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Red meat consumption, cooking mutagens, *NAT1/2* genotypes, and pancreatic cancer risk in two ethnically diverse prospective cohorts

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Abstract

There is limited evidence on the association between red meat consumption and pancreatic cancer among ethnic minorities. We assessed this relationship in two large prospective cohorts: the Multiethnic Cohort Study (MEC) and the Southern Community Cohort Study (SCCS). Demographic, dietary, and other risk factor data were collected at cohort entry. Red meat intake was assessed using cohort-specific validated food frequency questionnaires. Incident pancreatic cancer cases were identified via linkages to state cancer registries. Cox regression was used to calculate relative risks (RRs) and 95% confidence intervals (CIs) for the association of red meat intake with pancreatic cancer risk in each cohort. We performed additional analyses to evaluate cooking methods, mutagens and effect modification by *NAT1/2* genotypes. From a total of 184,542 (MEC) and 66,793 (SCCS) at-risk participants, we identified 1,618 (MEC) and 266 (SCCS) incident pancreatic cancer cases. Red meat consumption was associated with pancreatic cancer risk in the MEC (RR_{Q4vsQ1} 1.18, 95% CI 1.02–1.37) and with borderline statistical significance in the SCCS (RR_{Q4vsQ1} 1.31, 95% CI 0.93–1.86). This association was significant

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

ETHICS STATEMENT

This study was approved by the Institutional Review Boards of University of Hawaii Cancer Center, University of Southern California, and Vanderbilt University. Participants provided informed consent at cohort entry.

in African Americans (RR_{Q4vsQ1} 1.49, 95% CI 1.06–2.11) and Latinos (RR_{Q4vsQ1} 1.44, 95% CI 1.02–2.04) in the MEC, and among African Americans (RR_{Q4vsQ1} 1.55, 95% CI 1.03–2.33) in the SCCS. *NAT2* genotypes appeared to modify the relationship between red meat and pancreatic cancer in the MEC ($p_{interaction}$=0.03). Our findings suggest that the associations for red meat may be strongest in African Americans and Latinos. The mechanisms underlying the increased risk for these populations should be further investigated.

Keywords

cohort; diet; epidemiology; pancreatic ductal adenocarcinoma

INTRODUCTION

Pancreatic cancer is the third leading cause of cancer mortality in the United States [1] with a five-year survival rate of only 9%[1]. As these poor outcomes are mainly due to late diagnosis, understanding risk factors and biologic mechanisms may improve primary prevention and reduce disease burden. Consumption of red meat has been investigated as a potential risk factor for pancreatic cancer in several epidemiologic studies[2,3]. In a meta-analysis of 16 prospective cohorts and 8 case-control studies, summary statistics revealed positive associations of red meat and pancreatic cancer among case-control studies, but not among cohort studies[3]. Of the individual cohort studies, only five studies[4–8] found an increased risk of pancreatic cancer, including an earlier analysis in the Multiethnic Cohort (MEC) based on 500 cases[4]. However, this analysis was not sufficiently powered to evaluate each ethnic group separately[4]. Additionally, most of the other prior epidemiologic studies were conducted in predominantly white populations, with only the China Kadoorie Biobank[9] and the Black Women's Health Study[10] examining this relationship in minorities. Hence, the association between red meat and pancreatic cancer risk among ethnic minorities is still unclear.

Furthermore, it has been hypothesized that meat preparation and the production of cooking-related mutagens (e.g. heterocyclic aromatic amines [HAA]), may explain the association between red meat intake and pancreatic cancer. While past studies have found evidence of increased risks for grilled/barbecued meat[6,8,11,12], well-done meat[7,8], and some HAAs[6–8,13,14], these factors have not been well investigated in ethnically heterogeneous populations prospectively. Moreover, the carcinogenic impact of red meat and HAAs on the pancreas may be modified by their bioactivation or detoxification by N-acetyltransferase 1 and 2 (NAT1 and NAT2). The enzymatic activity of NAT1 and NAT2 can be assessed by genotyping common variants in *NAT1* and *NAT2*[15,16]. The interaction between meat intake/HAAs and *NAT1/2* genotypes have been examined in relation to other malignancies[17–20], but has not yet been evaluated for pancreatic cancer.

