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ORIGINAL ARTICLE

Phenotypic CYP2A6 Variation and the Risk of Pancreatic Cancer

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ABSTRACT

Objective Cytochrome P450 2A6 (CYP2A6) is an important metabolic enzyme capable of activating several procarcinogens, including dietary and tobacco-specific nitrosamines, which have been linked to pancreatic cancer. Positive associations between high CYP2A6 activity and lung and colorectal cancers have been reported. This is the first investigation of CYP2A6 activity and pancreatic cancer. **Design** In this case-control study of cancer of the exocrine pancreas, phenotypic CYP2A6 activity was measured using a ratio of urinary caffeine metabolites. Demographic, smoking, dietary and medical information were obtained by questionnaire. CYP2A6 phenotype, which is not influenced by smoking status, was measured for 90 cases and 470 controls. **Results** When modeled as a continuous variable, and adjusted for age, sex, race, education, current smoking status and chronic pancreatitis, the odds ratio (OR) per one unit of the natural log of the CYP2A6 ratio was 1.52 (95% confidence interval, CI: 1.09-2.12). In an adjusted categorical analysis, subjects in the uppermost quartile (based on controls) of CYP2A6 activity, when compared to the lower three quartiles, carried an 80% greater risk of pancreatic cancer (OR=1.80; 95% CI: 1.07-3.02). **Conclusions** High levels of CYP2A6 activity, as measured by a caffeine phenotyping assay, were positively associated with pancreatic cancer in this case-control study among a Midwestern U.S. population.

INTRODUCTION

Pancreatic cancer is a relatively rare malignancy but a significant cause of cancer mortality. Approximately 37,680 new cases will be diagnosed in the U.S. in 2008, where it is the fourth leading cause of cancer death among men and women. Prognosis is poor, with only 5% of patients surviving five years after diagnosis [1]. Established risk factors for cancer of the pancreas are age and smoking. Additional risk factors may include obesity, male sex, diabetes, chronic pancreatitis, p16 or BRCA2 germline mutations, and a diet high in meats (which may be linked to intake of well-done fried and grilled meats or, smoked and processed meats) [2, 3, 4].

As pancreatic cancer may be caused in part by nitrosamines from smoking and diet, CYP2A6 activity could play a role in pancreatic carcinogenesis [5]. The cytochrome P450 (CYP) family of enzymes metabolizes drugs and endogenous compounds. CYP2A6 metabolizes coumarin, nicotine and many procarcinogens [6, 7]. Dietary and tobacco-specific nitrosamines, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), are among the pro-carcinogens thought to be activated by CYP2A6-mediated oxidation/hydroxylation [8]. CYP2A6 activity is highly variable between individuals and ethnicities [9]. Additionally, CYP2A6 has been reported to affect smoking behavior [10, 11], though not consistently [12, 13]. Positive and inverse

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Abbreviations 17U: 1,7-dimethyluric acid; 17X: 1,7-dimethylxanthine; 95% CI: 95% confidence intervals; CYP2A6: cytochrome P450 2A6; FFQ: food frequency questionnaire; HPLC: high performance liquid chromatography; OR: odds ratio; NAT2: *N*-acetyltransferase 2; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

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associations of genotypes representing high CYP2A6 activity (fast metabolizers) have been reported with lung [9, 10, 14, 15], esophageal [15, 16] and colorectal cancer [17]. A positive association between colorectal cancer and a high CYP2A6 activity phenotype, measured by a caffeine metabolism assay, has been reported [18, 19, 20].

Previous pharmacogenetic studies have phenotyped subjects using coumarin or caffeine as probe drugs for CYP2A6 activity; [18, 19, 20, 21, 22, 23] here we used a well-characterized caffeine phenotyping assay, as previously described [19, 21]. CYP2A6 catalyzes the hydroxylation of the caffeine metabolite 1,7-dimethylxanthine (17X) to yield 1,7-dimethyluric acid (17U) [20]. The ratio of 17U:17X metabolites in the urine was used to assess CYP2A6 activity, with a higher ratio signifying higher metabolic activity [23, 24, 25].

