Colorectal cancer · Colorectal adenoma · Gene–diet interaction · Diet · Meat consumption

Introduction

Colorectal cancer (CRC) is a multifactorial disease determined jointly by genetic susceptibility and exposure to environmental factors. The National Cancer Institute estimates that at least two-thirds of CRCs are potentially

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preventable; as a result, there are tremendous opportunities to help people avoid this cancer (Platz et al. 2000). Epidemiologic evidence has provided support for the association between meat consumption and CRC risk. Extensive research in the fields of epidemiology, toxicology and population genetics has contributed to formulating the hypothesized biologic relationship between meat consumption and the risk of CRC through a focus on meat-derived carcinogens such as heterocyclic aromatic amines (HAAs).

HAAs are carcinogenic compounds formed when meat is cooked at high temperatures. In general, the use of high-temperature cooking methods (pan-frying, oven-broiling and grilling/barbecuing) and increasing degree of meat doneness produce the highest HAA concentrations (Turesky 2007). The three most abundant carcinogenic HAAs formed in meats are 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) (Gooderham et al. 2001; Turesky 2007). These three compounds have been detected in beef, lamb, pork, chicken and fish, especially when chargrilled, fried or roasted (Gooderham et al. 2001; Turesky 2007). PhIP and MeIQx are potent mutagens in bacterial and mammalian cell genotoxicity assays and induce colon tumors in rats; less genotoxicity data are available for DiMeIQx (Dingley et al. 1999; Magagnotti et al. 2000; Nishikawa et al. 2005; Turteltaub et al. 1999).

The underlying carcinogenic processes related to the HAA–CRC relationship can be conceptualized as consisting of several events that may predispose an individual to the development of CRC, including dietary exposure to HAAs through meat consumption, bioactivation of HAAs to form the ultimate carcinogenic metabolites, adduction of the ultimate carcinogenic metabolites to DNA and DNA mutations. Experimental and observational studies support the use of colorectal adenomas (adenomatous polyps) as surrogate end points for CRC; it has been estimated that most cases (70–90 %) of CRC are preceded by colorectal adenomas (Schatzkin and Gail 2002). Generally, increases in colorectal adenoma and/or CRC risk have been found among those who consume diets with high levels of HAAs and those who are genetically susceptible to the carcinogenic potential of HAAs (Cleary et al. 2010; Cotterchio et al. 2008; Ferrucci et al. 2009a; Landi et al. 2005; Trubicka et al. 2010).

Figure 1 presents a simplified model of the hypothesized role of HAAs in CRC etiology. It illustrates the numerous opportunities for polymorphisms within genes involved in the biotransformation of HAAs and DNA repair in contributing to the mutagenicity and carcinogenicity of HAAs. Bioactivation via CYP-mediated *N*-oxidation of HAAs

produces the *N*-hydroxy-HAA species (Turesky 2007). Subsequently, the *N*-hydroxy-HAA species may undergo conjugation to *N*-acetoxy-HAA by *N*-acetyltransferases (NATs) which results in the formation of the ultimate carcinogenic metabolites that react with DNA, resulting in covalent adducts (Dingley et al. 1999; Turesky 2007). DNA adducts are repaired primarily by components of the nucleotide excision repair pathway (NER: xeroderma pigmentosum group A (XPA) and XPD) and to a lesser extent by components in the base excision repair pathway (BER: X-ray repair cross-complementing group 1 (XRCC1)) (Yeh et al. 2005). Left unrepaired, DNA adducts can induce DNA damage; specifically, micronucleus formation, DNA strand breaks and sister chromatid exchanges are markers of DNA damage that have been associated with PhIP and MeIQx exposures, and associations have been observed between these markers and components of the NER (XPD and XPC), BER (XRCC1 and ADP-ribosyltransferase (ADPRT)) and homologous repair (XRCC3) pathways (Cornetta et al. 2006; Godderis et al. 2006; Wang et al. 2010).

The frequent consumption of meats has been linked to an increased risk of CRC. HAA exposure is a potential causal factor, given that epidemiologic studies have shown the highest CRC risk for individuals who consume meats cooked well-done and who harbor either elevated activities of enzymes that bioactivate HAAs or decreased activity of DNA repair proteins (Cleary et al. 2010; Cotterchio et al. 2008; Ferrucci et al. 2009a; Landi et al. 2005; Trubicka et al. 2010). However, the reported associations between dietary factors and genetic polymorphisms remain inconsistent, and the associations cannot confirm the relationship between specific chemical exposures and carcinogenesis. The purpose of this research was to assess the role of HAAs in colorectal carcinogenesis (specifically colorectal adenoma development) and whether this association is modified by variation in genetic susceptibility.