In this study, we sought to investigate the association between red meat consumption and pancreatic cancer risk in two prospective cohorts of ethnically diverse populations: the MEC and the Southern Community Cohort Study (SCCS). Compared to past prospective cohorts, the MEC and SCCS have larger populations of ethnic minorities with elevated pancreatic

cancer risks[21,22] who have been generally understudied in previous literature. The aims of our study were three-fold: first, to provide updated overall and ethnic-specific results in the MEC based on 10+ additional years of follow-up and over three times the number of cases; second, to compare findings with another minority cohort in a separate geographical region with different lifestyles; and lastly, to further elucidate whether cooking preparation, HAAs and *NAT1/2* genotypes contribute to the relationship between red meat and pancreatic cancer.

METHODS

Study population

The MEC and SCCS are prospective cohorts that were established to investigate risk factors and disparities in cancer and other chronic diseases. The MEC consists of 215,000 individuals aged 45–75 from Hawaii and Los Angeles County from five main ethnic groups: African Americans, Japanese Americans, Latinos, Native Hawaiians and whites. The SCCS comprises nearly 86,000 participants from the southeastern United States and has one of the highest proportions of African Americans (two-thirds of cohort) compared to other US-based prospective cohorts. During cohort entry (MEC: 1993–1996, SCCS: 2002–2009), participants completed a baseline questionnaire that included information on demographics, medical history, lifestyle factors, and diet. For the present study, individuals were excluded if they had prevalent cancer (N=21,609) or were missing cancer status (N=2,702) at baseline, were not in the main ethnicity groups (N=4,123), had implausible diet information (N=11,619), or were missing data on major pancreatic cancer risk factors (e.g. body mass index [BMI], smoking, and diabetes) (N=11,724).

Exposure assessment

Red meat consumption was evaluated from the validated baseline food frequency questionnaires (FFQ)[23,24] in both the MEC and SCCS. From the self-reported intake of several food items (Supplemental table 1), we calculated red meat intake densities (grams per 1,000 kcal/day) and analyzed this as both a categorical (cohort-specific quartiles) and continuous (per serving size of 85g/1,000 kcal/day) variable.

Cooking preparation and HAA exposure were assessed through a detailed cooked-meat module on the first follow-up questionnaire (1998–2002) in the MEC. In this module, participants were asked to report the cooking method (pan-fried, oven-broiled, grilled/barbecued), consumption frequency, and doneness level (light/medium/dark brown) of several red meat food items. We then integrated this information with the National Cancer Institute's CHARRED database[25] to estimate the intake of HAAs (2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline [DiMelQx], 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline [MelQx], and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine [PhIP]) for each response. For those who completed the cooked-meat module and were cancer-free at the time of the follow-up questionnaire, we investigated the consumption of meat prepared by each method, consumption of dark brown meat, and intake of HAAs (individual HAAs separately and combined total amount). Consumption of grilled/barbecued

and oven-broiled meat were assessed as dichotomous variables (any vs. none) while all other exposures were assessed as quartiles of intake.

NAT1 and *NAT2* genotypes

To assess *NAT1/2* genotypes, we conducted a nested case-control study of incident pancreatic cancer cases and controls among MEC/SCCS participants who provided a biospecimen sample. Controls were selected using incidence density sampling and matched to cases 1:1 on age at cohort entry, sex, and ethnicity. Genomic DNA was analyzed using the Illumina Multi-Ethnic Genotyping Array (MEGA) chip. Samples went through extensive quality control that included processes such as SNP and sample call rate filtering, intensity checks, assessments of inter- and intra-plate controls, and tests of Hardy-Weinberg equilibrium. Genotyping data were then imputed using ShapeIT v2[26], Minimac3[27] and the 1000 Genomes Project reference panel from the Haplotype Reference Consortium[28]. For two *NAT1/2* SNPs that were imputed, imputed dosages were converted to hard calls.

For NAT1, the *NAT1*10* "increased activity" allele was considered the risk allele and was defined as simultaneously having the variant alleles in two SNPs in *NAT1*: 1088T>A (rs1057126) and 1095C>A (rs15561)[16]. We categorized individuals as having 0, 1 or 2 *NAT*10* alleles.

For NAT2, we evaluated the number of risk slow acetylator alleles (*NAT2*5*, *NAT2*6*, *NAT2*7*, *NAT2*14*) using the signature SNP for each allele in *NAT2*: 341T>C (rs1801280) for *NAT2*5*, 590G>A (rs1799930) for *NAT2*6*, 857G>A (rs1799931) for *NAT2*7*, and 91G>A (rs1801279) for *NAT2*14*[15,29]. Individuals were classified as rapid, intermediate and slow acetylator genotypes if they had zero, one, or two slow *NAT2* alleles, respectively.