A case-control study was conducted to examine associations between meat and meat-derived carcinogen intake and pancreatic cancer [26]; a secondary objective was to examine differences in phase I and II metabolizing enzymes as potential risk factors for pancreatic cancer. The objective of this particular analysis was to investigate a potential association between pancreatic cancer and phenotype for CYP2A6, a phase I enzyme. To date, this relationship has not been explored.

MATERIALS AND METHODS

Study Design

A population-based case-control study of cancer of the exocrine pancreas was conducted within a source cohort of the Upper Midwest as previously described [3, 21, 26]. Cases were defined as subjects with cancer of the exocrine pancreas confirmed by histology or cytology or subjects with clinical symptoms consistent with pancreas cancer plus a finding of the "double duct sign" with endoscopic retrograde cholangiopancreatography (ERCP) or findings by computed tomography (CT) of a mass in the pancreas with evidence of liver metastases. Prior to analysis, all pathology reports of potential cases were evaluated for eligibility by a pathologist or confirmed through the Minnesota Cancer Surveillance System, a pathology-based cancer registry. Tumors of the endocrine pancreas, as well as unusual morphologies such as sarcomas and lymphomas, were not eligible. Incident cases were diagnosed between April 1994 and September 1998 and recruited from two sources: all hospitals in the seven-county metropolitan area of Minneapolis and St. Paul, MN, USA; and the Mayo Clinic (Rochester, MN, USA). Cases drawn from the Mayo Clinic included only subjects who lived in the Upper Midwest. Controls were frequency matched to cases by age and sex, and randomly selected from Minnesota driver's license lists for individuals 20-64 years, and from U.S. Health Care Financing Administration (now the Centers for Medicare and Medicaid Services) records for individuals 65 years and older. Of 460 eligible cases contacted, 258 (56.1%) participated in at least some portion of the study. Of these 258 case subjects, 90 (34.9%) participated in the caffeine assay. Of 1,141 eligible controls identified, 676 (59.2%) were enrolled. Of these 676 control subjects, 470 (69.5%) participated in the caffeine assay.

Data Collection

A detailed questionnaire was administered in person to participants. Data on demographics, smoking history, dietary intake and medical history were collected. Subjects were asked to identify themselves as current, former or never smokers. Never smokers were classified as those who had never smoked 100 cigarettes in their lifetime. Smokers were asked about lifetime episodes of smoking history: age at starting (and stopping, if relevant) and the average number of cigarettes smoked per day during each episode. Subjects were asked if they had ever been diagnosed with chronic pancreatitis, diabetes and other conditions. They were also asked for their age at diagnosis. If diagnosis of diabetes was within two years of the diagnosis date of pancreatic cancer among cases or the pseudo-diagnosis date among controls, then subjects were considered to be non-diabetic for analyses. A food frequency questionnaire (FFQ), similar to the Willett FFQ [27], was administered. In addition, information on portion size, preparation methods, and doneness preferences of specific meat items was gathered [26].

The caffeine phenotyping assay was performed as described by Butler *et al.* [28], with minor modifications [21]. The caffeine assay was completed in subjects' homes at a time after the initial consent process and interview; study staff subsequently collected frozen specimens. Subjects who had been told to avoid caffeine for medical reasons, those who reported a high sensitivity to caffeine, and refusals were excluded from the assay. Though the reason for refusal was not routinely collected, controls generally cited the time required for the assay as the reason. For cases, particularly those ascertained at the Mayo Clinic, the assay was not logistically practical. Participating subjects swallowed two 100 mg caffeine tablets following an overnight fast. Participants were instructed not to ingest caffeine- or methylxanthine-containing foods or beverages from midnight before until 5 hours after the caffeine dose. Subjects emptied their bladders at 4 and 5 hours following caffeine ingestion. At 5 hours, a urine specimen was collected and frozen at -4°C for a maximum of 7 days. Specimens were subsequently thawed, adjusted to a pH of 3.5, aliquoted, and stored at -70°C. High performance liquid chromatography (HPLC) was employed to detect and quantify the caffeine metabolites 17U and 17X [21].

ETHICS

Informed consent was obtained from all participants, and the Institutional Review Board of the University of

Minnesota approved this study. The study protocol adhered to the ethical guidelines set form in the World Medical Association Declaration of Helsinki, revised in Tokyo, 2004.