Materials and methods

Study participants recruitment

A cross-sectional study recruited a total of 444 male and female consenting patients, aged 40 to 65, undergoing a screening colonoscopy at a regional endoscopy centre at Hotel Dieu Hospital in Kingston, Ontario, Canada, between 2009 and 2012. Indications for colonoscopy included a positive family history of CRC or adenoma in a first- or second-degree relative, a positive fecal occult blood test result and average risk screening. Exclusion criteria included previous diagnosis of inflammatory bowel disease (ulcerative colitis or Crohn's disease) or known genetic

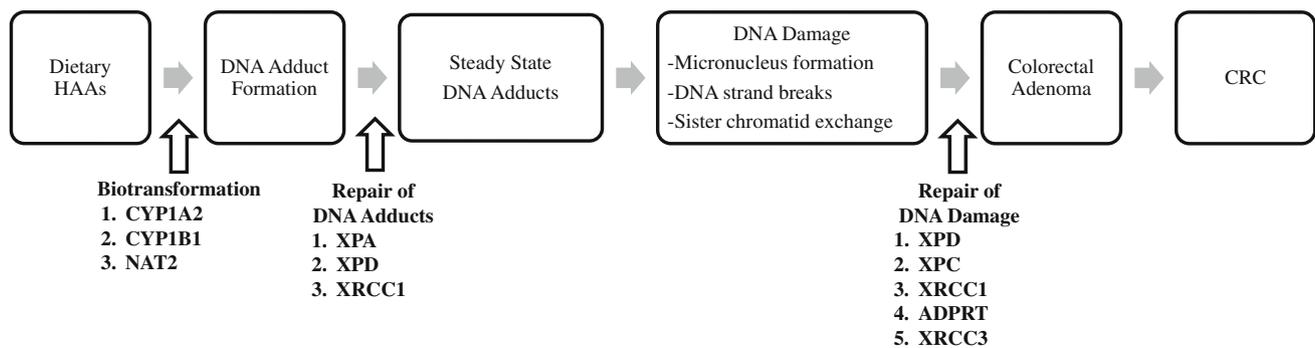


Fig. 1 Conceptual model of the role of HAAs in CRC etiology illustrating the potential gene–diet interactions between polymorphisms within genes involved in the biotransformation of HAAs and DNA repair

disorders that predispose to CRC (hereditary nonpolyposis CRC, familial adenomatous polyposis) or any gastrointestinal abnormality (adenoma, hyperplastic polyps or cancer) detected at a previous colonoscopy. As well, patients with a diagnosis of cancer in the last 5 years (except nonmelanoma skin cancer) were not recruited. Out of the 444 patients who consented to participate, 60 were excluded due to colonoscopy scheduling problems, incomplete colonoscopy (did not reach the cecum), or a diagnosis of inflammatory bowel disease or CRC based on current colonoscopy findings. Thirty-eight subjects with hyperplastic polyps at colonoscopy were also excluded from this study population, as hyperplastic polyps are considered to have less malignant potential than adenomas and may progress to CRC by means of a different pathway than adenomas, yielding a sample size of 346 participants (Liang et al. 2013). Finally, four subjects missing questionnaire data were excluded, yielding a final sample size of 342 participants for this study.

Data collection consisted of (1) a self-administered questionnaire, completed prior to the colonoscopy visit, which included a meat consumption module used to assess average HAA exposure as well as additional personal, lifestyle and dietary factors that have been shown to be related to the development of colorectal adenomas; (2) a fasting blood sample taken at the colonoscopy visit for genotyping and quantification of blood measures of albumin, folate and triglycerides, and (3) pathology reports for outcome assignment.

Exposure assessment of dietary HAAs

Participants completed a meat consumption questionnaire module that collected information on the number of times per day/per week/per month that different meat items were consumed over the previous year, according to the type of cooking method and the usual preferred level of doneness. Meat consumption was assessed separately for summer (April to October) and winter (November to March)

months. Meat items considered included beef steak, hamburger, pork and chicken. Dietary HAA intake over the previous year was then estimated by linking the responses in the meat consumption module to the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease (CHARRED) mutagen database developed by the National Institutes of Health (National Cancer Institute: Division of Cancer Epidemiology and Genetics 2006). In the development of the CHARRED mutagen database, multiple samples of commonly consumed meats were cooked by different methods and to varying degrees of doneness and analyzed for HAA content (PhIP, MeIQx and DiMeIQx) as well as total mutagenic activity. For the CHARRED mutagen database, meat mutagenicity (total mutagenic activity) was quantified using the Ames test (Ames et al. 1975). The Ames test is a widely accepted biologic assay that is used to assess the mutagenic potential of a substance by evaluating its ability to produce genetic damage that leads to gene mutations. Meat mutagenicity as an exposure measure incorporates the mutagenic potential of all classes of carcinogens found in cooked meats including HAAs. Specific estimates of PhIP, MeIQx and DiMeIQx concentrations (expressed in nanograms per gram of meat (ng/g)) and meat mutagenicity (expressed in number of revertant colonies per gram of meat) available in the CHARRED database are quantified into 120 categories that take into account different meat items, cooking methods and doneness levels (National Cancer Institute: Division of Cancer Epidemiology and Genetics 2006).

For each participant, the intake of dietary PhIP, MeIQx and DiMeIQx was derived for each meat item by multiplying the specific HAA content (ng/g) by the serving size (g) and by the frequency of intake per day. For meat mutagenicity, exposure was calculated by multiplying the specific mutagenic activity (expressed in number of revertant colonies per gram of meat) by the serving size (g) and by the frequency of intake per day for each meat item. Serving size information was not collected in this study; thus, the medium serving size for each meat item

provided in the CHARRED mutagen database was utilized in all calculations. Total dietary PhIP, MeIQx and DiMeIQx intake (expressed in ng per day) and meat mutagenicity exposure (expressed in number of revertant colonies per day) were estimated by summing intake across meat items separately for summer and winter months. Average HAA intake and meat mutagenicity exposure over the previous year was then calculated by taking an average of the measures derived from summer and winter months.