Outcome

Individuals were followed from cohort entry to pancreatic cancer diagnosis, death, or end of follow-up (MEC: 12/31/2014; SCCS: 12/31/2016). For the analyses of the MEC cooked-meat module exposures, participants were followed starting from the date of the follow-up questionnaire. Incident pancreatic cancer cases (International Classification of Diseases for Oncology, 3rd Edition [ICD-O-3] codes C25.0-C29.9) were identified using linkages with statewide Surveillance, Epidemiology and End Results (SEER) cancer registries. Mortality information was attained from state cancer registries, state death certificate files and the National Death Index.

Statistical analyses

We used Cox proportional hazards regression with time since cohort entry as the time metric to estimate hazard ratios (reported as relative risks [RRs]) for the associations between red meat consumption, cooking methods, and HAA intake and pancreatic cancer risk. Red meat consumption was assessed as cohort-specific quartiles (Q2, Q3, Q4 vs. Q1) and as number of servings (per 85g/1,000 kcal/day). Intake of red meat prepared by each cooking method was analyzed as quartiles of consumption (Q2, Q3, Q4 vs. Q1), except for grilled/barbecued and oven-broiled meat consumption which were analyzed as dichotomous variables (any vs. none). Intakes of total and each individual HAA were also analyzed as quartiles (Q2, Q3, Q4

vs. Q1). For exposures assessed on the MEC cooked-meat module, the analyses were limited to individuals who did not have cancer at the time of the follow-up questionnaire.

All models included the following covariates assessed at baseline: age (continuous), ethnicity, sex (male vs. female), BMI category (<18, 18–25, 25–30, ≥30 kg/m^2), smoking status (never, former, current), pack years of smoking (continuous), diabetes (yes vs. no), family history of pancreatic cancer (yes vs. no) and log transformed total calories (kcal/day). For the analyses of cooking preparation and HAAs, we further adjusted for total consumption of red meat (grams per 1,000 kcal/day).

For all exposures, we ran stratified analyses across all five ethnic subgroups. For total red meat consumption, we performed further analyses among subgroups defined by age group (<50, 50–59, 60–69, 70+) and sex. Heterogeneity was assessed using a separate model with an interaction term for the exposure and subgroup variable.

We performed a number of sensitivity analyses to minimize residual confounding. First, we ran models that adjusted for other potential confounders, such as alcohol consumption and total fat intake. We also ran models that replaced smoking pack years with smoking duration (years) and amount smoked (cigarettes per day) as separate variables. Results were unchanged in all sensitivity analyses; thus, we only present results from the original models.

For the genetic nested case-control study, only sets with one case and at least one matched control were included in the analysis. We estimated the odds ratios (ORs) for the independent associations of *NAT1/2* genotypes and the joint associations of *NAT1/2* and red meat consumption/HAAs on pancreatic cancer risk using conditional logistic regression. *NAT1* (1 or 2 vs. 0 alleles) and *NAT2* (intermediate or slow vs. rapid) were analyzed as categorical variables in the models evaluating the independent associations of the genotypes. For the interaction models, red meat intake and HAAs were dichotomized by the median value, *NAT1* was dichotomized as ≥1 vs. 0 increased activity alleles, and *NAT2* was dichotomized into intermediate/slow vs. rapid genotypes. Each combination of *NAT1/2* genotype and exposure was assessed in a separate model. All conditional logistic regression models included set number as a strata variable (to account for matching on age, sex and ethnicity) and BMI category, smoking status, smoking pack years, diabetes and family history of pancreatic cancer as covariates. We also adjusted for population stratification using six principal components, which captured most of the ancestry variation from the five ethnic groups[30]. The interaction models with red meat intake were further adjusted for log transformed total calories.

Schoenfeld residuals were used to verify the proportional hazards assumption. All analyses were two-sided (α=0.05) and performed using SAS 9.3 (Cary, NC). Our study was approved by the Institutional Review Boards of University of Hawaii Cancer Center, University of Southern California, and Vanderbilt University. Participants provided informed consent at cohort entry.

RESULTS

After exclusions, the study population consisted of 184,542 individuals from the MEC and 66,793 individuals from the SCCS. The largest ethnic groups in the MEC were Japanese Americans and Latinos, while African Americans comprised nearly 70% of SCCS participants. Compared to MEC participants, SCCS participants were younger and more likely to have pancreatic cancer risk factors (current smoking, obesity, diabetes) and had higher intakes of total calories and red meat (Table 1). There were 1,618 incident cases of pancreatic cancer in the MEC (average follow-up 17.5 years) and 266 cases in the SCCS (average follow-up 10.6).