STATISTICS

Cases and controls were compared by using the Fisher's test exact and the linear-by-linear association chi-square. The ratio of 17U:17X was used as an indicator of CYP2A6 activity. The distributions of 17U:17X and the natural logarithm (ln) of 17U:17X were tested for normality among controls by the Shapiro-Wilk test and the Shapiro-Francia test. The Wilcoxon rank sum test and the non-parametric trend test for differences across groups were used to evaluate the relationship between the ratio of 17U:17X and the characteristics of the control subjects. Unconditional logistic regression analyses were conducted, and odds ratios (OR) and 95% confidence intervals (95% CI) were calculated. Both 17U:17X and ln(17U:17X) were modeled as continuous variables; in addition, 17U:17X was modeled also as categorical variable, with category cut points defined by quartiles of the 17U:17X distribution among controls. Squared and cubed terms for 17U:17X and ln(17U:17X) were tested in the continuous models, but their addition did not improve the model fit sufficiently to be retained. Age-adjusted and multivariate-adjusted analyses were performed. Analyses were conducted using Stata analytical software (Stata Corp., College Station, TX, USA). All P values reported are two-sided.

Table 1. Characteristics of pancreatic cancer cases and controls phenotyped for CYP2A6.

	Cases (No. 90)	Controls (No. 470)	P value
Sex:			0.045 [c]
- Males	63 (70.0%)	274 (58.3%)	
- Females	27 (30.0%)	196 (41.7%)	
Race:			0.393 [c]
- White	85 (94.4%)	461 (98.1%)	
- Other	3 (3.3%)	8 (1.7%)	
- Unknown [a]	2 (2.2%)	1 (0.2%)	
Age:			0.350 [d]
- <50 years	11 (12.2%)	57 (12.1%)	
- 50-59 years	22 (24.4%)	85 (18.1%)	
- 60-69 years	19 (21.1%)	131 (27.9%)	
- 70-79 years	33 (36.7%)	140 (29.8%)	
- 80+ years	5 (5.6%)	57 (12.1%)	
Education:			0.004 [d]
- Less than high school	16 (17.8%)	49 (10.4%)	
- High school	30 (33.3%)	125 (26.6%)	
- More than high school	41 (45.6%)	295 (62.8%)	
- Unknown [a]	3 (3.3%)	1 (0.2%)	
Cigarette smoking:			0.013 [d]
- Never	31 (34.4%)	216 (46.0%)	
- Former	39 (43.3%)	204 (43.4%)	
- Current	17 (18.9%)	49 (10.4%)	
- Unknown [a]	3 (3.3%)	1 (0.2%)	
Total meat:			0.034 [d]
- <65 g/day	21 (23.3%)	171 (36.4%)	
- 65-110 g/day	32 (35.6%)	152 (32.3%)	
- >110 g/day	34 (37.8%)	146 (31.1%)	
- Unknown [a]	3 (3.3%)	1 (0.2%)	
Processed meat:			0.181 [d]
- Less than 1 servings/week	24 (26.7%)	135 (28.7%)	
- 1-2.5 servings/week	27 (30.0%)	181 (38.5%)	
- More than 2.5 servings/week	35 (38.9%)	137 (29.1%)	
Unknown [a]	4 (4.4%)	17 (3.6%)	
Diabetes: [b]			0.229 [c]
- No	82 (91.1%)	444 (94.5%)	
- Yes	8 (8.9%)	26 (5.5%)	

[a] Unknown data were excluded from the comparison between cases and controls

[b] Self-reported diabetes diagnosed more than two years prior to the diagnosis date of pancreatic cancer among cases or the pseudo-diagnosis date among controls.

[c] Fisher'e exact test

[d] Linear-by-linear association chi-square

RESULTS

Subject Characteristics

The risk factors observed in the subset of study subjects with phenotype data available for this analysis were very similar to those in the overall study (as previously published) [3, 26]. For example, the respective multivariate-adjusted ORs and 95% CIs in the overall study and current analysis for specified risk factors were as follows: current smoking: 2.0 (1.2-3.3) and 2.0 (1.0-3.9); diabetes: 1.9 (1.2-3.0) and 2.0 (0.9-4.8) and the highest to lowest quintile of mutagenic activity (a measure of meat-derived carcinogenic potential): 2.4 (1.3-4.3) and 2.7 (1.1-6.6). Distribution of potentially confounding characteristics among cases and controls were examined (Table 1). Again the distribution of these factors among phenotyped cases and controls were very similar to those in the overall study for each variable (data not shown). The study population was 97.5% Caucasian (cases: 94.4%, controls: 98.1%). The mean age was 65.5 years among controls and 65.0 years among cases. The proportion of females among controls was significantly greater than among cases and on average, controls were more highly educated and less likely to be smokers than cases. In addition, total meat intake was significantly higher in cases than in controls (P=0.034).

