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Polymorphisms of Cytochrome P4501A2 and N-acetyltransferase genes, Smoking, and Risk of Pancreatic Cancer¹

Donghui Li², Li Jiao, Yanan Li, Mark A. Doll, David W. Hein, Melissa L. Bondy, Douglas B. Evans, Robert A. Wolff, Renato Lenzi, Peter W. Pisters, James L. Abbruzzese, and Manal M. Hassan

Departments of Gastrointestinal Medical Oncology (D.L., L.J., Y.L., J.L.A., R.A.W., R.L., A.N., M.M.H.), Surgical Oncology (D.B.E., P.W.P.), and Epidemiology (M.L.B.), The University of Texas, M.D. Anderson Cancer Center, 1515 Holcombe Blvd. Houston, TX 77030. Department of Pharmacology and Toxicology and Brown Cancer Center (M.A.D, D.W.H), University of Louisville School of Medicine, Louisville, KY 40292

Abstract

To test the hypothesis that genetic variation in the metabolism of tobacco carcinogens, such as aromatic amines (AA) and heterocyclic amines (HCA), contributes to pancreatic cancer, we have examined genetic polymorphisms of three key enzymes, i.e. cytochrome P450 1A2 (*CYP1A2*) and N-acetyltransferase 1 and 2 (*NAT1* and *NAT2*), in a hospital-based case-control study of 365 patients with pancreatic adenocarcinoma and 379 frequency-matched healthy controls. Genotypes were determined using PCR-restriction fragment length polymorphism (RFLP) and Taqman methods. Smoking information was collected by personal interview. Adjusted odds ratio (AOR) and 95% confidence interval (CI) was estimated by unconditional multivariate logistic regression analysis. We found that the *NAT1* “rapid” alleles were associated with a 1.5-fold increased risk of pancreatic cancer (95% CI: 1.0–2.1) with adjustment of potential confounders. This effect was more prominent among never smokers (AOR: 2.4, 95% CI: 1.4–4.3) and females (AOR: 1.8, 95% CI: 1.0–3.1). Some genotypes were significantly associated with increased risk for pancreatic cancer among smokers, especially heavy smokers (> 20 pack years). For example, heavy smokers with the *CYP1A2***ID* (T-2467delT) delT, *CYP1A2***IF*(A-163C) C allele, *NAT1* “rapid” or *NAT2* “slow” alleles had an AOR (95% CI) of 1.4 (0.7–2.3), 1.9 (1.1–3.4), 3.0 (1.6–5.4) and 1.5 (0.8–2.6), respectively, compared with never smokers carrying the non at-risk alleles. These effects were more prominent in females than in males. The corresponding AOR (95% CI) was 3.1 (1.0–8.0), 3.8 (1.5–10.1), 4.5 (1.6–12.7), and 2.0 (0.8–5.1) for females versus 1.0 (0.4–1.9), 1.1 (0.5–2.4), 2.1 (1.0–4.6) and 1.1 (0.5–2.6) for males. A significant synergistic effect of *CYP1A2***IF* C allele and *NAT1* “rapid” alleles on the risk for pancreatic cancer was also detected among never smokers (AOR: 2.9, 95% CI: 1.2–6.9) and among females (AOR: 2.5, 95% CI: 1.1–5.7). These data suggest that polymorphisms of the *CYP1A2* and *NAT1* genes modify the risk of pancreatic cancer.

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²To whom requests for reprints should be addressed, at Department of Gastrointestinal Medical Oncology, The University of Texas, M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 426, Houston, TX, 77030. Phone (713) 792-2012; Fax: (713) 563-1195. E-mail: dli@mdanderson.org.

³The abbreviations used are: AA, aromatic amines; AOR: adjusted odds ratio; CYP, cytochrome P450; GST, glutathione S-transferase; HCA, heterocyclic amines; NAT, N-acetyltransferase; PAH, polycyclic aromatic hydrocarbon; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

Keywords

Cytochrome P450; NAT; polymorphism; genetic susceptibility; gene-environment interaction; pancreatic cancer

INTRODUCTION

In the United States, pancreatic cancer is the fourth leading cause of cancer death in both men and women (1). It is a lethal disease with a mortality rate approximately equals to its incidence rate. Its etiology is poorly understood, and the most consistent risk factor, as suggested by epidemiologic studies, is cigarette smoking, which is implicated in about 30% of the cases of pancreatic cancer (2).

Cigarette smoke is a major source of carcinogen exposure and individual variation in carcinogen metabolism has been considered as a risk factor for smoking-related cancers. Whereas many molecular epidemiologic studies have explored the genetic determinants involved in tobacco-related human cancers, few studies have been reported on pancreatic cancer. Three early reports, all with limited sample sizes reported no significant association between susceptibility to pancreatic cancer and polymorphisms of the cytochrome P450 (*CYP*) *1A1*, *2D6*, *2E1*, glutathione S-transferase (*GST*) *M1*, *GSTT1*, and N-acetyltransferase (*NAT*) genes (3–5). Nevertheless, a recently reported population-based case-control study found that the combination of heavy smoking and the presence of a *GSTT1* null genotype was significantly associated with an increased risk of pancreatic cancer, and the effect was more prominent among women than among men (6). The same study failed to demonstrate any significant main effect of *CYP1A1* on risk of pancreatic cancer or interaction with smoking (6).

While the role of polycyclic aromatic hydrocarbon (PAH) exposure and metabolism in pancreatic cancer need further investigation, several lines of evidence support a role of aromatic amine (AA) and heterocyclic amine (HCA) carcinogens in the pathogenesis of pancreatic cancer. First of all, the spectra of *p53* and *K-ras* mutations in pancreatic adenocarcinoma are more similar to that of bladder cancer and colorectal cancer than that of lung, head and neck, and esophageal cancers (7,8). The predominant G to A transition observed in the former resembles that seen in animals exposed to AA or nitrosamines, whereas the G to T transversion implicates exposure to PAHs. Secondly, AA-DNA adducts have been identified in human pancreatic tissues (9). Thirdly, the pancreas is highly susceptible to HCA-induced DNA damage (10–13). Fourthly, epidemiological studies have found that high consumption of cooked meat and fish increases the risk for pancreatic cancer (14,15).

