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Dietary Mutagen Exposure and Risk of Pancreatic Cancer

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Abstract

To investigate the association between dietary exposure to food mutagens and risk of pancreatic cancer, we conducted a hospital-based case-control study at the University of Texas M. D. Anderson Cancer Center during June 2002 to May 2006. Atotal of 626 cases and 530 noncancer controls were frequency matched for race, sex and age (±5 years). Dietary exposure information was collected via personal interview using a meat preparation questionnaire. A significantly greater portion of the cases than controls showed a preference to well-done pork, bacon, grilled chicken, and pan-fried chicken, but not to hamburger and steak. Cases had a higher daily intake of food mutagens and mutagenicity activity (revertants per gram of daily meat intake) than controls did. The daily intakes of 2-amino-3,4,8-trimethylimidazo[4,5—*f*]quinoxaline (DiMeIQx) and benzo(a)pyrene (BaP), as well as the mutagenic activity, were significant predictors for pancreatic cancer ($P = 0.008$, 0.031, and 0.029, respectively) with adjustment of other confounders. A significant trend of elevated cancer risk with increasing DiMeIQx intake was observed in quintile analysis ($P_{trend} = 0.024$). Ahigher intake of dietary mutagens (those in the two top quintiles) was associated with a 2-fold increased risk of pancreatic cancer among those without a family history of cancer but not among those with a family history of cancer. Apossible synergistic effect of dietary mutagen exposure and smoking was observed among individuals with the highest level of exposure (top 10%) to PhIP and BaP, $P_{interaction} = 0.09$ and 0.099, respectively. These data support the hypothesis that dietary mutagen exposure alone and in interaction with other factors contribute to the development of pancreatic cancer.

Introduction

Pancreatic cancer is the fourth leading cause of cancer death for both American men and women (1). Because diagnosis usually occurs late in the natural history of the tumor, the mortality rate of pancreatic cancer is approximately equal to its incidence rate (2). The etiology of pancreatic cancer is not well understood, which makes its prevention almost impossible. Novel approaches are needed to identify the genetic and environmental determinants of pancreatic cancer risk.

Cigarette smoking is the only established environmental risk factor for pancreatic cancer (3). Another important suspected risk factor is diet (4). Increased risks have been associated with

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higher consumption of animal protein and fat, and decreased risks have been associated with higher intake of vegetables, fruits, and dietary fibers (5-10). Diet is an important source of carcinogen exposure. For example, nitrosamines, heterocyclic amines (HCA), and polycyclic aromatic hydrocarbons (PAH) can be derived either from natural food or during the process of food preparation (11,12). Several epidemiologic studies have shown an association between higher consumption of smoked meat, grilled or barbecued meat, fried food, and dehydrated or preserved food and risk of pancreatic cancer (13-16). These studies raise the question of whether dietary exposure to nitrosamines, HCA, and PAH play a role in the etiology of pancreatic cancer.

Meat cooked at high temperature is a source of carcinogenic HCA and PAH. The formation of HCA and PAH depends on the meat type and is highest in meats cooked by high-temperature cooking methods, such as barbecuing and in well-done meats (17,18). The major subclass of HCAs found in the human diet comprise the aminoimidazoazaarenes 2-amino-3-methylimidazo[4,5—*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5—*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5—*f*]quinoxaline (MeIQx), 2-amino-3,4,8-trimethylimidazo[4,5—*f*]quinoxaline (DiMeIQx), and 2-amino-1-methyl-6-phenylimidazo [4,5 — *b*]pyridine (PhIP; refs. 19,20). All are mutagenic in Ames Salmonella tests, and most, except DiMeIQx, have been shown to be carcinogenic in animals (21,22). Other than HCA, grilled meats also contain PAH that forms when fat drips onto a heated surface and burns. The smoke formed coats the food with PAH. BaP is perhaps the most extensively studied PAH and is one of the most potent PAH animal carcinogens (23). Animal studies have shown that dietary intake of BaP causes increased levels of tumors at several sites, particularly in the gastrointestinal tract (24). Another PAH compound, dimethylbenzanthracene, has been shown to induce pancreatic ductal adenocarcinomas in rats, and the tumors are histologically similar to those seen in humans (25,26). Epidemiologic studies have associated dietary exposure to HCA and PAH with increased risk for cancers of the colon, breast, prostate, and pancreas (27-31).