CYP2A6 Activity

The caffeine metabolite ratio 17U:17X ranged from 0.24 to 24.47 (cases: 0.28-24.47, controls: 0.24-23.34). The distributions of 17U:17X and ln(17U:17X) were normal, although the log transformation tempered extreme values.

The mean and median CYP2A6 activities among controls, measured by the urinary 17U:17X ratios, were slightly higher among female *vs*. male controls, though the difference was not statistically significant (Table 2). There were too few non-Caucasians to make substantial conclusions about race and urinary 17U:17X. The 17U:17X ratio increased significantly and monotonically with age among controls (P<0.001). No statistically significant differences in 17U:17X were seen among never, former and current smokers (at the non-parametric analysis only), or among those reporting different levels of smoking intensity. The 17U:17X ratio was higher in diabetics *vs*. non-diabetics

Table 2. CYP2A6 activity, measured by the urinary caffeine metabolite ratio 17U:17X, by subject characteristics among controls.

	Mean	Geom. mean	Median	P [a]	P [b]
All controls (No. 470)	2.39	1.84	1.81	-	-
Sex:				0.113	0.139
- Males (No. 274)	2.27	1.76	1.75		
- Females (No. 196)	2.55	1.95	1.89		
Race: [c]				0.070	0.042
- White (No. 461)	2.41	1.85	1.81		
- Other (No. 8)	1.26	1.15	1.25		
Age:				<0.001	<0.001
- <50 years (No. 57)	1.93	1.59	1.57		
- 50-59 years (No. 85)	1.94	1.61	1.58		
- 60-69 years (No. 131)	2.11	1.69	1.70		
- 70-79 years (No. 140)	2.88	2.09	2.01		
- 80+ years (No. 57)	2.97	2.28	2.20		
Smoking status: [c]				0.029	0.088
- Never (No. 216)	2.26	1.72	1.70		
- Former (No. 204)	2.58	1.96	1.90		
- Current (No. 49)	2.18	1.87	1.89		
Pack/years: [d]				0.411	0.625
- <20 (No. 94)	2.58	2.02	1.81		
- 20-40 (No. 90)	2.47	1.89	1.95		
- >40 (No. 69)	2.46	1.91	1.89		
Diabetes: [a]				0.018	0.003
- No (No. 444)	2.34	1.80	1.75		
- Yes (No. 26)	3.22	2.67	2.42		

Geom.: geometric

[a] P values from linear regression of natural log of 17U:17X with all covariates

[b] Non-parametric analysis. For sex, race and diabetes, P value is from Wilcoxon rank sum test for comparison of two groups; for age, smoking status and pack/years, P value is from non-parametric trend test for differences across groups.

[c] Race and smoking status missing for one control subject.

[d] Among 253 currently or formerly smoking controls reporting pack/years.

among controls (Table 2), but this was not seen among cases (data not shown). Overall, cases had higher mean, median and geometric mean values as compared to controls (3.45 *vs*. 2.39, 2.02 *vs*. 1.81, and 2.19 *vs*. 1.84, respectively).

The age-adjusted OR for association between a 1 unit change ln(17U:17X) and pancreatic cancer was 1.40 (95% CI: 1.03-1.91; Table 3). A multivariate-adjusted model with age, sex, race, education, current smoking status and chronic pancreatitis yielded an OR of 1.52 (95% CI: 1.09-2.12) (Table 3). Based on this model, an individual at the 90th percentile of 17U:17X (4.58) *vs*. an individual at the 10th percentile (0.79) would carry a 4.91-fold greater risk of pancreatic cancer, all other variables being equal. Additional adjustment for diabetes, pack/years of smoking, pack/years squared, total meat intake, processed meat intake, vegetable intake, total energy intake, and physical activity did not substantively alter these findings (data not shown).