The carcinogenic action of AA and HCA requires metabolic activation resulting in electrophiles that bind to DNA. *CYP1A2* is the major enzyme involved in the *N*-hydroxylation activation of these compounds (16). The expression of *CYP1A2* is controlled by two mechanisms, i.e. constitutive expression and inducibility regulation. Although expressed mainly in the liver, expression of the *CYP1A2* enzyme in human pancreas has been detected (17,18). Large individual variations exist in the enzyme activities of *CYP1A2*, but the phenotype and genotype correlation is not well understood (19). The *CYP1A2* gene consists of 7 exons and is located at chromosome 15q22-qter. More than 40 single nucleotide polymorphisms (SNPs) of the *CYP1A2* gene have been discovered (20). The initial report from Japan (21) reported 4 SNPs of this gene, resulting in 4 alleles: *CYP1A2* A (G-3860A), B (T-2467delT), C (T-739G), and D (A-163C). The Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee subsequently named the A, B, C and D alleles as *CYP1A2*1B*, *1D*, *1E*, and *1F*, respectively (20). A later study of 13 *CYP1A2* SNPs suggested that only the

*CYP1A2*1D* and *CYP1A2*1F* need to be analyzed in the routine assessment of *CYP1A2* genotype (22). *CYP1A2*1F* possesses an intron 1 A-163C (aka A-164C and A-154C) polymorphism that appears to affect the inducibility of the enzyme (23,24). Whether *CYP1A2* polymorphisms modify susceptibility to human cancers is unknown.

Two other important enzymes involved in the metabolism of AA and HCA carcinogens are *N*-acetyltransferase 1 (NAT1) and *N*-acetyltransferase 2 (NAT2). The *N*-hydroxylation of AA or HCA catalyzed by *CYP1A2* may compete with *N*-acetylation catalyzed by NAT while the *N*-hydroxylation intermediates of AA or HCA may be further activated by *O*-acetylation to more DNA reactive species (25). NAT1 and NAT2 catalyze both *N*-acetylation and *O*-acetylation (26). *NAT1* and *NAT2* genes are located on chromosome 8p 23.1-p21.3 and 8p22, respectively, and both are encoded by single open reading frames of 870 base pairs that exhibit genetic polymorphism in human populations (27). Molecular epidemiological studies demonstrated that individuals with *NAT1* rapid acetylator genotypes or *NAT2* slow acetylator genotypes in the presence of known carcinogen exposures, such as cigarette smoking, dietary exposure to HCA or occupational exposure to AA, were at increased risk for various types of human cancers (28,29). The human pancreas predominantly expresses *NAT1* (30) whereas *NAT2* is predominantly expressed in the liver. We hypothesize that *NAT2* slow acetylator genotype may lead to a deficient hepatic detoxification of carcinogens while the higher local NAT1 activity contributes to the formation of highly reactive DNA damaging species in the pancreas, hence, the slow *NAT2* and rapid *NAT1* genotypes could increase an individual's risk for pancreatic cancer.

To our knowledge, no study has ever been conducted to investigate the role of *CYP1A2* gene in pancreatic cancer and the *NAT* genes have only been examined in a small study of 81 pancreatic cancer cases and 78 controls (4). Thus, we examined the frequencies of the *CYP1A2*1D* and **1F* alleles and *NAT1* and *NAT2* genotypes and the effect of these polymorphisms on risk for pancreatic cancer in a hospital based case-control study.

MATERIALS AND METHODS

Study Population

The study was approved by the Institutional Review Board of the University of Texas M. D. Anderson Cancer Center (M.D. Anderson). Cases were patients with pathologically confirmed pancreatic ductal adenocarcinomas who had been seen at M.D. Anderson from 2000 to 2004. There was no restriction in the recruitment of cases with respect to age, race, and sex. All study participants were residents of the United States and were able to communicate in English. The response rate of case recruitment was 78%. The common reasons for refusal to participate included the patients being too sick or too upset to participate and time constraints. There were no significant demographic differences between individuals who agreed or refused to participate in the study. Controls were recruited from spouses, friends and non-blood relatives of patients with various types of cancers other than pancreatic cancer. The eligible controls were identified by a 5-minute self-administered questionnaire acquiring demographic information and cancer history. Cases and controls were frequency-matched by age (± 5 years), sex, and race. The response rate of control recruitment was 77%. There were no significant differences between individuals who agreed or refused to participate in terms of age, sex, race, and state of residence.

Data Collection

A questionnaire was administered to study participants by personal interview to collect information on tobacco use, cigarette smoking, alcohol use, occupational history, medical history, and family history of cancer. Both cases and controls were interviewed by the same

study personnel. No proxy interviews were conducted. Those who smoked more than 100 cigarettes in their lifetime were defined as ever smokers. Smokers who have quit smoking for more than 1 year before recruitment were defined as former smokers. Those who consumed 4 alcoholic drinks per month for at least 6 months in their lifetime were defined as ever drinkers. A common portion size of each alcoholic beverage type (beer, wine, and liquor) was specified. Daily ethanol intake was calculated based on the type of drink, the frequency of use, and the amount consumed. The ethanol content of each type of drink was calculated assuming 13.2 g of ethanol for 12 oz of beer, 10.8 g for 4 oz of wine, and 15.1 g for 1.5 oz of liquor, according to the standard of the U.S. Department of Agriculture. The 75th percentile value of weekly alcohol intake (gram) of controls among alcohol drinkers was used as the criterion to define heavy versus light drinkers.

A blood sample was obtained from each participant along with consent for genotyping. The exclusion criteria for the final data analysis included: 1) failure to donate a blood sample; 2) failure to complete the risk questionnaire; 3) having a prior history of cancer (except for non-melanoma skin cancer); and 4) being misdiagnosed as pancreatic adenocarcinoma (case only).