Data from animal studies have shown that the pancreas is highly susceptible to HCA exposure. For example, in the rat and dog, oral administration of radiolabeled PhIP resulted in high levels of radioactivity bound to DNA in many organs, the level of DNA adducts being highest in the pancreas (32). In addition, i.v. injection of radiolabeled reactive metabolites of PhIP, *N*-hydroxy-PhIP, or *N*-acetoxy-PhIP into rats resulted in high levels of DNA-PhIP adducts in the pancreas (33). Several HCA compounds have been shown to induce pancreatic tumors or promote tumor growth in experimental animals (34,35). Our previous studies have shown the presence of bulky aromatic DNA adducts and PhIP-DNA adducts in human pancreatic tissues and a higher level of such adducts inpatients with pancreatic cancer compared with those without cancer (36,37). This experimental evidence strongly supports a role of HCA carcinogens in pancreatic carcinogenesis.

To further investigate the associations between dietary exposure to carcinogens and risk of pancreatic cancer, we conducted a hospital-based case-control study at the University of Texas M. D. Anderson Cancer Center during June 2002 to May 2006. We measured the daily intake of three HCAs (MeIQx, DiMeIQx, and PhIP), a PAH (BaP), and a mutagenic index (revertants per gram of daily meat intake) using a previously validated meat preparation questionnaire. The association between dietary mutagen exposure and risk of pancreatic cancer was examined.

Materials and Methods

Study Design and Study Population

The present study is a hospital-based case-control study. Cases were individuals with newly diagnosed and pathologically confirmed primary pancreatic ductal adenocarcinoma

(International Classification of Diseases for Oncology code C25.3, WHO, 2000) and were identified from the Gastrointestinal Center at the University of Texas M. D. Anderson Cancer Center (M. D. Anderson). Controls were noncancer individuals recruited from friends and genetically unrelated family members (usually spouses or in-laws) of patients who were diagnosed with cancers other than pancreatic cancer. Eligible controls were identified in various clinics of M. D. Anderson using a brief screening questionnaire collecting information on demographics, cancer history, state of residence, relationship to the accompanied patient, and willingness to participate in a research project. Cases and controls were recruited consecutively during the period of January 2002 and June 2006. All study participants were U.S. residents who had no prior cancer history (except for non -melanoma skin cancer). Cases and controls were frequency matched by age (± 5 years), gender, and race. Informed consent was obtained from each participant for interview and for a blood sample. The study was approved by the Institutional Review Board of M. D. Anderson.

During the study period (January 2002 to June 2006), a total of 1,294 primary pancreatic adenocarcinoma cases were registered at M. D. Anderson tumor registry, including 593 cases registered during the period of July 2004 to June 2006 and 701 cases in previous years. For our study, a total of 890 potential patients were approached, and 785 (88.2%) consented to participate. The major reasons for nonparticipation included lack of interest (57%), not eligible because of non-English speaking or foreign residency (20%), too sick or too upset to participate (14%), and time constraints (11%). Among the 785 enrolled patients, 76 were excluded because they were later diagnosed with other types of pancreatic disease or other types of cancer; 35 were excluded because of their prior history of cancer; and 48 were excluded because of missing meat preparation questionnaire (response rate: 93.8%). Among the 626 cases included in the current study, 506 were recruited during July 2004 to June 2006. The recruitment rate was low at the initial stage of the pilot study. In the last 2 years, we have reached a recruitment rate of 85% (506 recruited/593 eligible). A total of 702 potential controls were approached, and 540 (76.9%) consented to participate in the study. The major reason for nonparticipation was unwillingness to subject to venipuncture for the blood sample and time constraint. A total of 530 (98.1%) consented control participants completed the meat preparation questionnaire. As a result, a total of 626 eligible cases and 530 controls were ascertained for this study. There is no significant difference in the demographics between the study participants and refusals.

Data Collection

Three questionnaires were used, a structured risk factor questionnaire, a meat preparation questionnaire, and a food frequency questionnaire (FFQ). The first two questionnaires were administered by personal interview in the clinic when study participants were recruited, and the FFQ was self-administered and returned with a postage-paid envelope.

The risk factor questionnaire collected information on demographics, smoking and alcohol exposure, medical history (including pancreatitis and type II diabetes), occupational history, and family history of cancer.