To further evaluate potential confounding, we repeated analyses excluding particular subjects. For example, subjects that were non-white and those for whom information on race was not provided were excluded and the results were virtually identical and remained statistically significant. Similarly, exclusion of subjects with chronic pancreatitis or with unknown pancreatitis history, or exclusion of diabetics, did not substantively change results (data not shown).

In a categorical analysis, the dataset was divided into quartiles based on 17U:17X among controls. Although evidence for a linear dose-response relationship was lacking (P=0.112) there was a positive association when comparing subjects in the uppermost category *vs*. the lowest category of CYP2A6 activity (Table 3; multivariate-adjusted OR=1.75; 95% CI: 0.90-3.40), but statistical significance was not reached.

It was of interest to assess potential interactions between CYP2A6 activity and both processed meat intake and cigarette smoking. Processed meat intake alone did not appear to have a significant effect on pancreatic cancer in this dataset (high *vs*. low processed meat intake of the overall sample population: multivariate-adjusted OR=0.91; 95% CI: 0.56-1.50). Although smoking was associated with an increased risk of pancreatic cancer, of the effect of CYP2A6 activity among those with less than 10 pack/years were similar to the effect in those with 10 or more pack years (Table 4; multivariate-adjusted ORs = 2.91; 95% CI: 1.39-6.08; and 2.69; 95% CI: 1.20-6.00, respectively.

DISCUSSION

Our results indicate an increased risk of pancreatic cancer among the highest levels of CYP2A6 metabolic activity, as quantified by a caffeine phenotyping assay in this case-control study. This association was evident in a significant association with ln(17U:17X) modeled as a continuous variable, and also in a non-significant trend in a categorical model of quartiles of activity. We found no evidence for interaction between processed meat intake, smoking, or diabetes and CYP2A6 activity, but our statistical power for these analyses was limited.

Table 3. CYP2A6 phenotype among pancreatic cancer cases and controls.

	Cases	Controls	OR [a] (95% CI)	OR [b] (95% CI)
ln(17U:17X)	-	-	1.40 (1.03-1.91) [c]	1.52 (1.09-2.12) [c]
Quartiles (17U:17X):				
- First (<1.07)	21 (23.3%)	118 (25.1%)	1.00 (Reference)	1.00 (Reference)
- Second (1.07-1.80)	17 (18.9%)	117 (24.9%)	0.82 (0.41-1.63)	0.95 (0.46-1.97)
- Third (1.81-2.76)	19 (21.1%)	117 (24.9%)	0.94 (0.48-1.84)	0.97 (0.47-1.99)
- Fourth (>2.76)	33 (36.7%)	118 (25.1%)	1.61 (0.88-2.97)	1.75 (0.90-3.40)

[a] Adjusted for age (continuous).

[b] Adjusted for age (continuous), education (less than high school, high school, more than high school), gender (male, female), race (white, nonwhite), current smoking status (yes, no), and chronic pancreatitis (yes, no).

[c] OR per 1 unit change of ln(17U:17X)

The caffeine phenotype assay has been used by other investigators to assess activity of metabolic enzymes [18, 19, 20, 21, 23]. Coumarin has also been used as probe drug for metabolic activity and has revealed wide interindividual variation in CYP2A6 phenotype [22], which has not been well explained by genetic polymorphisms [7, 29]. Therefore phenotype assays remain an important tool for the quantification of CYP activity. It has been suggested that the conversion of 17X to 17U may be catalyzed in part by CYP1A2 [23], in which case the assay would not specifically measure CYP2A6 activity. However, CYP1A2 activity is heavily influenced by smoking status [30, 31], whereas we and others have found no association between CYP2A6 (17U:17X) and smoking status [19]. This argues that CYP1A2 does not have an appreciable role in 17X to 17U hydroxylation. Furthermore, Nowell *et al*. reported that CYP2A6 was the only CYP capable of the 17X to 17U conversion at a substrate concentration of 0.1 mM, which closely approximates *in vivo* conditions [19]. Of note, a high level of concordance (98%) between genotype and phenotype status has been reported in an *N*-acetyltransferase 2 (NAT2) model using the caffeine assay [21], giving credence to the assertion that this assay can be a valid marker of genetically determined metabolic enzyme activity.