Detection of *CYP1A2*1D* and *CYP1A2*1F* Polymorphisms

DNA was extracted from peripheral blood lymphocytes using a Flexigene DNA kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The *CYP1A2* polymorphisms were detected by PCR-RFLP (21). The case-control status of the samples was blinded to the laboratory personnel. More than 10% of samples were analyzed in duplicate and were 100% concordant.

Detection of *NAT1* and *NAT2* polymorphisms

Eight SNPs of the *NAT1* gene (A-40T, C-344T, G445A, G459A, G560A, T640G, T1088A, and C1095A) and 6 SNPs of the *NAT2* gene (C282T, T341C, C481T, G590A, A803G, G857A) were determined using the MassCode method by Qiagen (Valencia CA) in the first batch of 300 samples with equal number of cases and controls. The second batch of 400 samples was genotyped for *NAT1* (31) and *NAT2* (32) using Taqman-based methods at the University of Louisville. The SNPs included *NAT1* C97T, C190T, G445A, C559T, G560A, A752T, T1088A, and C1095T and *NAT2* G191A, C282T, T341C, C481T, G590A, A803G, and G857A. The underlined SNPs are those that were overlapped in both batches of DNA samples. About 10% of the samples were also analyzed using a PCR-RFLP method (33,34).

Statistical Analysis—Chi-square (χ^2) tests were used to compare the distribution of categorical variables and genotype frequencies between cases and controls. Tests for Hardy-Weinberg equilibrium were conducted using a χ^2 test with 1 degree of freedom. Risk assessment was restricted to non-Hispanic whites because of the known ethnic variations in genotype distribution and pancreatic cancer risk as well as the small sample size of the minority groups. Unconditional logistic regression analysis was used to calculate ORs and 95% CIs. Any variables that showed a significant risk modifying effect in univariate analyses were included in the multivariate analyses. The minor *CYP1A2*1D* delT allele and the *CYP1A2*1F* C allele was considered as the at-risk allele. Individuals homozygous or heterozygous for *NAT1*10* or *NAT1*11* alleles were considered “rapid” acetylator genotypes. Individuals homozygous for *NAT1*3* or *NAT1*4* were considered the reference genotype. The low frequency *NAT1*14* allele (slow acetylator allele) was not included in the risk assessment. *NAT2*4*, **12A*, **B*, **C*, and **13* are rapid acetylator *NAT2* alleles and all others are slow acetylator *NAT2* alleles (26,29). Individuals homozygous for slow *NAT2* alleles are slow acetylator genotype. The *NAT1* rapid and *NAT2* slow acetylator genotypes were considered as “at-risk”. For detection of possible interactions between genotypes and smoking, never smokers with the non at-risk genotype was used as the reference group and AORs for never

smokers with the at-risk genotype (OR_{10}), smokers with non at-risk genotype (OR_{01}), and smokers with the at-risk genotype (OR_{11}) were estimated using unconditional logistic regression. The magnitude of an interaction effect was assessed by evaluating departure from additive effects using the synergism index (S index) (35,36). A synergistic effect was suggested when OR_{11} was greater than the sum of OR_{10} and OR_{01} . The S index and 95% CI was calculated as $OR_{11} - 1 / OR_{01} + OR_{10} - 2$. All statistical analyses were performed using STATA and SPSS software. P values less than 0.05 were indicative of statistical significance.

RESULTS

The study involved 365 cases of pathologically confirmed pancreatic adenocarcinoma and 379 healthy controls. The distributions of sex and race between the two groups were approximately equal: 58.1% of cases versus 54.4% of controls were men and 41.9% of the cases versus 45.6% of the controls were women ($P = 0.31$); and 87.4% of the cases and 88.4% of the controls were non-Hispanic whites ($P = 0.91$). Hispanics and African Americans consisted 6% and 5% of the study population, respectively. Because of the small number of minorities enrolled in this study and the known racial differences in pancreatic cancer risk and genotypes, all risk estimates were restricted only to non-Hispanic whites (319 cases and 335 controls). The mean \pm SD age of cases and controls was 62.3 ± 10.4 and 60.4 ± 11.1 years, respectively, ($P = 0.01$). As shown in Table I, controls were overrepresented with individuals younger than 52 years of age. Fifty-two percent of the cases and 58% of the controls were from Texas and the remaining were from 39 other U.S. states. Family history of cancer (in the first degree relatives) was not associated with the risk for pancreatic cancer (data not shown), whereas family history of pancreatic cancer was non-significantly associated with an increased risk for pancreatic cancer. Because diabetes and pancreatitis can be a manifestation of pancreatic cancer, risk estimation was performed separately in individuals with a history of these diseases, subdivided by the length of time these conditions diagnosed relative to the time of their cancer diagnosis or recruitment into this study (< 3 years versus ≥ 3 years). In both subgroups, diabetes was associated with a significantly increased risk of pancreatic cancer. It is also notable that 90% of the self-reported pancreatitis cases occurred within 3 years of the cancer diagnosis (Table I). Smoking and alcohol consumption was not associated with the history of pancreatitis. Although all 4 controls with pancreatitis were ever smokers, the frequency of pancreatitis was 8.6% in never smokers and 11.1% in ever smokers among cases ($P = 0.46$). The frequency of pancreatitis among cases was 9.5%, 3.7% and 12.5% among never drinkers, light drinkers and heavy drinkers, respectively. Alcohol use, in general, did not appear to affect the risk for pancreatic cancer. Cases tended to have consumed a larger volume of alcohol than controls; the median weekly alcohol consumption was 168 g versus 86 g in cases and controls, respectively ($P=0.002$, Mann Whitney's test). However, heavy alcohol consumption (>263 g/wk) did not translate into increased risk of pancreatic cancer, and light drinking (≤ 263 g/wk) actually showed a protective effect in this study population.