The University of Arizona Cancer Center provided the meat preparation questionnaire used in the current study. Initially, Dr. Rashmi Sinha of the National Cancer Institute (NCI) developed a database of HCA concentrations in commonly consumed meat items cooked by various techniques and degrees of doneness[6] and a questionnaire with some color photographs showing varying degrees of cooked meat, poultry, and fish (38,39). Dr. Elena Martinez from the University of Arizona Cancer Center adopted the NCI questionnaire and modified it into a short, 4-page, 50-item, self-administered, scannable instrument that uses the NCI′s color

[6] http://charred.cancer.gov/

photographs. The output from the questionnaire provides daily consumption of MeIQx, PhIP, DiMeIQx, and BaP, as well as a mutagenic index (revertant colonies/grams of daily meat intake), which is calculated based on the previous laboratory information on the mutagenic activity of sample extracts in the standard plate incorporation assay with *Salmonella typhimurium strain* TA98 (40). It is a measure of total mutagenic potential from meat and, therefore, incorporates all meat-related mutagens. Responses also provide frequency of consumption of meats by doneness. The completed questionnaires were mailed to Arizona for scanning, and the output data were electronically sent back to M. D. Anderson for final analysis.

The FFQ was developed and validated by the Department of Nutrition at the School of Public Health of Harvard University (41). This scannable questionnaire covers responses including 84 foods and 19 questions about food habits and dietary supplements. It also considers the intake of several vitamins and supplements (years of intake and doses per day). The completed questionnaires were mailed to Harvard, and responses to the items were calculated for macronutrients and 76 micronutrients. Because this questionnaire was not implemented at the beginning of our study, 431 of the 626 cases (69%) and 420 of the 530 controls (79%) in the current study had completed the FFQ with a response rate of 70%.

For both the meat preparation questionnaire and FFQ, the recall period for dietary intake was 1 year before their cancer diagnosis for cases or recruitment to the study for controls.

Statistical Analysis

Demographic and risk factor information were compared between cases and controls using Pearson χ^2tests (for categorical variables) and Mann-Whitney rank test (for continuous variables). The daily intake of each dietary mutagen was natural log transformed to achieve a normal distribution before the ANOVA was applied. The correlations between each dietary mutagen and well-done meat item were analyzed using a Spearman correlation.

The associations between dietary mutagen exposure and risk for pancreatic cancer were estimated using unconditional logistic regression with the dietary mutagen variables in the models either as continuous variables or as categorical variables using the quintiles in the control group as cutoffs and the lowest quintile group as the reference. The likelihood ratio test was used to test for the linear trends by comparing models with or without the exposure variables. The individual HCAs and BaP were first modeled independently of each other with the other adjusted non-dietary risk factors. Stepwise regression (backward selection) of all exposure variables with a P value of 0.25 was then run to generate the final multivariable model. The 60th percentile (the 3rd quintile) was selected as the cutoff to dichotomize the dietary mutagen variables. This cutoff was selected based on the Receiver Operating Characteristics curve for the highest sensitivity and specificity to distinguish the cases and controls. The exposure variables were also dichotomized using the 90th percentile control values as the cutoff. All statistical models were adjusted for gender, race (white, Hispanics, African American, and others), age (<50, 51-60, 61-70, >70 years), education (≤high school, college, graduate), smoking (ever versus never smokers), alcohol consumption (never, ≤ or >60 mL of ethanol/day), history of diabetes (yes or no) and pancreatitis (yes or no), and family history of cancer in the first-degree relatives (yes or no). Additionally, in the subgroup of study participants with the FFQ data (431 cases and 420 controls), total caloric intake (kJ/day), total intake (g/day) of protein, animal fat, or total fat was included in the model, as a continuous variable, to test whether there is any measurement error associated with overreporting or underreporting of dietary components.

For detection of possible interactions between HCA exposure and other risk factors, interaction terms were generated using the product of the exposure variable and other risk factor variables. Likelihood ratio tests were used to compare models with and without the interaction terms. All

statistical analyses used SPSS 12.0 (SPSS Inc., Chicago, IL) and Stata 9.0 (College Station, TX) software programs.

Results

The mean (±SD) age of the cases (n = 626) and controls (n = 530) was 62.0±9.9 and 61.4±10.0 years, respectively (P = 0.30). As shown in Table 1, cases and controls were similar in age distribution, but there were slightly less minorities among controls and more males compared with cases. Compared with cases, the controls had fewer persons with less than high school education (9.7% versus 2.5%) and more individuals with a college education (48.7% versus 57.7%). Ever smoking, heavy drinking (daily alcohol consumption >60 mL of ethanol), history of diabetes, history of pancreatitis, and family history of cancer in first-degree relatives were all significantly associated with having pancreatic cancer in this study population (Table 1).