Outcome assessment of colorectal adenomas

Any abnormal tissue removed during colonoscopy was assessed using standard diagnostic criteria by an expert gastrointestinal pathologist. Patients with one or more pathologically confirmed tubular, tubulo-villous, serrated or villous adenoma(s) comprised the event group. Patients without any abnormality identified during colonoscopy were assigned to the non-event group.

Genetic polymorphisms

At the colonoscopy visit, a fasting venous blood sample was collected in an ethylenediaminetetraacetic acid-containing vacutainer which was immediately placed on ice and centrifuged within 45 min of the blood draw; the buffy coat (leukocytes) was removed and stored at -20°C . DNA was isolated using 5 Prime ArchivePureTM DNA Blood Kits. Concentration and purity of isolated DNA were determined by Quant-iTTM Picogreen[®] dsDNA assay kit; 327 of 342 participants had adequate DNA for genetic analysis.

Polymorphisms in genes involved in the metabolism of HAAs and DNA repair were genotyped utilizing DNA isolated from blood leukocytes using the MassARRAY[®] iPLEX[®] Gold-SNP Genotyping assay by Genome Québec in Montréal, Québec, Canada. Selection of polymorphisms for genotyping was based on the following priorities: (1) identified polymorphisms with demonstrated effects on biomarkers of HAAs (i.e., HAA-DNA adducts, bulky DNA adducts and HAAs in urine); (2) identified polymorphisms in genetic factors related to the repair of DNA damage induced by HAAs and selecting those with evidence of either a main effect or an interaction with dietary HAAs/meat consumption on CRC risk and; (3) selected polymorphisms with functional evidence demonstrating an effect on protein activity in vitro/in vivo. Due to the limited sample size of this study, candidate polymorphisms were only genotyped if their minor allele frequency was greater than 10 %. Polymorphisms in *CYP1A2* (rs726551), *CYP1B1**2 (rs10012, rs1056827), *NAT2**13 (rs1041983), *XPA* (rs1801280), *XPB* (rs13181, rs1799793), *XRCC1* (rs25487), *ADPRT* (rs1136410), *XPC* (rs2228001) and

XRCC3 (rs861539) were genotyped. Differences between observed and expected genotype frequencies according to Hardy-Weinberg Equilibrium were tested using chi-squared tests among non-events; all polymorphisms with the exception of *CYP1A2* (rs726551), *CYP1B1**2 (rs10012, rs1056827), *XPA* (rs1801280) and *XPB* (rs13181) were in accordance with the expected Hardy-Weinberg Equilibrium distributions ($p > 0.05$). Polymorphisms under investigation in this research may deviate from Hardy-Weinberg Equilibrium, since participants were recruited from an underlying CRC screening cohort that would overly represent those with a family history of CRC.

Statistical analysis

Logistic regression was utilized as the basic framework for the outcome contrast of event (adenoma) versus non-event (healthy) groups. Analyses assessed whether exposure to (a) dietary HAAs and (b) meat mutagenicity, differed between patients with adenomas and individuals with no abnormality detected during colonoscopy.

Categorization of exposures using equal-distant categories was selected as the most appropriate representation. Exposure variables had non-normal distributions (which yielded no natural cut-points), and because of the skew of the distribution, quantile representation would yield categories that may not be meaningful. For each exposure variable, four equal-distant categories were created based on the distribution of the non-event group while ensuring a minimum of 15 % of the study population within each category. Using dietary PhIP as an example, the highest category of PhIP was created by categorizing those above the 75th percentile of the study population as the highest category of PhIP intake (PhIP >348.91 ng/day). Three equal-distant categories were then created by dividing the value of the 75th percentile (348.91 ng/day) by three, yielding three PhIP exposure categories (0 to 116.30, 116.31 to 232.61 and 232.62 to 348.91 ng/day).

Potential covariates were considered if they have been shown in the literature to be related to the development of colorectal adenomas and included sex; age; smoking; physical activity; body mass index (BMI); family history of CRC; alcohol consumption; dietary intakes of energy; fiber; fruit and vegetables; and blood measures of albumin, folate and triglycerides. The selection of a categorical versus continuous representation for continuous covariates was determined by a qualitative assessment of whether the odds ratio (OR) across covariate categories followed a dose-response trend predicting colorectal adenoma risk; when the dose-response trend was considered linear, covariates were modeled utilizing continuous representations; otherwise, categorical representations were selected. With this strategy, continuous representations were chosen

for BMI, dietary fiber intake and serum triglycerides. For age, intake of alcohol, total energy, and fruit and vegetables, physical activity, and serum albumin and folate categorical representations were utilized. Stepwise selection was the main variable selection strategy used to create a parsimonious model of covariates that was predictive of colorectal adenoma risk using a liberal p value of 0.15. Sex, smoking status, dietary intakes of fiber, and fruits and vegetables, and blood levels of albumin and folate were found to be predictive of colorectal adenomas. The main effects of dietary exposure to (a) HAAs and (b) meat mutagenicity, on colorectal adenoma risk, while controlling for the identified predictors, were assessed in separate models using multivariable logistic regression analysis to estimate adjusted ORs and corresponding 95 % confidence intervals (CI).