The association between cigarette smoking and risk for pancreatic cancer in the study population is summarized in Table II. Ever smokers comprised 61.9% of the cases and 53.2% of the controls. If individuals who consumed pipe, cigar, snuff, and/or chewing tobacco for more than a year are included as smokers, 66.0% of the cases and 57.3% of the controls would be classified as ever smokers. Males had a higher smoking prevalence (63% of controls and 65% of cases) than females (40% of controls and 58% of cases) but female smokers had a greater risk of developing pancreatic cancer (AOR: 2.0, 95% CI: 1.2–3.5) than male smokers (AOR: 1.0, 95% CI: 0.6–1.5). Overall, ever smokers had a 30% increased risk for pancreatic cancer (95% CI: 0.9–1.9). Former smokers had a 2.2-fold increased risk for pancreatic cancer among females and but not among males. A dose-response relationship was observed between the intensity (cigarettes smoked per day) and duration (years smoked) of smoking, as well as the product of intensity and duration (pack years) of smoking and the risk of pancreatic cancer

among women. The median and 75th percentile of pack years smoked was 20 and 40 among controls compared to 25 and 48 among cases, respectively.

The genotype frequencies in non-Hispanic whites are presented in Table III. The distribution of *CYP1A2*1D* and *CYP1A2*1F* genotypes was in agreement with the Hardy-Weinberg equilibrium. Among the 19 *NAT* gene SNPs tested, all but two, i.e. *NAT1* G560A and *NAT2* G857A, were in agreement with the Hardy-Weinberg equilibrium. The genotype/allele frequencies of *CYP1A2* were quite comparable between cases and controls (all *P* values >0.05) and no significant main effect on the risk for pancreatic cancer was observed. Hispanic controls (n=22) had a higher frequency of the *CYP1A2*1D* delT allele (0.62), *CYP1A2*1F* C allele (0.35), and *NAT1* rapid genotype (54%), but a lower frequency of *NAT2* slow genotype (45%) than non-Hispanic whites. African American controls (n=18) showed the same trend as Hispanics, with the corresponding allele/genotype frequencies of 0.42, 0.50, 56% and 22%, respectively. Because of the limited sample size, no risk estimate was made in these minority groups.

The frequencies of major *NAT1* genotypes among non-Hispanic white controls was: **4/*4*: 0.60, **4/*10*: 0.27, **10/*10*: 0.03, **4/*3*: 0.03 and **4*11*: 0.02. *NAT1* alleles were further classified into rapid (**10* and **11*) and reference (**3* and **4*) alleles and the frequencies of the rapid versus reference genotypes stratified by racial groups are presented in Table III. A borderline significant difference in the distribution of *NAT1* rapid alleles between cases and controls was observed among non-Hispanic whites (*P* = 0.06). Logistic regression analysis demonstrated that the *NAT1* rapid alleles were associated with a 1.5-fold increased risk of pancreatic cancer (95% CI: 1.0 – 2.1) after adjusting for age, diabetes, and pancreatitis. This association was more prominent in never smokers (AOR: 2.42, 95% CI: 1.4–4.3) and females (AOR: 1.8, 95% CI: 1.0–3.1) than in smokers (AOR: 1.1, 95% CI: 0.7–1.8) and males (AOR: 1.3, 95% CI: 0.8–2.2).

The *NAT2* allele frequencies detected in the non-Hispanic white control subjects of the current study are comparable to those reported in a large pool of Caucasian controls (37). The observed (versus previously reported) frequencies of the most common *NAT2* genotypes were: **5/*6*: 0.23 (0.26), **5/*5*: 0.22 (0.22), **4/*5*: 0.17 (0.21), **4/*6*: 0.11 (0.13), **4/*4*: 0.05 (0.07), and **6/*6*: 0.08 (0.07). The distribution of the rapid, intermediate and slow acetylator *NAT2* genotypes in cases and controls are shown in Table III. There was no significant difference in the distribution of *NAT2* genotypes between cases and controls within the non-Hispanic white group. The distribution of the *NAT1* rapid genotype was higher among individuals with the *NAT2* rapid than those with the *NAT2* slow genotypes. The frequency of *NAT1* rapid was 41.5% in *NAT2* rapid and 25.3% in *NAT2* slow controls, 51.8% in *NAT2* rapid and 32.5% in *NAT2* slow cases.

Next, we examined the association between these genotypes and the risk for pancreatic cancer in relation to cigarette smoking. Ever smokers carrying the *CYP1A2*1F* C allele or *NAT1* rapid genotype, both reported to confer a higher inducibility or enzyme activity, showed a 1.6 to 1.9-fold increased risk for pancreatic cancer compared to never smokers with the low inducibility/activity alleles (Table IV). The magnitude of this effect was greater in women than in men. Women with the at-risk genotypes and were ever smokers had a 3-fold increased risk for pancreatic cancer compared to women carrying the non at-risk genotypes and who never smoked. There was a significant additive interaction between the presence of *CYP1A2*1F* allele and smoking on the risk of pancreatic cancer among women (S index = 4.0, 95% CI: 1.5–6.5). For the same comparison, men with the at-risk genotypes and were ever smokers had an AOR of 0.9 and 1.2 only. On the other hand, using never smokers with the non at-risk genotypes as the reference group, *CYP1A2*1D* allele (AOR: 1.2, 95% CI: 0.8–2.0) or *NAT2*

slow genotype (AOR: 1.3, 95% CI: 0.8-2.2) were not statistically associated with risk for pancreatic cancer among smokers.