Cases, when compared with controls, had a significantly higher frequency of consuming well-done pork, bacon, grilled chicken, and pan-fried chicken (Table 2). For example, 56.6% of the cases versus 48.5% of the controls had their bacon well done (P = 0.009) and 38.1% of the cases versus 26.7% of the controls preferred their grilled chicken well-done (P < 0.001). There were no significant differences in the doneness for steak and hamburger between cases and controls.

The dietary mutagens consumed in the highest quantity within the control group were PhIP and then BaP and MeIQx (Table 3). The median level of PhIP, MeIQx, BaP, and DiMeIQx among controls was 118.4, 32.6, 37.3, and 1.8 ng/day, respectively, which were comparable to previously reported values in studies conducted among U.S. populations (26-30). The daily intake of the various dietary mutagens and the mutagenic index were significantly correlated. The Spearman correlation coefficients between MeIQx, DiMeIQx, PhIP, BaP, and mutagenic index ranged between 0.27 and 0.84 (all with P < 0.001, data not shown). Within the HCAs, DiMeIQx and MeIQx were highly correlated (r = 0.84), and their correlation with PhIP was 0.49 and 0.53, respectively. BaP intake was highly correlated with PhIP intake (r = 0.72). DiMeIQxand MeIQxwere most highly correlated with well-done hamburger intake (r = 0.28 and 0.35, respectively). PhIP and BaP were highly correlated with consumption of well-done grilled chicken (r = 0.20 and 0.17, respectively). Cases tended to have a higher level of intake of all four dietary mutagens and a higher mutagenic index than controls did, but the difference was statistically significant for DiMeIQx only (Table 3). Among this study population, FFQ data were available from 420 controls and 431 cases. No significant difference in the intakes of total calories, protein, and animal fat or total fat was observed between cases and controls (Table 3).

In multivariate-adjusted regression analyses, the daily intakes of BaP (P = 0.031) and DiMeIQx (P = 0.008), and total mutagenic activity (P = 0.029) as continuous variables, were each associated with a linear increased risk of pancreatic cancer (data not shown). This association was not statistically significant for PhIP (P = 0.206) and MeIQx(P = 0.089). Using stepwise regression (backward selection) at a P value of 0.25, all individual HCAs and BaP were included in the final model; DiMeIQx(P = 0.003) and BaP (P = 0.031) remained as a significant independent predictor for cancer risk with adjustment of all other confounders (data not shown).

Quintile analysis showed that increasing intake of DiMeIQx across quintiles was significantly associated with increased risk of pancreatic cancer (P_{trend}= 0.024), and the AOR (95% CI) was 1.52 (1.03-2.25) for the highest quintile group compared with the lowest quintile group (Table 4). There was a 30% increased risk for pancreatic cancer in the highest quartile of PhIP and BaP intake compared with the lowest quartile, but the difference was not statistically significant (Table 4). The combined mutagenicity of these meat-related mutagens was assessed using a

mutagenic index; this index showed a possible trend of association with pancreatic cancer (P_{trend}= 0.099). Additional adjustment for consumption of total calories, proteins, animal fat, or total fat did not significantly alter the OR estimations; therefore, these variables were not included in the final models.

When food mutagen and mutagenicity variables were dichotomized using the 60th percentiles of control values, those distributed in the top 40% range of exposure (except for PhIP) were each associated with a significantly increased risk for pancreatic cancer (Table 5). Interestingly, a differential effect of food mutagen exposure on risk of pancreatic cancer by the status of family history of cancer was observed. A 2-fold increased risk of cancer was observed among those without a family history of cancer, but not among those with a family history of cancer (Table 5). No significant interaction was observed between food mutagen exposure and other risk factors. However, a possible synergistic effect of food mutagen exposure and smoking was observed among individuals with the top 10% high intake of PhIP and BaP ($P_{interaction}$= 0.090 and 0.099, respectively; data not shown).