Given the available sample size, interactions were exploratory since this research had limited statistical power to elucidate an interaction if one existed. First, an interaction analysis with sex was undertaken due to an emerging hypothesis of the inhibiting effects of estrogens on genetic factors involved in the biotransformation of HAAs (Le Marchand et al. 1997; Pollock et al. 1999). Second, gene–diet interactions were examined between (a) dietary HAAs intake and (b) meat mutagenicity exposure, and polymorphisms in genes involved in the biotransformation of HAAs and DNA repair on colorectal adenoma risk. For all genetic polymorphisms, heterozygotes were grouped with the homozygote variant category (based on the dominant model) to maximize statistical efficiency. Polymorphisms in *CYP1B1* (rs10012 and rs1056827) are genetically linked and *XPD* (rs1799793 and rs13181) polymorphisms are in linkage disequilibrium ($p < 0.01$). Thus, the haplotypes of *CYP1B1*, combining genotypes of rs10012 ‘CG or GG’ with rs1056827 ‘GT or TT,’ and *XPD*, combining genotypes rs1799793 ‘AG or AA’ with rs13181 ‘CA or CC,’ were examined in all analyses.

In gene–diet interaction analyses, exposures were represented as dichotomous variables by combining the highest two categories of HAA/meat mutagenicity exposures (e.g., PhIP >232.61 ng/day) and utilizing the lowest two categories of HAA/meat mutagenicity exposures as the referent (e.g., PhIP ≤ 232.61 ng/day). All interactions were explored with the inclusion of cross-product terms in the multivariable logistic regression models.

Results

This study included 342 participants, and the distributions of categorical covariates among those with and without colorectal adenomas were compared and presented in Table 1. Out of the 342 participants, information was

missing for physical activity ($N = 2$), serum albumin ($N = 6$), serum folate ($N = 6$), BMI ($N = 3$) and serum triglycerides ($N = 6$); thus, in bivariate analyses including these variables, analyses are conducted based on the sample size given in Table 1. Statistically significant relationships with adenoma risk were observed for sex, smoking status, serum albumin and folate. For continuous covariates, differences in means were contrasted, and statistically significant differences were observed for dietary intake of fiber and serum triglycerides between non-event and event groups (Table 1).

The multivariable relationships between (a) dietary HAAs and (b) meat mutagenicity exposure, on colorectal adenoma risk, were examined among 336 participants with complete data, while controlling for predictors of colorectal adenoma identified using stepwise regression (Table 2). In the total study population, dietary HAAs or meat mutagenicity exposures were not significantly associated with colorectal adenoma risk. However, positive associations between dietary HAAs/meat mutagenicity exposures and colorectal adenoma risk were suggestive of a relationship among males. For example, higher exposure to meat mutagenicity was associated with a non-significant increase in risk of colorectal adenomas among males but not females (p value for interaction for sex = 0.14).

The total sample size available for the genetic analysis included 327 participants; genotype information was missing for *CYP1A2* ($N = 14$), *CYP1B1**2 (rs10012: $N = 15$; rs1056827 $N = 1$), *NAT2**13 ($N = 2$), *XPD* ($N = 16$), *XRCC1* ($N = 10$), *ADPRT* ($N = 1$), *XPC* ($N = 2$) and *XRCC3* ($N = 10$). In multivariable analyses of the genetic factors related to colorectal adenoma risk, associations were observed with the haplotype representation of *CYP1B1**2 (rs10012 and rs1056827; OR 2.28, 95 % CI 1.29–4.03) (Table 3).

Table 4 presents the gene–diet interaction results for PhIP, DiMeIQx and meat mutagenicity exposures assessed in separate multivariable logistic regression models. For PhIP, interactions with the *CYP1B1**2 (rs10012 and rs1056827; p value for interaction = 0.01) allele and *XPD* polymorphisms (rs13181 and rs1799793; p value for interaction <0.01) on colorectal adenoma risk were observed. Specifically, among those with the *CYP1B1* rs10012 ‘CG or GG’ and rs1056827 ‘GT or TT’ genotypes that confer higher bioactivation activity, higher PhIP intake was associated with an increased risk of colorectal adenomas (OR 1.91; 95 % CI 0.85–4.26); in contrast, among those with *CYP1B1* genotypes which confer lower bioactivation activity (i.e., *CYP1B1* rs10012 ‘CC’ and rs1056827 ‘GG’), higher PhIP intake was associated with a protective effect on colorectal adenoma risk. For the *XPD* (rs13181 and rs1799793) polymorphisms, among genotypes which confer lower DNA repair activity (*XPD*

Table 1 Characteristics of study population among colorectal adenoma events and non-events