Red meat intake and pancreatic cancer risk

Red meat intake was highest in Native Hawaiians, Latinos, and African Americans in the MEC, and among whites in the SCCS (Supplemental table 2). In the MEC, the highest quartile of red meat consumption was associated with an elevated risk of pancreatic cancer (RR_{Q4vsQ1} 1.18, 95% CI 1.02–1.37) for all ethnic groups combined (Table 2). While there was no overall heterogeneity across ethnicity ($p_{interaction}$=0.42), the strongest associations were observed in African Americans (RR_{Q4vsQ1} 1.49, 95% CI 1.06–2.11) and Latinos (RR_{Q4vsQ1} 1.44, 95% CI 1.02–2.04). In the SCCS, there was a non-significant positive association between the highest quartile of red meat intake and pancreatic cancer (RR_{Q4vsQ1} 1.31, 95% CI 0.93–1.86). This association was significant for African Americans (RR_{Q4vsQ1} 1.55, 95% CI 1.03–2.33) and not in whites (RR_{Q4vsQ1} 0.82, 95% CI 0.41–1.60), but the test for heterogeneity was not statistically significant ($p_{interaction}$=0.65). Similar patterns of association were detected when examining servings of red meat (Table 2). Moreover, we did not observe significant heterogeneity across age subgroups or sex in either cohort (Supplemental table 3).

Cooking preparation, heterocyclic aromatic amines and pancreatic cancer risk

In the MEC, 146,192 individuals completed the cooked-meat module and were at-risk for pancreatic cancer at the time of the follow-up questionnaire. Among these individuals, there were 1,165 incident cases of pancreatic cancer (average follow-up 12.9 years). Consumption of pan-fried meat was the most common across all participants, while intake of dark brown meat and HAAs was highest among Latinos, Native Hawaiians, and African Americans (Supplemental table 4).

We observed no significant associations for higher intakes of red meat prepared by any of the cooking methods or HAAs for the entire cohort (Table 3). When evaluating these relationships by ethnicity, we found that pan-fried meat intake was associated with increased pancreatic cancer risk only among African Americans ($p_{interaction}$=0.02). Furthermore, African Americans generally had more pronounced associations across all HAAs, with the strongest associations for DiMeIQx (RR_{Q4vsQ1} 1.51, 95% CI 0.99–2.29, $p_{interaction}$=0.09). The associations for some HAAs among Latinos and Native Hawaiians were also elevated, but were all non-significant (Supplemental table 5).

NAT1, *NAT2* and pancreatic cancer risk

The nested case-control study of participants with genetic data included 724 individuals (362 cases/362 controls) in the MEC and 473 individuals (166 cases/307 controls) in the SCCS. Overall, all *NAT1/2* genotype frequencies were similar to that of prior literature[18,20]. Japanese Americans and Native Hawaiians were more likely to have more copies of *NAT1*10* and the rapid *NAT2* genotype (Supplemental table 6).

We did not detect any independent associations of the *NAT1*10* or *NAT2* genotypes and pancreatic cancer in either the MEC or the SCCS (Table 4). However, we observed a significant interaction between the *NAT2* genotypes and red meat in the MEC ($p_{interaction}$=0.03). Compared to those with the *NAT2* rapid genotype and lower (Q1-Q2) red meat consumption, there was a non-significant reduced risk among individuals with the *NAT2* rapid genotype and higher (Q3-Q4) red meat intake (OR 0.92, 95% CI 0.77–1.10), and a non-significant increased risk for those with the *NAT2* intermediate/slow genotypes and both levels of red meat intake (Q1-Q2: OR 1.04, 95% CI 0.84–1.29; Q3-Q4: OR 1.15, 95% CI 0.87–1.52). There were no other significant interactions with *NAT1/2* and the other exposures (Table 4).

DISCUSSION

In our study, we investigated the relationship between red meat consumption and pancreatic cancer risk in two ethnically diverse prospective cohorts with distinct lifestyle factors and characteristics. We observed that red meat intake was associated with pancreatic cancer in both the MEC and SCCS, namely among African Americans and Latinos. While we did not find any overall association between cooking methods and HAA intake on pancreatic cancer risk in the MEC, African Americans did have positive associations for pan-fried meat and DiMeIQx. Furthermore, we found that the *NAT2* genotype had a significant interaction with red meat consumption in the MEC.