The genotype effect on the risk for pancreatic cancer was more prominent among heavy smokers and among females. Using the median of the control values (20 pack years) as the criterion, heavy smokers (>20 pack years) with the at-risk genotypes of *CYP1A2*1D*, *CYP1A2*1F*, *NAT1* or *NAT2* had an AOR (95% CI) of 1.4 (0.7–2.3), 1.9 (1.1–3.4), 3.0 (1.6–5.4), and 1.5 (0.8–2.6), respectively, compared with never smokers carrying the non at-risk genotypes (Table V). When we evaluated the association between these genotypes and smoking by sex we found that women had a higher AOR than men for all four genotypes. Compared to women who never smoked and carrying the non at-risk genotypes, women who smoked more than 20 pack years and carrying the *CYP1A2*1D delT* allele, *CYP1A2*1F C* allele, *NAT1* rapid or *NAT2* slow genotypes had an AOR (95% CI) of 3.1 (1.0–8.0), 3.8 (1.5–10.1), 4.5 (1.6–12.7), and 2.0 (0.8–5.1), respectively. The corresponding AOR (95% CI) was 1.0 (0.4–1.9), 1.1 (0.5–2.4), 2.1 (1.0–4.6) and 1.1 (0.5–2.6) among men. A weak interaction on an additive scale was observed between heavy smoking and the *CYP1A2*1D* and *CYP1A2*1F* alleles among females, the estimated S index (95% CI) was 5.6 (0.3–10.9) and 2.8 (0.8–4.7), respectively.

Finally, we attempted to examine the joint effect of different genotypes. We observed a significant joint effect of the *CYP1A2*1F C* allele and *NAT1* rapid genotype on risk for pancreatic cancer among never smokers and females (Table VI). The overall AOR (95% CI) was 1.8 (1.1–3.1) for individuals carrying the *NAT1* rapid and *CYP1A2*1F C* alleles compared to individuals carrying the *NAT1* reference and *CYP1A2*1F AA/AC* genotypes. This effect was more prominent in never smokers (AOR: 2.9, 95% CI: 1.2–6.9) versus smokers (AOR: 1.5, 95% CI: 0.8–2.8) and in females (AOR: 2.5, 95% CI: 1.1–5.7) versus males (AOR: 1.5, 95% CI: 0.8–2.9). Individuals having either of these two at-risk alleles alone did not show a significantly higher risk of pancreatic cancer. The S index (95% CI) for interaction in females was 2.3 (0.12–4.43). No significant joint effect of other gene or allele combinations was observed (data not shown). A significantly increased cancer risk was observed among never smokers and among females having both rapid *NAT1* and *NAT2*. This effect was predominantly caused by *NAT1* genotype because individuals with rapid *NAT1* and slow *NAT2* had similar AORs as those with both rapid *NAT1* and *NAT2*.

DISCUSSION

To our knowledge, the current study is the first to report a significant main effect of the *NAT1* gene and interactions of *NAT1* and *CYP1A2* genotypes with smoking on risk for pancreatic cancer. We have shown that the *NAT1* rapid acetylator genotype was associated with a significantly increased risk of pancreatic cancer among never smokers and among females. We have also shown that *CYP1A2*1F C* allele and *NAT1* rapid acetylator genotypes in combination with heavy smoking were positively associated with an increased risk for pancreatic cancer among females. These observations support our hypothesis that the *CYP1A2* and *NAT* gene polymorphisms modify the risk for smoking-related pancreatic adenocarcinomas, by altering the metabolism of AA and HCA tobacco carcinogens.

The frequency of the *CYP1A2*1F* polymorphism in several different populations has been reported. Among populations in Britain (22), Germany (23), Denmark (38), Egypt (39), and China (40), the C allele frequency was about 0.31–0.34. The frequency was relatively higher among Japanese (0.39)(20) and Ethiopians (0.40) (41). In the United States the only reported study was conducted among Hawaiian women, and the frequency was 0.30 (42). The C allele frequency among non-Hispanic white controls in our study was 0.31, which is quite comparable to the reported frequencies.

The association between the *CYP1A2*1F* polymorphism and risk of cancer has previously been investigated in two studies. Neither the study of 49 colon cancer cases and 65 controls (22) nor the study of 164 ovarian cancer cases and 194 controls (42) demonstrated a significant association between this polymorphism and risk for cancer. The lack of cancer association in these studies could be related to their small sample sizes or the lack of exposure assessment because only the main effect was examined. Phenotype studies have suggested possible associations between higher CYP1A2 activity and risk for bladder cancer, hepatocellular carcinoma, and colon cancer (19,43,44). Higher CYP1A2 activity has also been found to positively influence urinary mutagenicity among smokers and after consumption of pan-fried meat, a major source of HCA compounds (45). However, the functional significance of the *CYP1A2*1F* allele is not clear at present. One study found a higher enzyme activity associated with the A allele among smokers (22), another study found no difference in the enzyme activity and inducibility between the A and C alleles (41). Our study demonstrated that the C allele is the “at-risk” allele. Whether and how the C allele affects enzyme activity and inducibility of CYP1A2 needs to be further investigated. It is possible that the *CYP1A2*1F* polymorphism modifies pancreatic cancer risk not through altered enzyme activity but rather via linkage disequilibria with other genes that play an important role in tumorigenesis. As shown in a latest study (39), there are several other intron 1 polymorphisms of the *CYP1A2* gene. Even though the A-164C polymorphism alone did not show any functional significance, significantly decreased enzyme activity and inducibility was observed among individuals with the haplotype of -164A, -740G, and -730T, compared to those with other haplotypes, such as -164C, -740T, and -730C; -164A and -740G; and -164A only (41). Information on the frequency and functional significance of the *CYP1A2*1D* allele is limited. We observed a significant interaction of the variant allele with heavy smoking among women in this study. Further investigation of the haplotypes and other SNPs of the *CYP1A2* gene in our study population may help us better understand the mechanisms underlying the association between *CYP1A2* gene and increased cancer risk.