Thirteen allelic variants of *CYP2A6* have been identified [9]. Three are fully functional, five have decreased enzymatic function, three are inactive, one is hyperactive, and one is likely a misclassified artifact of a CYP pseudogene [9, 32]. Among Caucasians, the wild-type sequences *CYP2A6*1A* and **1B* account for nearly 97% of alleles [9, 33]. However, a wide phenotypic range between individuals within ethnic populations has been observed [6, 9, 22] indicating that environmental factors and/or unrecognized genetic influences may have a role in CYP2A6 phenotypic expression. CYP2A6 genotype distributions and phenotype activity differ considerably between Caucasian, African-American, Chinese and Japanese populations [34, 35]. Our study population consisted largely of Caucasian subjects; therefore, we cannot address potential differential effects between ethnic populations.

A number of studies have investigated a relationship between CYP2A6 phenotype or genotype and cancers of the lung or colon, though results have not shown a consistent pattern of risk. A case-control study in a Japanese population reported that the proportion of inactive, whole deletion alleles was significantly greater in controls vs. cases of lung cancer [14]. Another Japanese case-control study reported decreased risk of lung cancer among subjects with a homozygous *CYP2A6* deletion (*CYP2A6*4/*4*) *vs*. homozygous wild-type (**1A/*1A*; OR=0.23; 95% CI: 0.08-0.67), and also noted that none of their case subjects with squamous cell carcinoma (No. 105) or small cell carcinoma (No. 44), tumor morphologies believed to be caused by smoking, carried the homozygous deletion genotype [10]. Conversely, a Chinese case-control study reported an increased risk of lung cancer among carriers of a *CYP2A6* deletion (**4*) when compared to subjects without the deletion (OR=2.0; 95% CI: 1.2-3.2) [15]. However, the controls in that study may not have been representative, as the frequency of the deletion allele was much lower among controls (8.6%) than had previously been reported in a Chinese population (15.1%) [15, 36]. A case-control study of lung cancer in a French population found no association with carriers of inactive CYP2A6 alleles **2* and **4 vs*. wild type (**1/*1*; OR=1.1; 95% CI: 0.7-1.9) [37], though it may have had insufficient statistical power.

A British case-control study reported that carriers of an inactive CYP2A6 variant (**2*) *vs*. subjects without **2* may be partially protected from colorectal cancer (OR=0.51; 95% CI: 0.28-1.06) [17]. A phenotype study, similar to the present study, showed that high CYP2A6 activity was associated with an increased risk of colorectal cancer (highest tertile *vs*. lowest tertile of CYP2A6 activity: OR=2.9; 95% CI: 1.6-5.0) [18, 19, 20]. Positive associations between CYP2A6 activity and esophageal [16] and oral cancer [38] have also been reported, and a link to liver cancer has been proposed [9]. However, inverse associations with esophageal [15] and gastric cancer [39] have been reported.

N-nitroso compounds have been suggested as human pancreatic carcinogens and several nitrosamines have induced tumor development in pancreatic cells in animal and human cell culture models [2], thus a causal association between elevated CYP2A6 activity and

Table 4. CYP2A6 phenotypes by consumption of processed meat and smoking history among pancreatic cancer cases and controls.

	17U:17X <2.764 (Lower 3 quartiles)			17U:17X >2.764 (Upper quartile)		
	Cases	**Controls**	**OR (95% CI)**	**Cases**	**Controls**	**OR (95% CI)**
Processed meat [a]						
- <1 servings /week	19 (34.5%)	111 (32.7%)	1.00 (Reference)	5 (16.1%)	24 (21.1%)	1.59 (0.53-4.82)
- 1-2.5 servings /week	14 (25.5%)	131 (38.6%)	0.56 (0.26-1.22)	13 (41.9%)	50 (43.9%)	1.37 (0.60-3.12)
- >2.5 servings /week	22 (40.0%)	97 (28.6%)	0.93 (0.45-1.95)	13 (41.9%)	40 (35.1%)	1.61 (0.69-3.78)
Smoking [b]						
- <10 pack/year	19 (33.9%)	206 (58.7%)	1.00 (Reference)	17 (56.7%)	67 (56.8%)	2.91 (1.39-6.08)
- ≥10 pack/year	37 (66.1%)	145 (41.3%)	2.31 (1.22-4.37)	13 (43.3%)	51 (43.2%)	2.69 (1.20-6.00)

[a] For the 539 subjects who completed the food frequency questionnaire. ORs were adjusted for age (continuous), education (less than high school, high school, more than high school), gender (male, female), race (white, nonwhite), current smoking status (yes, no), and chronic pancreatitis (yes, no).