Our findings are consistent with the increased risk for red meat observed in our previous analysis in the MEC[4]. Compared to our past study, the present analysis has over ten additional years of follow-up and a three-fold increase in the number of cases. This allowed us to perform ethnic-specific analyses with greater statistical power, which illustrated that the overall association in the MEC was driven mainly by the 44–49% increased risk among African Americans and Latinos. Red meat intake was also associated with an elevated risk of similar magnitude (55%) among African Americans in the SCCS, providing further evidence of an association among this particular ethnic group. Furthermore, a recent analysis from the prospective Black Women's Health Study detected a 65% higher risk of pancreatic cancer for red meat intake among older African American women[10]. To our knowledge, our study is the first and largest cohort to show an elevated risk among a large sample of African American and Latino men and women.

We also evaluated the associations between cooking practices and HAAs and pancreatic cancer risk, which has been previously investigated in mainly white populations with conflicting results. Of the ten studies that assessed cooking methods[6–8,11,12,14,31–34], four observed an increased risk for grilled/barbecued meat[6,8,11,12], one detected a non-significant

elevated risk for pan-fried meat[11], and two[7,8] found an association for well-done meat. In regard to HAAs, one case-control study found no association[33], while several others observed increased risks for DiMelQx, MelQx and overall mutagenetic activity[6–8,13,14]. In our current analysis, we observed a higher risk for pan-fried red meat and stronger associations for DiMeIQx among African Americans in the MEC. These results indicate that the elevated pancreatic cancer risk for red meat among African Americans could perhaps be due to mechanisms involving cooking-related mutagens. Though Latinos had the highest intake of HAAs, the associations between HAAs and pancreatic cancer were inconsistent and non-significant. Hence, the biological pathway underlying the increased risk from red meat for this population remains unclear.

In the nested case-control analysis, we found that the *NAT2* genotype had a significant interaction with red meat consumption in the MEC. NAT1 and NAT2 are enzymes that are involved in the bioactivation and detoxification of heterocyclic amines and other carcinogens through N-acetylation and O-acetylation[35]. It has been suggested that individuals with increased activity *NAT1* genotypes and slow acetylator *NAT2* genotypes are at higher risk of several cancers[15]. *NAT1/2* and pancreatic cancer have only been previously evaluated in two prior case-control studies, which observed independent associations of rapid *NAT1* alleles, but not *NAT2* alleles with pancreatic cancer[36,37]. Our present study is the first to evaluate effect modification by *NAT1/2* genotypes for red meat intake and HAAs and pancreatic cancer. The observed interaction for *NAT2* and red meat in the MEC suggests that the harmful influence of red meat consumption may be stronger among those with slower acetylator *NAT2* genotypes. This finding was not replicated in the SCCS, likely because the MEC had a much higher prevalence of the reference *NAT2* rapid genotype from Japanese Americans and Native Hawaiians. As our genetic analysis was limited to a much smaller sample of participants with biospecimens, our results should be validated in larger studies of ethnically diverse individuals.

The major strengths of the current study are the large, ethnically diverse prospective cohorts from distinct regions of the United States. This allowed us to not only minimize recall and selection bias, but also compare associations across multiple minorities with varying lifestyle factors and dietary patterns. In fact, our results show that the positive association for red meat intake is present across two populations despite differences such as the higher BMI and greater intake of calories and fat among SCCS participants. We were also able to update our results from our previous MEC study and provide further information about the demographics and risk factors driving the associations. Furthermore, by investigating cooking variables, HAAs and genetics, we were able to conduct a comprehensive assessment of the potential mechanisms involved in the relationship between red meat and pancreatic cancer. However, all of the dietary, cooking-related and HAA information was self-reported, so measurement error may be a potential issue. Nevertheless, it should lead to non-differential misclassification since the information was collected before cancer diagnosis. We also did not have information on other cooking-associated mutagens that have been associated with pancreatic cancer (e.g. polycyclic aromatic hydrocarbons and advanced glycation end products)[13,14,38]. Lastly, the genetic analysis was based on a much smaller sample and was not adequately powered for ethnic-specific analyses.