Discussion

In this large-scale, hospital-based case-control study, we have shown a significant association between risk of pancreatic cancer and well-done meat consumption, daily intake of food mutagen DiMeIQxand BaP, as well as the mutagenic activity index, a biologically relevant and integrated measure of mutagenicity. Furthermore, we have shown, for the first time, that HCA exposure was associated with increased risk of pancreatic cancer among individuals with a negative family history of cancer, but not among those with a positive family history of cancer. These observations support the hypothesis that dietary mutagen exposure is a possible risk factor for pancreatic cancer.

Previous epidemiologic studies have associated a higher intake of meat products and certain meat cooking methods, e.g., frying or grilling, with increased risk for pancreatic cancer (14-17). The current study and the study conducted by Anderson et al. (31) are the first to show that dietary HCA and PAH carcinogens derived from meat cooked at high temperature are partially responsible for the increased risk of pancreatic cancer. This information has a significant public health impact because the cooking method is a modifiable factor to reduce the risk of cancer.

There are several strengths of the current study. It is one of the largest ongoing case-control studies of pancreatic cancer in the United States. The current sample size (626 cases and 530 controls) provides sufficient statistical power for the detection of an OR of 1.5. It also provides an opportunity to examine the potential interactions between food mutagen exposure and other risk factors. The study has detailed risk factor and dietary exposure information collected by personal interview, and no proxy interview was involved. Although the FFQ was completed in only 75% of the cases and 87% of the controls in this study population, the intakes of total calorie, protein, and fat had only a minimal effect on the risk estimates of dietary exposure to HCA and PAH and were not part of the final model. Because most of the pancreatic cases are diagnosed at late stage, patients are quite ill at diagnosis and many die quickly afterward (within 3 months from the date of diagnosis). A population-based study usually can catch 30% of the total cases, which might introduce some selection bias by missing patients with more severe disease. The current hospital-based case-control study enrolled more than 85% of the patients that are evaluated at M. D. Anderson, which may have a minimal selection bias. However, because M. D. Anderson is a tertiary referral hospital, the patient population of this study may not be the best representative of the U.S. population. Thus, our data need to be confirmed in other study populations.

The current study is subject to the inherited recall bias of any case-control study. Because cancer cases are recruited after their cancer diagnosis, they may recall their usual diet differently from controls. For example, patients with pancreatic cancer may have digestive problem with greasy food, and they may change their diet due to the illness. Therefore, some cancer cases may have reduced consumption of meat and fat after the start of their disease. However, pancreatic cancer is a rapidly progressing disease without any symptom noticeable long before the cancer diagnosis. Thus, dietary information collected 1 year before the cancer diagnosis for cases and 1 year before recruitment to the study for controls should help to reduce the recall bias. Furthermore, we do not believe that the risk estimate would be significantly affected by recall bias in our study because the recall bias could both be toward or away from the null value. We also do not have any reason to believe that the cancer cases would overreport their preference to well-done meat or the method of cooking over the controls. Another source of selection bias could be the potential differences between responders and nonresponders. Our response rates were higher as compared with most studies of this disease. Previous studies in breast cancer have suggested that differences in educational level, race, or dietary factors between responder and nonresponder cases and controls were unlikely to be an important source of bias (42,43), but we could not completely exclude this possibility in our study. Finally, our control subjects had a higher level of education, younger age, and fewer minorities compared with the cases; these differences could be related to a dietary habit of less meat/fat consumption that leads to an overestimated risk of HCA exposure. However, no significant difference in the intakes of total calories, protein, and animal fat or total fat was actually observed between cases and controls in our study. Adjustment of these variables did not significantly alter the OR estimations.

Laboratory studies have shown that the formation of HCA during cooking can be decreased by natural and synthetic antioxidants, by tryptophan or proline, by removing the essential creatine through marinating or brief microwave cooking before frying or broiling, and by decreasing the cooking temperature and increasing the frequency of flipping the meat during cooking (44-46). These sources of HCA variability were not measured in our study and may lead to nondifferential misclassification of HCA exposure, yielding biased estimates in either direction. However, the major sources of exposure variability were measured (e.g., doneness and cooking method), making it unlikely that the association with DiMeIQx or BaP exposure were due to misclassification.