Covariate	Categories	$N_{\text{Non-event}} (\%)^a$, Mean \pm SD	$N_{\text{Event}} (\%)^a$, Mean \pm SD	p^b
Sex	Female	151 (69.9)	47 (37.3)	<0.01
	Male	65 (30.1)	79 (62.7)	
Age	39–50	42 (19.4)	19 (15.1)	0.56
	50–60	124 (57.4)	74 (58.7)	
	60+	50 (23.2)	33 (26.2)	
Smoking status	Never	130 (60.2)	53 (42.1)	<0.01
	Past	65 (30.1)	50 (39.7)	
	Current	21 (9.7)	23 (18.2)	
Family history of CRC	Yes	142 (65.7)	72 (57.1)	0.11
	No	74 (34.3)	54 (42.9)	
Physical activity (MET min/week)	$\leq 1,865$	54 (25.0)	35 (28.2)	0.30
	1,866–3,913	54 (25.0)	35 (28.2)	
	3,914–6,363	54 (25.0)	20 (16.2)	
	$\geq 6,364$	54 (25.0)	34 (26.4)	
Alcohol intake (g/day)	Abstainers	37 (17.1)	17 (13.5)	0.38
	Low	118 (54.6)	65 (51.6)	
	Moderate	61 (28.3)	44 (34.9)	
Dietary energy intake (kcal/day)	$\leq 1,017.0$	54 (25.0)	38 (30.2)	0.62
	1,017.0–1,388.5	54 (25.0)	33 (26.2)	
	1,388.5–1,781.6	54 (25.0)	30 (23.8)	
	$\geq 1,781.6$	54 (25.0)	25 (19.8)	
Fruit and vegetable intake (servings per day)	≤ 2.9	55 (25.5)	33 (26.2)	0.07
	3.0–4.1	54 (25.0)	26 (20.6)	
	4.2–6.0	53 (24.5)	46 (36.5)	
	≥ 6.1	54 (25.0)	21 (16.7)	
Serum albumin (g/L)	≤ 40.0	58 (27.2)	33 (26.8)	<0.01
	40.1–42.0	63 (29.6)	18 (14.6)	
	42.1–44.0	52 (24.4)	35 (28.5)	
	≥ 44.1	40 (18.8)	37 (30.1)	
Serum folate (nmol/L)	≤ 26.6	55 (25.8)	28 (22.8)	0.02
	26.7–37.3	52 (24.4)	40 (32.5)	
	37.4–45.3	32 (15.0)	29 (23.6)	
	≥ 45.4	74 (34.7)	26 (21.1)	
Body mass index (kg/m ²)		27.9 \pm 6.2	29.2 \pm 5.9	0.07
Dietary fiber intake (g/day)		27.95 \pm 16.10	24.4 \pm 12.0	0.03
Serum triglycerides (mmol/L)		1.23 \pm 0.71	1.47 \pm 0.89	<0.01

^a Percentages are calculated based on the N of each covariate; missing information on physical activity ($N = 2$), serum albumin ($N = 6$), serum folate ($N = 6$), BMI ($N = 3$) and serum triglycerides ($N = 6$)

^b p value for differences in proportions calculated using chi-squared tests and differences in means were calculated using t tests

rs13181 ‘CC or CA’ and *XPD* rs1799793 ‘AA or AG’ genotypes), higher PhIP intake was associated with a higher risk of colorectal adenomas (OR 1.84; 95 % CI 0.88–3.84). Conversely, protective associations with colorectal risk were observed among those with higher PhIP intake and *XPD* polymorphisms which conferred higher DNA repair activity (i.e., *XPD* rs13181 ‘AA’ and rs1799793 ‘GG’ genotypes). Similar gene–diet interactions were observed for DiMeIQx and *XPD* polymorphisms (p value for interaction = 0.05); for meat mutagenicity, gene–diet interactions were found with the *CYP1B1**2 allele (rs10012 and rs1056827: p value for interaction

<0.01). No significant interactions were observed for MeIQx (data not shown).

Discussion

The findings of this research support the roles of dietary HAA intake, meat mutagenicity exposure and genetic polymorphisms relevant to the carcinogenic potential of HAAs, as contributors to colorectal adenoma risk. First, there were suggestive positive associations between dietary HAAs/meat mutagenicity exposures and colorectal

Table 2 Colorectal adenoma risk associated with dietary HAA intake and exposure to meat mutagenicity in the total study population and separately among females and males

	Categories ^a	N ^b	Total adjusted OR ^c (95 % CI)	N ^b	Females adjusted OR ^c (95 % CI)	N ^b	Males adjusted OR ^c (95 % CI)
PhIP	≤116.30	55/36	Referent	37/19	Referent	18/17	Referent
	116.31–232.61	66/32	0.73 (0.37, 1.44)	43/8	0.44 (0.16, 1.20)	23/24	1.34 (0.52, 3.50)
	232.62–348.91	39/18	0.60 (0.26, 1.35)	27/5	0.32 (0.10, 1.04)	12/13	1.27 (0.39, 4.10)
	>348.92	53/37	0.99 (0.49, 1.97)	41/13	0.56 (0.23, 1.38)	12/24	2.14 (0.74, 6.51)
MeIQx	≤18.54	75/32	Referent	54/12	Referent	21/20	Referent
	18.55–37.09	56/32	1.38 (0.68, 2.78)	38/13	1.94 (0.74, 5.07)	18/19	0.97 (0.36, 2.61)
	37.10–55.63	29/26	2.18 (1.02, 4.68)	19/10	2.39 (0.81, 7.08)	10/16	2.02 (0.68, 5.94)
	>55.64	53/33	1.25 (0.62, 2.50)	37/10	0.94 (0.34, 2.55)	16/23	1.67 (0.63, 4.51)
DiMeIQx	≤2.06	73/37	Referent	54/16	Referent	19/21	Referent
	2.07–4.12	58/29	0.78 (0.39, 1.58)	38/10	0.79 (0.30, 2.08)	20/19	0.79 (0.30, 2.13)
	4.13–6.18	29/22	1.24 (0.57, 2.71)	18/10	1.58 (0.56, 4.46)	11/12	0.97 (0.32, 2.98)
	>6.19	53/35	1.17 (0.59, 2.30)	38/9	0.68 (0.26, 1.79)	15/26	1.98 (0.73, 5.37)
Meat mutagenicity	≤3,589.83	54/29	Referent	40/13	Referent	14/16	Referent
	3,589.84–7,179.67	71/33	0.88 (0.44, 1.78)	47/14	0.96 (0.38, 2.44)	24/19	0.75 (0.31, 2.39)
	7,179.68–10,769.50	35/26	0.98 (0.44, 2.15)	19/8	1.09 (0.36, 3.33)	16/18	1.01 (0.34, 2.98)
	>10,769.51	53/35	1.17 (0.57, 2.42)	42/10	0.63 (0.23, 1.71)	11/25	2.58 (0.84, 7.94)