Our study provides evidence of an association for red meat intake and pancreatic cancer, particularly among African Americans and Latinos. Pan fried meat and HAAs were also associated with elevated risks for African Americans, indicating a potential carcinogenic mechanism involving cooking mutagens for this population. We further observed that *NAT2* acetylator genotypes may perhaps modify the association between red meat intake and pancreatic cancer. These findings could be useful in developing targeted dietary recommendations for these populations, especially African Americans who have an elevated risk for pancreatic cancer[22]. Further investigations on other biomarkers and genetic pathways are warranted to better elucidate the mechanisms involved in the relationship between red meat consumption and pancreatic cancer incidence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

The de-identified data that support the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations:

BMI	body mass index
CI	confidence intervals
DiMelQx	2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline
FFQ	food frequency questionnaire
HAA	heterocyclic aromatic amines
ICD-O-3	International Classification of Diseases for Oncology, 3rd Edition
MEC	Multiethnic Cohort Study
MEGA	Multi-Ethnic Genotyping Array
MelQx	2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline
NAT1	N-acetyltransferase 1
NAT2	N-acetyltransferase 2
OR	odds ratio

PhIP	2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
RR	relative risk
SCCS	Southern Community Cohort Study
SEER	Surveillance, Epidemiology and End Results

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NOVELTY AND IMPACT

Prior studies in white populations have reported an association between red meat consumption and pancreatic cancer risk. In this study of two ethnically diverse prospective cohorts, we observed that the positive association between red meat and pancreatic cancer was strongest in African Americans and Latinos. The increased risk for African Americans may be explained by higher intakes of cooking-associated mutagens. Furthermore, *NAT2* genotypes appeared to modify the relationship between red meat and pancreatic cancer.

Table 1.

Baseline characteristics of participants from the Multiethnic Cohort (MEC) and the Southern Community Cohort Study (SCCS)

	MEC (N=184,542)	SCCS (N=66,793)
N cases	1,618	266
Follow-up years, mean ± SD	17.5 ± 5.5	10.6 ± 3.0
Age at enrollment, mean ± SD	59.9 ± 8.9	51.9 ± 8.7
Sex, N (%)		
Male	83,586 (45.3)	27,492 (41.2)
Female	100,956 (54.7)	39,301 (58.8)
Ethnicity, N (%)		
White	46,354 (25.1)	20,476 (30.7)
African American	30,728 (16.7)	46,317 (69.3)
Japanese American	53,423 (29.0)	-
Latino	40,626 (22.0)	-
Native Hawaiian	13,411 (7.3)	-
Smoking status, N (%)		
Never	82,847 (44.9)	24,485 (36.7)
Former <20 pack-years	53,364 (28.9)	8,808 (13.2)
Former ≥20 pack-years	18,713 (10.1)	5,756 (8.6)
Current <20 pack-years	15,957 (8.7)	15,158 (22.7)
Current ≥20 pack-years	13,661 (7.4)	12,586 (18.8)
Body mass index, N (%)		
Underweight (<18 kg/m^2)	3,236 (1.8)	809 (1.2)
Normal (18–25 kg/m^2)	73,727 (40.0)	16,221 (24.3)
Overweight (25–30 kg/m^2)	70,860 (38.4)	19,866 (29.7)
Obese (≥30 kg/m^2)	36,719 (19.9)	29,897 (44.8)
Diabetes, N (%)	21,558 (11.7)	14,137 (21.2)
Family history of pancreatic cancer, N (%)	3,174 (1.7)	943 (1.4)
Alcohol intake (drinks/day), N (%)		
0	94,435 (51.2)	30,721 (46.0)
≤ 1 drink	55,315 (30.0)	21,894 (32.8)
> 1 drink	34,792 (18.9)	14,178 (21.2)
Total calories (kcal/day), mean ± SD	2,176.9 ± 1,058.1	2,577.6 ± 1,456.9
Total fat intake (g per 1,000 kcal/day), mean ± SD	33.5 ± 7.8	38.0 ± 7.2
Red meat intake (g per 1,000 kcal/day), mean ± SD	26.03 ± 16.07	48.42 ± 31.38
Red meat intake (g per 1,000 kcal/day), quartile cutoffs		
Quartile 1 (N = MEC: 46,135 | SCCS: 16,698)	0.0 – 14.1	0.0 – 26.3
Quartile 2 (N = MEC: 46,136 | SCCS: 16,698)	14.1 – 23.9	26.3 – 43.3
Quartile 3 (N = MEC: 46,136 | SCCS: 16,699)	23.9 – 35.2	43.3 – 64.5
Quartile 4 (N = MEC: 46,135 | SCCS: 16,698)	35.2 – 216.5	64.5 – 428.7

Table 2.