The daily intake levels of MeIQx, DiMeIQx, and BaP in our study population were quite comparable to those reported in studies conducted in other U.S. populations (27-32). The level of PhIP intake was much higher than that reported in the population-based case-control study of pancreatic cancer (31), but was comparable to those reported in studies of other human cancers (27-30). The intake level of individual HCAs and BaP was highly correlated. The strongest association for individual food mutagens with increased risk of pancreatic cancer was seen for DiMeIQx and BaP. The level of DiMeIQx exposure in this study was most related to the doneness of hamburger, whereas BaP was mainly associated with doneness of grilled chicken. DiMeIQx has not been shown to be carcinogenic in animals, and BaP is ubiquitously present in the human environment. It is also known that there are other dietary HCA and PAH carcinogens that are not measured by this study instrument. The current study could not implicate any meat item as the major source of carcinogens or any meat-derived carcinogen as pancreatic carcinogen.

Experimental evidence from laboratory animals and from human pancreatic tissues suggest that the pancreas is highly susceptible to HCA and PAH exposure. For example, both HCA and PAH can induce or promote pancreatic tumors in animals (25,26,34,35). High level of HCA-induced DNA adducts have been detected in pancreatic tissues from animals exposed to HCA (32,33) and from individuals with unknown exposure to HCA (37). Susceptibility of the

pancreas to HCA exposure may be partially determined by the presence of carcinogen-metabolizing enzymes, such as *N*-acetyltransferases, in this tissue (47). We have recently shown, in the same study population used in the current study, that there were significant interactions of CYP1A2 and *N*-acetyltransferase gene polymorphisms with cigarette smoking in modifying the risk for pancreatic cancer (48). In fact, the same enzymatic pathways metabolize the tobacco aromatic amines and the dietary HCAs. It is conceivable that genetic variations in carcinogen metabolism may influence the individual susceptibility to dietary HCA and PAH exposure. The current study observed a possible (not statistically significant) synergistic effect between cigarette smoking and high-level exposure to dietary PhIP and BaP, but not with other HCAs. Both PhIP and BaP are known cigarette carcinogens. Whether the observed association between risk of cancer and exposure to PhIP or BaP in smokers is indeed an addictive dose response effect or a residual effect of smoking needs further investigation.

The observation that food mutagen exposure increased the risk of pancreatic cancer among individuals without a family history of cancer but not among those with a family history of cancer is intriguing. There is no reason to believe that individuals with a family history of cancer would be less susceptible to food mutagen exposure. It is possible that the association between food mutagen exposure and risk of pancreatic cancer may be masked by some unidentified genetic factors in those with a family history of cancer. Another possibility would be that individuals with a family history of cancer were more careful with their diet and, thus, had less exposure to dietary mutagens. However, when we compared the dietary mutagen exposure levels between individuals with and without a family history of cancer, no significant difference was observed among control subjects for any of the exposure variable. In contrast, cases with a family history of cancer had significantly less exposures to dietary mutagens than those without such a history. Thus, it remains unexplained why dietary mutagen exposure did not increase the risk of pancreatic cancer among people with a family history of cancer.

In conclusion, data from this case-control study suggest that HCA and PAH carcinogens derived from meat cooked at high temperature are associated with an increased risk of pancreatic cancer. These observations need to be confirmed in other study populations using similar measures to quantify carcinogen type and food source.

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Table 1

Distribution of selected variables among patients and controls

Variable	Patients (*N* = 626) *n* (%)	Controls (*N* = 530) *n* (%)	*P* value (χ^2)
Age at recruitment (y)			0.35
≤50	82 (13.1)	74 (14.0)	
51-60	176 (28.1)	169 (31.9)	
61-70	242 (38.7)	180 (34.0)	
>70	126 (20.1)	107 (20.2)	
Gender			0.03
Female	282 (45.0)	205 (38.7)	
Male	344 (55.0)	325 (61.3)	
Race			<0.01
Non-Hispanic white	542 (86.6)	492 (92.8)	
Hispanic	33 (5.3)	21 (4.0)	
African American	43 (6.9)	15 (2.8)	
Others	8 (1.3)	2 (0.4)	
Education *			<0.01
<High school	60 (9.7)	13 (2.5)	
High school	159 (25.7)	119 (22.5)	
College	301 (48.7)	306 (57.7)	
Graduate degree	98 (15.9)	92 (17.4)	
Family history of cancer †			<0.01
No	218 (35.1)	235 (44.4)	
Yes	403 (64.9)	293 (55.4)	
History of pancreatitis			<0.01
No	573 (91.5)	524 (98.9)	
Yes	53 (8.5)	6 (1.1)	
History of diabetes			<0.01
No	480 (76.7)	474 (89.4)	
Yes	146 (23.3)	56 (10.6)	
Smoking status			<0.01
Never	259 (41.4)	269 (50.8)	
Ever	367 (58.6)	261 (49.2)	
Alcohol consumption			0.05
Never	268 (42.8)	248 (47.0)	
≤60 g/day	307 (49.0)	255 (48.3)	
>60 g/day	51 (8.1)	25 (4.7)	

* Information on education was missing from eight cases.