Bolded text indicate statistically significant relationships

^a Units: ng/day for HAAs and number of revertant colonies/day for meat mutagenicity

^b N for non-events/N for events; total of 336 participants with complete data

^c Adjusted for sex, smoking status, fruit and vegetable intake, dietary fiber intake and biomarker levels of albumin and folate

adenoma risk among males. Second, polymorphisms in *CYP1B1* (rs10012 and rs1056827) were found to be associated with colorectal adenoma risk. Furthermore, gene-diet interactions between dietary exposure to HAAs (PhIP and DiMeIQx) or meat mutagenicity, and polymorphisms in *CYP1B1* (rs10012 and rs1056827) or *XPB* (rs13181 and rs1799793) were observed. Overall, a higher risk of colorectal adenomas was related to higher HAA or meat mutagenicity exposures among those with genetic polymorphisms that confer higher activity for bioactivation of HAAs and/or a lower ability to repair damaged DNA.

Nine studies have evaluated the relationship between dietary exposure to HAAs and colorectal adenoma risk (Ferrucci et al. 2009b; Fu et al. 2011; Gunter et al. 2005; Rohrmann et al. 2009; Shin et al. 2007; Sinha et al. 2001, 2005; Wang et al. 2011a; Wu et al. 2006); five of these studies support a positive relationship and are inconsistent with our results that do not support an independent role of dietary exposure to HAAs in colorectal adenoma development in overall unstratified sex-specific analyses (Ferrucci et al. 2009b; Fu et al. 2011; Rohrmann et al. 2009; Sinha et al. 2001; Wang et al. 2011b). Among the nine studies, six have also investigated the association between meat mutagenicity and CRC/colorectal adenoma risk (Ferrucci et al. 2009b; Fu et al. 2011; Shin et al. 2007; Sinha et al. 2001, 2005; Wu et al. 2006); two support a

positive association, also inconsistent with our results (Fu et al. 2011; Sinha et al. 2001). The lack of an association may indicate a true lack of relationship. Alternatively, null findings may be a result of methodological limitations involved in exposure assessment, or they may be suggestive of the role of effect modification by other dietary constituents, or individual characteristics that obscured the HAA/meat mutagenicity–colorectal adenoma relationships (Rohrmann et al. 2009).

The sex-specific difference in the dietary HAA–CRC relationship has been previously investigated by four studies (Butler et al. 2003; Cross et al. 2010; Le Marchand et al. 2002; Nothlings et al. 2009). Only one case–control study, conducted in Hawaii among 727 matched pairs of CRC cases and controls, reported an interaction with sex with risks being higher among males (Le Marchand et al. 2002). To our knowledge, this study is the first to investigate a sex-specific difference in the relationship between dietary HAA/meat mutagenicity exposures and colorectal adenoma risk; results are suggestive of a positive association among males.

Sex-specific analysis was undertaken due to an emerging hypothesis related to the inhibiting effects of estrogens on genetic factors like *CYP1A2* (Le Marchand et al. 1997; Pollock et al. 1999). Data on the biologic plausibility of the sex-specific associations observed are scarce. However,

Table 3 Colorectal adenoma risk associated with genetic factors

Gene	rs	Genotype category	Functional effect	N ^a	Adjusted OR ^b (95 % CI) ^b	p
CYP1A2	762551	AC/CC	Referent	99/64	Referent	0.36
		AA	Higher bioactivation	108/54	1.28 (0.75–2.17)	
CYP1B1	10012; 1056827	CC + GG	Referent	113/48	Referent	<0.01
		CG/GG + GT/TT	Higher bioactivation	79/59	2.28 (1.29–4.03)	
NAT2	1041983	TT	Intermediate/Slow	109/57	Referent	0.45
		CC/CT	Rapid	99/61	1.22 (0.73–2.06)	
XPA	1800975	CT/TT	Referent	102/59	Referent	0.24
		CC	Lower DNA repair	101/55	0.79 (0.46–1.34)	
XPD	13181;1799793	AA + GG	Referent	102/54	Referent	0.71
		CC/CA + AA/GA	Lower DNA repair	106/64	1.11 (0.66–1.87)	
XRCC1	25487	GG	Referent	87/56	Referent	0.80
		AA/AG	Lower DNA repair	116/58	0.94 (0.55–1.59)	
ADPRT	1136410	TT	Referent	141/78	Referent	0.96
		CC/CT	Lower DNA repair	67/40	1.02 (0.58, 1.77)	
XPC	2228001	AA	Referent	84/33	Referent	0.07
		CC/CA	Lower DNA repair	124/84	1.68 (0.97–2.90)	
XRCC3	861539	CC	Referent	74/47	Referent	0.33
		TT/TC	Lower DNA repair	129/67	0.76 (0.44–1.31)	

Bolded text indicate statistically significant relationships

^a N for non-events/N for events

^b Adjusted for sex, smoking status, fruit and vegetable intake, dietary fiber intake and biomarker levels of albumin and folate

differences in sex-specific risks of CRC associated with cigarette smoking have been reported in the literature. Specifically, the heightened risk of CRC associated with cigarette smoking is predominately restricted to males (Cleary et al. 2010). Estrogen has also been posited to play a protective role against constituents within cigarette smoke, which encompasses genotoxic agents like HAAs, providing support and some emerging hypotheses underlying the sex-specific differences suggested in this research (Slattery et al. 2001, 2003).