Association between red meat intake and pancreatic cancer in the MEC and SCCS, among entire cohort and by ethnicity

	Quartile									
	Q1		Q2		Q3		Q4			Per serving
Subgroup	**N cases**	**RR (95% CI)[1]**	**N cases**	**RR (95% CI)[1]**	**N cases**	**RR (95% CI)[1]**	**N cases**	**RR (95% CI)[1]**	**p_{trend}[2]**	**RR (95% CI)[3]**
Multiethnic Cohort										
Entire cohort	361	1 (ref)	453	1.25 (1.08–1.43)	410	1.16 (1.00–1.34)	394	1.18 (1.02–1.37)	0.08	1.33 (1.02–1.74)
Ethnicity										
White	90	1 (ref)	94	1.28 (0.96–1.71)	75	1.20 (0.88–1.64)	65	1.25 (0.89–1.74)	0.23	1.53 (0.82–2.87)
African American	59	1 (ref)	70	1.34 (0.95–1.90)	87	1.71 (1.22–2.38)	81	1.49 (1.06–2.11)	0.01	1.94 (1.14–3.28)
Japanese American	135	1 (ref)	181	1.22 (0.98–1.53)	139	0.97 (0.76–1.23)	109	0.98 (0.76–1.28)	0.47	0.84 (0.49–1.43)
Latino	50	1 (ref)	73	1.29 (0.90–1.85)	69	1.15 (0.80–1.67)	102	1.44 (1.02–2.04)	0.08	1.63 (0.94–2.82)
Native Hawaiian	27	1 (ref)	35	0.93 (0.56–1.54)	40	0.91 (0.55–1.49)	37	0.76 (0.45–1.26)	0.27	0.81 (0.32–2.09)
$p_{interaction}$	0.42									0.48
Southern Community Cohort Study										
Entire cohort	64	1 (ref)	59	0.97 (0.68–1.38)	71	1.20 (0.85–1.70)	72	1.31 (0.93–1.86)	0.07	1.20 (0.86–1.67)
Ethnicity										
White	19	1 (ref)	16	0.82 (0.42–1.61)	18	0.90 (0.47–1.74)	18	0.82 (0.41–1.60)	0.63	0.79 (0.40–1.54)
African American	45	1 (ref)	43	1.02 (0.67–1.56)	53	1.33 (0.89–1.99)	54	1.55 (1.03–2.33)	0.02	1.38 (0.95–2.02)
$p_{interaction}$	0.65									0.27

All Cox models adjusted for age at enrollment, ethnicity, sex, BMI, smoking status, pack years of smoking, pre-existing diabetes, family history of pancreatic cancer and log transformed total calories.

[1] From a model with cohort-specific quartiles as main exposure

[2] From a model treating quartiles as continuous variable coded as consecutive numbers (e.g. 1, 2, 3).

[3] From a model with number of servings of red meat (per 85g/1,000kcal/day) as main exposure

Table 3.

Relative risks for pancreatic cancer for consumption of red meat by cooking method and intake of heterocyclic aromatic amines, among MEC participants who completed the cooked-meat module

	N Cases	RR (95% CI)[1]
Cooking preparation		
Grilled/barbecued meat consumption[2]		
0	641	1 (ref)
0.1 – 471.4	524	1.00 (0.89–1.13)
Oven-broiled meat consumption[2]		
0	652	1 (ref)
0.3 – 685.8	513	1.10 (0.98–1.24)
Pan-fried meat consumption[2]		
Q1 (0.0 – 2.2)	256	1 (ref)
Q2 (2.2 – 7.5)	298	1.08 (0.91–1.28)
Q3 (7.5 – 15.5)	329	1.14 (0.96–1.36)
Q4 (15.5 – 711.1)	282	0.98 (0.82–1.19)
p_{trend}		0.95
Meat doneness		
Dark brown meat consumption[2,3]		
Q1 (0.0 – 1.4)	182	1 (ref)
Q2 (1.4 – 5.5)	207	1.12 (0.91–1.36)
Q3 (5.5 – 13.7)	185	0.98 (0.80–1.21)
Q4 (13.7– 989.9)	167	0.91 (0.73–1.13)
p_{trend}		0.23
Heterocyclic amines		
DiMeIQx[4]		
Q1 (0.0 – 0.0)	307	1 (ref)
Q2 (0.0 – 0.7)	280	1.21 (1.03–1.43)
Q3 (0.7 – 2.3)	288	1.03 (0.87–1.22)
Q4 (2.3 – 398.5)	290	1.06 (0.90–1.26)
p_{trend}		0.88
MeIQx[4]		
Q1 (0.0 – 2.8)	270	1 (ref)
Q2 (2.8 – 17.1)	308	1.09 (0.92–1.29)
Q3 (17.1 – 45.0)	324	1.12 (0.95–1.33)
Q4 (45.0 – 3111.6)	263	0.93 (0.77–1.11)
p_{trend}		0.49
PhIP[4]		
Q1 (0.0 – 3.6)	285	1 (ref)