† Information is missing from five cases and two controls because of adopted family.

Table 2

Doneness preference for various meat items in cases and controls

Meat Item	Case, *n* (%)	Control, *n* (%)	*P* (χ^2)
Hamburger			
Rare	57 (9.1)	47 (9.9)	
Medium	229 (36.6)	165 (34.3)	
Well-done	339 (54.2)	273 (55.8)	0.70
Steak			
Rare	82 (13.1)	52 (9.9)	
Medium	325 (51.9)	298 (56.7)	
Well-done	219 (35.0)	176 (33.5)	0.14
Pork			
Rare	132 (21.1)	123 (23.3)	
Medium	221 (35.2)	216 (41.0)	
Well-done	273 (43.6)	188 (35.7)	0.02
Bacon			
Rare	98 (15.7)	83 (15.8)	
Medium	173 (27.7)	187 (35.7)	
Well-done	354 (56.6)	254 (48.5)	0.01
Grilled chicken			
Rare	93 (14.9)	105 (20.0)	
Medium	294 (47.0)	280 (53.3)	
Well-done	238 (38.1)	140 (26.7)	<0.01
Pan-fried chicken			
Rare	159 (25.4)	125 (23.8)	
Medium	238 (38.0)	240 (45.6)	
Well-done	229 (36.6)	161 (30.6)	0.03

NOTE: Information was missing from three to seven study participants for different meat items.

Table 3

Dietary mutagen intake and mutagenic activity index in cases and controls

Variable	Cases (*N* = 626)	Controls (*N* = 530)	*P* value*
MeIQx(ng/day)			
Mean ± SD	68.4 ± 92.2	55.8 ± 71.9	0.13
Median (range)	38.6 (16.3-82.3)	32.6 (16.4-65.9)	0.07
DiMeIQx(ng/day)			
Mean ± SD	4.2 ± 7.0	2.9 ± 4.1	0.01
Median (range)	2.0 (0.7-4.8)	1.8 (0.6-3.6)	0.02
PhIP (ng/day)			
Mean ± SD	236.3 ± 448.4	203.5 ± 285.2	0.27
Median (range)	126.4 (58.0-283.7)	118.4 (50.5-251.5)	0.20
BaP (ng/day)			
Mean ± SD	69.9 ± 92.5	61.2 ± 74.9	0.64
Median (range)	42.3 (11.6-91.8)	37.3 (11.6-77.9)	0.19
Mutagenicity (revertants/g/day)			
Mean ± SD	10,658.5 ± 18,445.3	8,108.1 ± 8,948.5	0.14
Median (range)	6,000.1 (2,997.9-11,933.8)	5,465.2 (2,926.1-9,971.6)	0.07
Calories (kcal)			
Mean ± SD	1,879.6 ± 851.2	1,948.4 ± 738.7	0.21
Median (range)	1,789.0 (1,380.9-2,375.8)	1,760.5 (1,378.4-2,258.9)	0.47
Protein (g)			
Mean ± SD	85.5 ± 38.9	83.7 ± 36.3	0.48
Median (range)	78.9 (60.3-101.7)	78.3 (58.7-101.4)	0.60
Animal fat (g)			
Mean ± SD	40.9 ± 24.1	38.8 ± 20.7	0.17
Median (range)	36.4 (25.9-50.9)	34.0 (24.1-49.2)	0.18
Total fat (g)			
Mean ± SD	74.0 ± 37.4	74.0 ± 34.2	1.00
Median (range)	65.9 (50.2-91.3)	68.0 (49.8-90.9)	0.68

* *P* values for comparison of the means were from ANOVA of natural log-transformed HCA, BaP, and mutagenicity values. *P* values for comparison of the medians were from Mann-Whitney test.