Four studies have investigated polymorphisms in *CYP1B1* and CRC risk (Cleary et al. 2010; Cotterchio et al. 2008; Landi et al. 2005; Trubicka et al. 2010), two of which reported an association (Landi et al. 2005; Trubicka et al. 2010). However, it is difficult to compare our results to these two studies since one of the studies did not evaluate *CYP1B1* rs1056827 (Landi et al. 2005) and the other had a different categorization of *CYP1B1* genotypes (Trubicka et al. 2010). This study found effect modification by polymorphisms in *CYP1B1* and *XPD* on the HAA/meat mutagenicity–colorectal adenoma relationships. Specifically, few studies have investigated the modifying effects of this comprehensive subset of polymorphisms on the HAA/meat mutagenicity–colorectal adenoma associations. Five studies with varied overlap in the genetic factors of interest were identified but none investigated *CYP1B1**2 or

XPD polymorphisms (Barbir et al. 2012; Gilsing et al. 2012; Lilla et al. 2006; Tiemersma et al. 2004; Wang et al. 2011c).

The directionalities of the genetic associations observed in this study are consistent with the functional evidence of each polymorphism, hence providing some mechanistic support for the role of HAAs in CRC development through an influence on colorectal adenoma risk. However, the inverse associations observed in gene–diet interaction analysis between higher dietary HAA/meat mutagenicity exposure and colorectal adenoma risk among *CYP1B1* and *XPD* genotypes that conferred lower bioactivation and higher DNA repair activity were unexpected. *CYP1B1* plays a role in the biotransformation of HAAs, and the *CYP1B1**2 (rs10012 and rs1056827) allele have been shown to be associated with lymphocyte DNA adducts (Georgiadis et al. 2004). Specifically, *CYP1B1* functions in the bioactivation of HAAs in extrahepatic tissues leading to the formation of *N*-hydroxy-HAAs which can undergo conjugation to form the ultimate carcinogenic metabolites. Reactive electrophilic metabolites that escape detoxification can form DNA adducts. DNA adducts are repaired predominately by the NER pathway. *XPD* encodes a helicase enzyme that participates in the unwinding of the helix in the region of damaged DNA during NER. The *XPD* polymorphisms (rs13181 and rs1799793) have been

Table 4 Colorectal adenoma risk associated with dietary PhIP and DiMeIQx intake, and meat mutagenicity exposure stratified by genetic factors

Gene	rs	Genotype category	PhIP intake > 232.61 ^a Adjusted OR (95 % CI) ^b	DiMeIQx intake > 4.12 ^a Adjusted OR (95 % CI) ^b	Meat mutagenicity > 7,179.67 ^a Adjusted OR (95 % CI) ^b
CYP1A2	762551	AC/CC	1.50 (0.70–3.23)	1.79 (0.84–3.83)	1.59 (0.75–3.39)
		AA	0.58 (0.27–1.25)	1.08 (0.51–2.27)	0.84 (0.39–1.79)
CYP1B1	10012; 1056827	CC + GG	0.42 (0.18–0.96)^c	0.93 (0.42–2.07)	0.51 (0.22–1.16)^c
		CG/GG + GT/TT	1.91 (0.85–4.26)^c	1.77 (0.80–3.89)	2.68 (1.18–6.09)^c
NAT2	1041983	TT	1.11 (0.53–2.34)	1.36 (0.65–2.85)	1.27 (0.61–2.67)
		CC/CT	0.77 (0.37–1.64)	1.41 (0.66–2.98)	1.04 (0.50–2.18)
XPA	1800975	CT/TT	0.92 (0.46–1.83)	0.95 (0.47–1.90)	1.18 (0.59–2.34)
		CC	0.98 (0.45–2.15)	2.02 (0.94–4.36)	1.09 (0.50–2.39)
XPD	13181;1799793	AA + GG	0.42 (0.19–0.94)^c	0.77 (0.35–1.69)^c	0.75 (0.35–1.63)
		CC/CA + AA/GA	1.84 (0.88–3.84)^c	2.28 (1.01–4.74)^c	1.68 (0.82–3.48)
XRCC1	25487	GG	0.80 (0.36–1.78)	1.83 (0.83–4.05)	1.48 (0.67–3.26)
		AA/AG	1.07 (0.51–2.24)	1.06 (0.52–2.19)	0.97 (0.47–2.01)
ADPRT	1136410	TT	0.92 (0.48–1.77)	1.75 (0.91–3.36)	1.02 (0.53–1.96)
		CC/CT	0.97 (0.39–2.40)	0.89 (0.36–2.22)	1.45 (0.59–3.63)
XPC	2228001	AA	0.90 (0.37–2.22)	1.20 (0.48–2.97)	1.08 (0.43–2.70)
		CC/CA	1.04 (0.53–2.02)	1.58 (0.82–3.05)	1.28 (0.67–2.48)
XRCC3	861539	CC	1.50 (0.62–3.60)	1.25 (0.53–2.96)	2.31 (0.96–5.57)
		TT/TC	0.77 (0.39–1.55)	1.40 (0.71–2.76)	0.85 (0.43–1.69)