	N Cases	RR (95% CI)[1]
Q2 (3.6 – 24.3)	298	0.98 (0.83–1.16)
Q3 (24.3 – 77.6)	300	0.99 (0.84–1.18)
Q4 (77.6 – 9771.9)	282	0.97 (0.82–1.16)
p_{trend}		0.81
Total HAAs[4]		
Q1 (0.0 – 8.8)	272	1 (ref)
Q2 (8.8 – 44.9)	305	1.06 (0.90–1.26)
Q3 (44.9 – 126.4)	316	1.10 (0.93–1.30)
Q4 (126.4 – 12105.4)	272	0.98 (0.82–1.17)
p_{trend}		0.91

[1] Cox models adjusted for age at enrollment, ethnicity, sex, BMI, smoking status, pack years of smoking, pre-existing diabetes, family history of pancreatic cancer, log transformed total calories and total red meat intake.

[2] Analyses for meat doneness only performed among individuals who reported this information in the questionnaires

[3] g/100 kcal/day

[4] ng/1000 kcal/day

Table 4.

Odds ratios for the independent effects of *NAT1/2* genotypes and the interaction with red meat intake and HAAs on pancreatic cancer risk

		MEC (N=724)		SCCS (N=473)	
Genotype		**Cases/Controls**	**OR (95% CI)[1]**	**Cases/Controls**	**OR (95% CI)[1]**
Independent effect					
*NAT1*10*					
0		141/145	1 (ref)	58/95	1 (ref)
1		160/163	0.96 (0.68–1.35)	73/153	0.82 (0.50–1.34)
2		61/54	1.12 (0.69–1.80)	35/59	0.92 (0.50–1.69)
p_{trend}			0.76		0.73
NAT2					
Rapid		93/90	1 (ref)	20/26	1 (ref)
Intermediate		147/163	0.95 (0.61–1.47)	65/141	0.62 (0.31–1.24)
Slow		122/109	1.25 (0.77–2.02)	81/140	0.84 (0.41–1.70)
p_{trend}			0.28		0.75
Interaction with red meat					
*NAT1*10*	Red meat				
0	Q1-Q2	71/76	1 (ref)	31/45	1 (ref)
0	Q3-Q4	70/69	1.02 (0.87–1.20)	27/50	1.05 (0.82–1.34)
1 or 2	Q1-Q2	114/111	0.99 (0.84–1.17)	48/114	0.92 (0.73–1.17)
1 or 2	Q3-Q4	107/106	0.95 (0.73–1.23)	60/98	1.10 (0.78–1.55)
$p_{interaction}$			0.45		0.24
NAT2	Red meat				
Rapid	Q1-Q2	55/42	1 (ref)	10/11	1 (ref)
Rapid	Q3-Q4	38/48	0.92 (0.77–1.10)	10/15	0.95 (0.67–1.35)
Intermediate/slow	Q1-Q2	130/145	1.04 (0.84–1.29)	69/148	0.84 (0.60–1.18)
Intermediate/slow	Q3-Q4	139/127	1.15 (0.87–1.52)	77/133	0.97 (0.63–1.48)
$p_{interaction}$			0.03		0.29
Interaction with HAAs[2]					
*NAT1*10*	Total HAAs				
0	Q1-Q2	58/70	1 (ref)		
0	Q3-Q4	66/61	1.01 (0.86–1.20)		
1 or 2	Q1-Q2	97/79	1.02 (0.86–1.22)		
1 or 2	Q3-Q4	101/112	0.89 (0.68–1.16)		
$p_{interaction}$			0.08		
NAT2	Total HAAs				
Rapid	Q1-Q2	43/32	1 (ref)		
Rapid	Q3-Q4	40/49	0.90 (0.74–1.09)		
Intermediate/slow	Q1-Q2	112/117	1.01 (0.81–1.27)		

		MEC (N=724)		SCCS (N=473)	
Genotype		**Cases/Controls**	**OR (95% CI)[1]**	**Cases/Controls**	**OR (95% CI)[1]**
Intermediate/slow	Q3-Q4	127/124	1.08 (0.81–1.46)		
$P_{interaction}$			0.10		

[1]Conditional logistic regression models with set number as a strata variable (to account for matching on age, sex, and ethnicity), and BMI, smoking status, pack years of smoking, pre-existing diabetes, family history of pancreatic cancer, and six principal components as covariates. Interaction models are further adjusted for log transformed total calories.

[2]HAA information only available for MEC participants.