The current study observed that *NAT1* rapid genotypes were associated with a significantly higher risk of pancreatic cancer among all study subjects, especially among never smokers and among females. On the other hand, rapid *NAT1* genotypes in combination with heavy smoking resulted in a 3.0-fold increased risk for pancreatic cancer compared with never smokers with the reference *NAT1* genotypes. Previous studies have shown that *NAT1* is the predominant NAT expressed in the human pancreas (30), however, the relationship between *NAT1* genotype and phenotype is not well understood (26,28,29). Some studies suggest that *NAT1*10* may be a rapid acetylator allele that has been associated with slight increases in NAT activity while other studies did not find such an association (26,28,29). Molecular epidemiological studies have found positive associations between the *NAT1* rapid alleles (*NAT1*10* and *NAT1*11*) and increased risk for bladder (46,47), colon (48–50), breast (51,52), prostate (53,54), gastric cancer (55) and lung cancer (56). Results from the current study have added pancreatic cancer to this list. The increased cancer risk among *NAT1* rapid allele carriers with heavy smoking can be explained by possible higher metabolic activation of tobacco carcinogens and consequently more reactive DNA damaging species in those individuals. The higher risk of cancer among never smokers associated with the *NAT1* rapid allele suggests that carcinogen exposure other than cigarette smoking may be also involved in human pancreatic cancer. This notion is supported by epidemiological findings that higher consumptions of grilled/barbequed meats were associated with increased risk for pancreatic cancer (14,15), which implies a role of HCA exposure in this disease.

The current study observed a differential distribution of *NAT1* rapid genotype among slow and rapid NAT2 acetylators, i.e. 41.5% and 25.3% in *NAT2* rapid and slow controls, 51.8% and 32.5% in *NAT2* rapid and slow cases, respectively. The higher frequency of *NAT1*10* allele in *NAT2* rapid acetylators has previously reported in several studies (57–59). Because of the

short distance between the two genes on the same chromosome, such a cosegregation of defined *NAT1/NAT2* traits is not unlikely. Results of linkage analysis and haplotype analysis of *NAT1/NAT2* genes are beyond the scope of the current manuscript and will be reported separately in the near future.

We also observed a higher risk for smoking-induced pancreatic cancer and a stronger interaction between *CYP1A2/NAT* genes and smoking in women than in men, suggesting that hormones or other gender-specific factors may play a role in mediating the effects of cigarette smoking on pancreatic carcinogenesis. Consistent with our findings, a previous study reported a stronger effect of *GSTT1*-null and heavy smoking on the risk for pancreatic cancer among women than men (6). In addition, epidemiologic studies have observed higher smoking-related relative risks of pancreatic cancer among women than among men (60,61). The mechanisms responsible for the sex-difference in susceptibility to smoking-related pancreatic cancer need further investigation.

There are some inherent limitations in this hospital-based case-control study. Since M. D. Anderson is a tertiary referral hospital and pancreatic cancer is rare, our control population was limited to patient companions from all over the country rather than a random sample from a defined population, which could potentially introduce selection bias. In addition, recall bias is another inherent limitation of the current study. Even though direct interview may reduce the information bias, the accuracy of assessments on cigarette smoking and alcohol consumption may still be subject to recall bias. Therefore, our observations need to be confirmed in a larger scale study and in another study population. If confirmed, our data support the hypothesis that individual variation in the metabolic activation of tobacco carcinogens poses an increased risk for pancreatic cancer, and women are more susceptible to such an effect than men.

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Table I

Risk factors for pancreatic cancer

Variable	Case n=319 (%)	Control n=335 (%)	OR (95% CI)*
Age			
≤52	56 (17.6)	83 (24.8)	1.0
53–62	97 (30.4)	90 (26.9)	1.60 (1.02–2.49)
63–69	76 (23.8)	81 (24.2)	1.39 (0.88–2.21)
≥70	90 (28.2)	81 (24.2)	1.65 (1.05–2.59)
Family History of Pancreatic Cancer			
No	301 (94.4)	322 (97.0)	1.0
Yes	18 (5.6)	10 (3.0)	1.93 (0.87–4.24)
Diabetes			
No	254 (80.6)	294 (91.9)	1.0
≤ 3 years	36 (11.4)	14 (4.4)	2.98 (1.57–5.64)
> 3 years	25 (7.9)	12 (3.8)	2.41 (1.19–4.90)
Pancreatitis			
No	291 (91.2)	333 (99.4)	1.0
≤ 3 years	22 (6.9)	0 (0)	
> 3 years	6 (1.9)	2 (0.6)	3.43 (0.69–17.1)
Alcohol use			
Never	148 (46.5)	136 (43.4)	1.0
Ever	170 (53.5)	185 (57.6)	0.84 (0.62–1.15)
Ethanol/week (g) [†]			
Never	148 (48.4)	136 (43.0)	1.0
≤ 263 g/wk	96 (31.4)	135 (42.7)	0.65 (0.46–0.93)
>263 g/wk	62 (20.3)	45 (14.2)	1.27 (0.81–1.98)

* Estimated by unconditional logistic regression.

[†] Detailed information on alcohol consumption was missing from some study subjects. The 75th percentile value of control alcohol drinkers was used as the cutoff.

Table II

Cigarette smoking and risk of pancreatic cancer*

Variable	Case/Con (%/%)	Male AOR (95% CI)	Case/Con	Female AOR (95% CI)	All AOR (95% CI) [†]
Smoking					
Never	65/65 (35/37)	1.0 (referent)	55/87 (42/60)	1.0 (referent)	1.0 (referent)
Former	90/91 (47/51)	0.9 (0.5 – 1.4)	56/37 (43/26)	2.2 (1.2 – 3.9)	1.3 (0.9 – 1.8)
Current	33/21 (18/12)	1.4 (0.7 – 2.8)	20/20 (15/14)	1.9 (0.9 – 4.1)	1.6 (1.0 – 2.7)
Ever	123/112 (65/63)	1.0 (0.6 – 1.5)	76/57 (58/40)	2.0 (1.2 – 3.5)	1.3 (0.9 – 1.9)
<i>P</i> (trend)		0.27		0.02	0.01
Cigarettes per day					
≤20	74/68 (40/38)	1.0 (0.6 – 1.6)	57/45 (44/31)	2.0 (1.2 – 3.6)	1.4 (1.0 – 2.0)
>20	48/44 (26/25)	0.9 (0.5 – 1.5)	17/12 (13/8)	2.1 (0.9 – 5.0)	1.3 (0.8 – 2.1)
<i>P</i> (trend)		0.73		0.01	0.04
Years of smoking					
≤20	41/54 (22/31)	0.7 (0.4 – 1.2)	29/25 (23/17)	1.7 (0.9 – 3.4)	1.0 (0.7 – 1.6)
>20	81/58 (43/33)	1.2 (0.7 – 2.0)	45/32 (35/22)	2.3 (1.3 – 4.2)	1.6 (1.1 – 2.3)
<i>P</i> (trend)		0.17		0.004	0.002
Pack years					
≤20	40/50 (22/28)	0.7 (0.4 – 1.3)	36/33 (28/23)	1.7 (0.9 – 3.3)	1.1 (0.7 – 1.7)
>20	81/62 (44/35)	1.1 (0.7 – 1.9)	39/24 (30/17)	2.6 (1.3 – 4.9)	1.6 (1.1 – 2.3)
<i>P</i> (trend)		0.26		0.002	0.003