Table 4

Quintile analysis of dietary mutagen intake and risk of pancreatic cancer

	1*	2	3	4	5	P_{trend}
MeIQx						0.14
Median (case/control)	7.46/7.61	19.42/18.98	35.33/32.60	57.82/55.43	129.77/129.48	
Range (control)	0-13.96	14.13-25.26	25.41-43.64	44.11-78.67	79.34-679.06	
No. (case/control)	138/106	95/106	107/106	120/106	166/106	
OR(95% CI)†	1.00	0.62 (0.42-0.94)	0.73 (0.49-1.09)	0.81 (0.54-1.20)	1.11 (0.75-1.65)	
DiMeIQx						0.02
Median (case/control)	0/0	0.78/0.85	1.74/1.80	3.08/3.02	7.63/7.15	
Range (control)	0-0.34	0.35-1.31	1.31-2.26	2.27-4.03	4.04-33.60	
No. (case/control)	115/107	127/105	91/107	109/105	184/106	
OR (95% CI)	1.00	1.09 (0.73-1.62)	0.73 (0.48-1.10)	0.86 (0.57-1.30)	1.52 (1.03-2.25)	
PhIP						0.22
Median (case/control)	20.10/24.22	64.67/61.48	111.68/118.44	219.76/221.54	496.88/431.48	
Range (control)	0-41.63	41.64-84.41	84.78-158.64	158.93-304.99	305.1-2,667.07	
No. (case/control)	127/106	92/106	138/106	127/106	142/106	
OR (95% CI)	1.00	0.84 (0.56-1.27)	1.37 (0.92-2.02)	1.10 (0.74-1.64)	1.30 (0.87-1.94)	
BaP						0.19
Median (case/control)	1.23/1.55	15.45/16.57	36.98/37.32	68.62/63.56	164.56/156.65	
Range (control)	0-6.35	6.78-26.67	26.94-48.12	48.87-95.21	95.62-695.27	
No. (case/control)	122/106	118/106	100/106	141/106	145/106	
OR (95% CI)	1.00	0.93 (0.62-1.39)	0.80 (0.53-1.21)	1.35 (0.91-2.00)	1.32 (0.89-1.97)	
Mutagenicity						0.10
Median (case/control)	1,419/1,676	3,342/3,392	5,427/5,465	9,119/8,716	18,611/17,006	
Range (control)	0-2,433	2,459-4,332	4,340-6,794	6,903-11,920	11,934-76,495	
No. (case/control)	125/106	105/106	109/106	131/106	156/106	
OR (95% CI)	1.00	0.74 (0.49-1.11)	0.87 (0.58-1.30)	1.03 (0.70-1.54)	1.17 (0.78-1.76)	

*Quintiles of consumption from control group.

†OR adjusted for age, race, sex, education, smoking, alcohol consumption, diabetes, pancreatitis, and family history of cancer.

Table 5

Risk modification of dietary mutagen exposure and family history of cancer (FHxCA)

Variable*	Case/con (*n*/*n*)	All	Negative FHxCA		Positive FHxCA		$P_{interaction}$
		OR(95% CI)†	Case/con	OR (*n*/*n*; 95% CI)	Case/con (*n*/*n*)	OR (95% CI)	
MeIQx(ng/day)							
≤55.43	386/369	1.0	118/161	1.0	268/208	1.0	
>55.43	235/159	1.48 (1.12-1.95)	100/74	1.95 (1.28-2.97)	135/85	1.17 (0.80-1.72)	0.16
DiMeIQx(ng/day)							
≤3.02	421/395	1.0	119/167	1.0	302/228	1.0	
>3.02	238/158	1.39 (1.05-1.83)	99/68	2.17 (1.41-3.34)	139/90	0.99 (0.68-1.94)	0.02
PhIP (ng/day)							
≤221.54	421/370	1.0	139/171	1.0	282/199	1.0	
>221.54	200/158	1.12 (0.85-1.48)	79/64	1.62 (1.05-2.50)	121/94	0.84 (0.58-1.21)	0.05
BaP (ng/day)							
≤63.56	395/371	1.0	127/174	1.0	268/197	1.0	
>63.56	226/157	1.51 (1.15-1.98)	91/61	2.32 (1.50-3.60)	135/96	1.16 (0.81-1.67)	0.02
Mutagenicity (revertants/g/day)							
≤8,716	391/369	1.0	121/169	1.0	270/200	1.0	
>8,716	230/159	1.41 (1.07-1.85)	97/66	2.06 (1.35-3.16)	133/93	1.00 (0.69-1.45)	0.03

*The 60th percentile of the control value was used as the cutoff.

†OR adjusted for age, race, sex, education, smoking, alcohol consumption, diabetes, and pancreatitis.