[b] For the 555 subjects who reported information on smoking history. ORs were adjusted for age (continuous), education (< high school, high school, > high school), gender (male, female), race (white, nonwhite), and chronic pancreatitis (yes, no).

pancreatic cancer is biologically plausible. Cigarette smoking is a significant risk factor for pancreatic cancer [1, 5] and CYP2A6 activates a number of tobacco-specific nitrosamines, including NNK, N-nitrosonornicotine (NNN), and *N*-nitrosodiethylamine (NDEA) into potentially carcinogenic forms [40]. These hydroxylated nitrosamines in turn induce DNA adducts and appear to stimulate DNA synthesis in the pancreatic ductular epithelium [41]. While CYP2A6 is not expressed in the pancreas, the activated products of its enzymatic activity are carried to the pancreas *via* the circulatory system. In addition to cigarette smoking, epidemiologic studies have reported positive associations between pancreatic cancer and sources of dietary nitrosamines, such as processed or smoked meats [2, 5].

CYP2A6 activity has been reported to influence smoking behavior. A number of studies have reported that individuals with inactive CYP2A6 alleles were less likely to smoke [11, 42] and those who did smoke, smoked fewer cigarettes [10, 11, 42], although other studies did not confirm these findings [13, 34, 43]. Nowell *et al*. found no association between phenotypic CYP2A6 activity and smoking status or pack/years of smoking [19], in agreement with the findings presented here.

There are limitations to consider when interpreting our results. Although the number of controls was large (No. 470), there were only 90 cases phenotyped. This limited our statistical power, particularly in the assessment of effect modification. Participation in the caffeine assay was lower than in the overall study and it was hampered by the required time commitment, logistics, and/or the health status of eligible participants. Importantly, the subject characteristics and primary findings in the sub-study and the parent study were very similar; indicating that lack of participation was not likely to introduce bias. In addition, a previous analysis in these subjects indicated that those who provided blood but refused the urine-based caffeine assay when compared to subjects who provided both blood and urine did not differ by NAT2 genotype distribution (P=0.28) [21].

The poor prognosis for patients with pancreatic cancer (median survival is approximately 3 months) makes it difficult to enroll potential case subjects before they are too ill to participate or they die. However, we have no evidence to indicate that CYP2A6 activity is affected by this potential selection bias especially considering prognosis is almost always poor with a median survival of approximately 3 months [1].

This study was conducted in a population that was almost entirely Caucasian, and as noted, there has been evidence of wide interethnic variation in CYP2A6 activity. Therefore, generalizability is limited, and the relatively low genetic variability of CYP2A6 in the Caucasian population may be masking a larger effect than our results indicate.

Lastly, temporality is an issue in any case-control study, and this relationship should be examined in a prospective study. There is evidence that CYP2A6 activity is elevated in response to inflammation [44, 45] and therefore, it is possible that CYP2A6 activity is higher in pancreatic cancer cases compared to controls as a result of the disease and the accompanying inflammation. However, null data from a case-control analysis of breast cancer and CYP2A6 phenotype activity using the same assay that yielded a positive association in the colorectal cancer case-control study indicate that CYP2A6 phenotype activity is not simply elevated in response to carcinogenesis [19].

Our study was strong in that it was designed to examine metabolic phenotypes in relation to this cancer and because we gathered extensive information on numerous exposures relevant to pancreatic cancer thus allowing us to adjust for potential confounding. To our knowledge, this is the first investigation of the relationship between CYP2A6 metabolic activity and pancreatic cancer. In this population-based case-control study of cancer of the exocrine pancreas in the Midwestern U.S., we found high levels of CYP2A6 phenotypic activity to be positively associated with pancreatic cancer. More research will be necessary to elucidate the true nature of this association. Studies conducted in more ethnically diverse populations, and studies coupling CYP2A6 genotype and phenotype data would be of particular interest.

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