* Information on smoking was missing from 16 controls. Never smoker was used as the reference group for all comparisons in this table.

[†] OR was adjusted for age, diabetes, pancreatitis, and ethanol intake (all as categorical variables).

Table III
Distribution of *CYP1A2*, *NAT1*, and *NAT2* genotypes*

Genotype	Cases n (%)	Controls n (%)
<i>CYP1A2</i>*<i>ID</i>		
TT	166 (54)	166 (50)
T/delT	107 (35)	127 (39)
delT/delT	34 (11)	36 (11)
Total	307	329
delT allele frequency	0.29	0.31
<i>CYP1A2</i>*<i>IF</i>		
AA	129 (42)	162 (49)
AC	146 (48)	139 (41)
CC	32 (10)	32 (10)
Total	307	333
C allele frequency	0.34	0.31
<i>NAT1</i>		
Reference	182 (60)	213 (66)
Rapid	117 (38)	100 (31)
Slow	5 (2)	9 (3)
Total	304	322
<i>NAT2</i>		
Rapid	17 (6)	27 (9)
Intermediate	97 (35)	105 (33)
Slow	167 (59)	185 (58)
Total	281	317

* *P* values from χ^2 test for genotype frequencies between cases and controls were all greater than 0.05.

Table IV

Interaction of genotypes with cigarette smoking

Genotype	Smoke	Case/ Con n/n	MALE	Case/ Con n/n	FEMALE	ALL
			AOR (95% * CI)		AOR (95% * CI)	AOR (95% * CI)
<i>CYP1A2</i> * <i>ID</i>						
T/T	Never	36/33	1.0 (referent)	28/41	1.0 (referent)	1.0 (referent)
T/delT & delT/delT	Never	27/31	0.8 (0.4 – 1.6)	22/43	0.8 (0.4 – 1.7)	0.8 (0.5 – 1.3)
T/T	Ever	66/58	0.9 (0.5 – 1.7)	36/26	1.9 (0.9 – 4.2)	1.2 (0.8 – 2.0)
T/delT & delT/delT	Ever	56/54	0.9 (0.5 – 1.7)	36/30	1.9 (0.9 – 4.1)	1.2 (0.8 – 2.0)
<i>CYP1A2</i> * <i>IF</i>						
CC	Never	26/27	1.0 (referent)	25/48	1.0 (referent)	1.0 (referent)
AC/AA	Never	36/38	0.8 (0.4 – 1.8)	26/38	1.3 (0.6 – 2.8)	1.1 (0.7 – 1.9)
CC	Ever	50/51	0.9 (0.4 – 1.8)	28/30	2.1 (1.0 – 4.5)	1.4 (0.8 – 2.3)
AC/AA	Ever	71/61	0.9 (0.5 – 1.8)	45/27	3.0 (1.4 – 6.6)	1.6 (1.0 – 2.7)
<i>NAT1</i>						
Reference	Never	37/46	1.0 (referent)	28/58	1.0 (referent)	1.0 (referent)
Rapid	Never	25/15	2.1 (0.9 – 4.9)	24/24	2.3 (1.0 – 4.9)	2.2 (1.3 – 3.8)
Reference	Ever	75/70	1.2 (0.6 – 2.0)	45/36	2.5 (1.2 – 5.2)	1.7 (1.1 – 2.6)
Rapid	Ever	42/38	1.2 (0.6 – 2.4)	29/20	3.4 (1.5 – 7.6)	1.9 (1.1 – 3.1)
<i>NAT2</i>						
Rapid & Intermediate	Never	23/23	1.0 (referent)	24/35	1.0 (referent)	1.0 (referent)
Slow	Never	32/35	1.0 (0.4 – 2.1)	28/47	0.9 (0.4 – 2.0)	0.9 (0.5 – 1.6)
Rapid & Intermediate	Ever	42/47	0.8 (0.4 – 1.7)	25/22	1.6 (0.7 – 3.7)	1.1 (0.6 – 1.9)
Slow	Ever	63/61	1.0 (0.5 – 2.0)	44/33	2.0 (0.9 – 4.3)	1.3 (0.8 – 2.2)

* Obtained from a logistical regression model with adjustment for age, diabetes, pancreatitis and ethanol intake (all as categorical variables).