Bolded text indicate statistically significant relationships

^a Referent categories: PhIP intake ≤ 232.61 ng/day; DiMeIQx intake ≤ 4.12 ng/day; meat mutagenicity intake $\leq 7,179.67$ number of revertant colonies/day

^b Adjusted for sex, smoking status, fruit and vegetable intake, dietary fiber intake and biomarker levels of albumin and folate

^c p -Interaction ≤ 0.05

investigated in relation to bulky DNA adducts with evidence of an association (Hou et al. 2002; Ketelslegers et al. 2006; Palli et al. 2001); thereby providing support for the role of DNA repair pathways in modifying the HAA–colorectal adenoma relationships.

Limitations and strengths

Potential sources of information bias and the limited statistical power of this research deserve consideration in the interpretation of study findings. First, it is well recognized that questionnaires do not capture diet without some error based on the inaccuracy of recall of past exposures. Limitations in exposure assessment that may have contributed to exposure misclassification include the lack of information on serving size and the limited number of meat items assessed in the study questionnaire. As well, other factors including frequency of turning the meat over during the cooking process, meat thickness, cut of meat, use of marinade or thawing meat in the microwave were not captured in the questionnaire and may have contributed to exposure variability. However, exposure assessment was conducted prior to knowledge of colonoscopy findings; thus, misclassification of exposure would be non-differential.

Second, though one of the main strengths of this research was the inclusion of colonoscopy-confirmed colorectal adenoma status for all participants, the use of colonoscopy findings still has the potential for outcome misclassification. Specifically, adenomas that are smaller are more likely to be missed; thus, the inclusion of patients with small adenomas within the non-event group would introduce non-differential misclassification. To mitigate the effects of outcome misclassification, participants with an incomplete colonoscopy (did not reach the cecum) or for whom the bowel was not sufficiently cleansed (which would have reduced the physician's ability to detect an abnormality) were excluded from this study.

The number of colorectal adenoma cases was smaller among females versus males which may have affected the ability to observe main effects with dietary HAAs and meat mutagenicity exposures in sex-stratified analyses. In addition, it is acknowledged that this study had limited statistical power to examine gene–diet interactions. Though significant interactions between dietary HAAs/meat mutagenicity and polymorphisms in *CYP1B1* and *XPD* on colorectal adenoma risk were observed, our study may have had limited statistical power to investigate additional interactions of smaller magnitudes. In addition, this study

observed suggestive sex-specific differences in the relationship between dietary exposure to HAAs/meat mutagenicity and colorectal adenoma risk. Sex-specific differences in risk may additionally vary according to genetic susceptibility; however, the size of the study sample precluded the ability to assess gene–diet interactions separately among males and females. In this study, no adjustments were made to account for the multiple comparisons involving the evaluation of the role of specific HAAs, meat mutagenicity and genetic risk factors on colorectal adenoma risk. However, the statistical comparisons examined in this research were based on a priori hypotheses driven by experimental and epidemiologic evidence. Although the possibility of chance findings cannot be entirely excluded, the patterns of association observed were consistent across genotype strata and previously reported associations; therefore, the effect of chance related to multiple comparisons cannot entirely explain the study findings.

The study population encompassed patients aged 40 to 65 undergoing a screening colonoscopy; the majority of the participants were white, which may limit the generalizability of the study results. However, this research demonstrated that colorectal adenomas are determined in part through the interaction between diet and genetic predisposition; if the appropriate genetic factors are considered in populations with different ethnic distributions, it is likely that the findings of this research are generalizable. Finally, the use of colorectal adenomas as a surrogate endpoint to CRC has a number of merits and implications. First, adenomas are the precursor to the vast majority of CRCs, and thus, the relationships observed in this study may be generalizable to CRC etiology. Second, adenomas are less symptomatic than CRC, and hence, the cross-sectional measures of dietary HAAs and meat mutagenicity exposures may be less likely to be affected by the disease process and may be more representative of past exposures. Third, the removal of adenomas can prevent future occurrences of CRC. Therefore, understanding the etiology of colorectal adenomas may have the ability to prevent future cases of CRC.

Conclusion

This research provides evidence supporting the role of dietary HAAs and genetic susceptibility to the risk of developing colorectal adenomas. The associations observed in this study are important given the high prevalence of colorectal adenomas in the Canadian population and worldwide. The relationships between HAA exposures and the use of high temperature cooking methods or a preference for higher doneness levels are well-established; thus, changes in meat preparation practices may be more

successful as a public health message for colorectal adenoma and CRC prevention than previous recommendations for general reduction of meat in the diet.

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Conflict of interest Vikki Ho, Sarah Peacock, Thomas E. Massey, Janet E. Ashbury, Stephen J. Vanner, Will D. King declare that they have no conflict of interests.

Compliance with ethics guidelines All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients included in the study.

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