Table V

Interaction between genotype and smoking by gender

Genotype	Smoking (pky)	Male		Female		All Subjects*	
		Case/Con n/n	OR (95% CI)	Case/Con n/n	OR (95% CI)	Case/Con n/n	OR (95% CI)
CYP1A2*ID							
T/T	NS	36/33	1.0	28/41	1.0	1.0	1.0
T/delT & delT/delT	NS	27/31	0.9 (0.4-1.6)	22/43	0.8 (0.4-1.8)	0.8 (0.4-1.3)	0.8 (0.4-1.3)
T/T	≤20	20/29	0.6 (0.3-1.4)	18/13	2.2 (0.8-5.5)	1.1 (0.6-1.9)	1.1 (0.6-1.9)
T/delT & delT/delT	≤20	19/21	0.7 (0.3-1.5)	17/20	1.3 (0.5-3.2)	1.0 (0.5-1.7)	1.0 (0.5-1.7)
T/T	>20	45/29	1.1 (0.5-2.3)	17/13	1.7 (0.6-4.3)	1.4 (0.8-2.4)	1.4 (0.8-2.4)
T/delT & delT/delT	>20	36/33	1.0 (0.4-1.9)	19/10	3.1 (1.0-8.0)	1.4 (0.7-2.3)	1.4 (0.7-2.3)
CYP1A2*IF							
AA	NS	26/27	1.0	25/48	1.0	1.0	1.0
AC/CC	NS	36/38	0.9 (0.4-1.8)	26/38	1.3 (0.6-2.9)	1.1 (0.7-1.9)	1.1 (0.7-1.9)
AA	≤20	16/23	0.6 (0.3-1.5)	15/17	2.1 (0.8-5.2)	1.2 (0.6-2.2)	1.2 (0.6-2.2)
AC/CC	≤20	22/27	0.7 (0.3-1.6)	21/16	2.5 (1.0-6.2)	1.3 (0.7-2.3)	1.3 (0.7-2.3)
AA	>20	34/28	1.1 (0.5-2.3)	13/13	2.0 (0.7-5.6)	1.5 (0.8-2.7)	1.5 (0.8-2.7)
AC/CC	>20	47/34	1.1 (0.5-2.4)	23/11	3.8 (1.5-10.1)	1.9 (1.1-3.4)	1.9 (1.1-3.4)
NAT1							
Reference	NS	37/46	1.0	28/58	1.0	1.0	1.0
Rapid	NS	25/15	2.1 (0.9-4.7)	24/24	2.2 (1.0-4.9)	2.2 (1.2-3.7)	2.2 (1.2-3.7)
Reference	≤20	27/27	1.2 (0.6-2.4)	21/21	2.1 (0.8-5.1)	1.7 (0.9-2.8)	1.7 (0.9-2.8)
Rapid	≤20	10/21	0.4 (0.1-1.1)	13/12	2.6 (1.0-7.1)	1.0 (0.5-2.0)	1.0 (0.5-2.0)
Reference	>20	47/43	1.1 (0.6-2.1)	23/15	3.0 (1.2-7.3)	1.7 (1.0-2.7)	1.7 (1.0-2.7)
Rapid	>20	31/17	2.1 (1.0-4.6)	16/8	4.5 (1.6-12.7)	3.0 (1.6-5.4)	3.0 (1.6-5.4)
NAT2							
Rapid & intermediate	NS	23/23	1.0	24/35	1.0	1.0	1.0
Slow	NS	32/35	1.0 (0.4-2.1)	28/47	0.9 (0.4-2.0)	0.9 (0.5-1.6)	0.9 (0.5-1.6)
Rapid & intermediate	≤20	12/21	0.5 (0.2-1.3)	11/13	1.2 (0.4-3.6)	0.7 (0.4-1.5)	0.7 (0.4-1.5)
Slow	≤20	20/25	0.7 (0.3-1.8)	22/19	1.9 (0.7-4.3)	1.2 (0.6-2.2)	1.2 (0.6-2.2)
Rapid & intermediate	>20	29/26	1.0 (0.5-2.4)	14/9	2.2 (0.7-6.7)	1.5 (0.8-2.7)	1.5 (0.8-2.7)
Slow	>20	42/36	1.1 (0.5-2.6)	21/14	2.0 (0.8-5.1)	1.5 (0.8-2.6)	1.5 (0.8-2.6)

* Obtained from a logistical regression model with adjustment for age, diabetes, pancreatitis and ethanol intake (all as categorical variables).

Table VI
Joint effect of *NAT* and *CYP1A2*1F* on risk of pancreatic cancer

Genotype		Case/Con	* AOR (95% CI)	Case/Con	AOR (95% CI)
<i>NAT1</i>	<i>CYP1A2*1F</i>		Never smokers		Ever smokers
Reference	AA/AC	30/45	1.0	47/49	1.0
Reference	CC	33/58	0.7 (0.4–1.5)	69/57	1.1 (0.6–1.9)
Rapid	AA/AC	17/24	1.3 (0.6–2.9)	27/29	1.0 (0.5–1.9)
Rapid	CC	28/15	2.9 (1.2–6.9)	43/29	1.5 (0.8–2.8)
			Male		Female
Reference	AA/AC	49/49	1.0	28/48	1.0
Reference	CC	59/71	0.7 (0.4–1.3)	43/48	1.3 (0.6–2.7)
Rapid	AA/AC	22/28	0.9 (0.4–1.8)	22/28	1.5 (0.7–3.3)
Rapid	CC	44/29	1.5 (0.8–2.9)	27/18	2.5 (1.1–5.7)
<i>NAT1</i>	<i>NAT2</i>		Never smokers		Ever smokers
Reference	Rapid	19/33	1.0	35/37	1.0
Reference	Slow	40/63	1.1 (0.5–2.4)	72/67	1.3 (0.7–2.4)
Rapid	Rapid	26/21	2.7 (1.1–6.5)	32/27	1.3 (0.6–2.8)
Rapid	Slow	20/16	2.4 (1.0–6.1)	34/27	1.4 (0.7–2.9)
			Male		Female
Reference	Rapid	33/39	1.0	21/33	1.0
Reference	Slow	65/75	1.0 (0.6–1.9)	47/61	1.5 (0.7–3.3)
Rapid	Rapid	31/28	1.2 (0.6–2.6)	27/23	2.5 (1.1–6.1)
Rapid	Slow	30/25	1.5 (0.7–3.1)	24/21	2.2 (0.9–5.5)

* Adjusted for age, diabetes, pancreatitis, ethanol intake (all as categorical variables), and smoking status (in comparisons by sex).