Polymorphisms of cytochrome P4501A2 and N-acetyltransferase genes, smoking, and risk of pancreatic cancer

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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 across smokers, especially heavy smokers (>20 pack years). For example, heavy smokers with the CYP1A2*1D (T-2467delT) delT, CYP1A2*1F (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*1F C allele and NAT1*1rapid’ 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.

Introduction

In the USA, pancreatic cancer is the fourth leading cause of cancer death in both men and women (1). It is a lethal disease: the mortality rate of which approximately equals 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 ~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) IA1, 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. Second, AA-DNA adducts have been identified in human pancreatic tissues (9). Third, the pancreas is highly susceptible to HCA-induced DNA damage (10–13). Fourth, epidemiologic 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 (available at www.imm.ki.se/CYPalleles/cyp1a2.htm). The initial report from Japan (20) reported four SNPs of this gene, resulting in four 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 (available at www.imm.ki.se/CYPalleles/cyp1a2.htm). 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 (21). CYP1A2*1F possesses an intron 1 A-163C (aka A-164C and A-154C) polymorphism that appears to affect the inducibility of the enzyme (22,23). 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 (24). NAT1 and NAT2 catalyze both N-acetylation and O-acetylation (25). 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 bp that exhibit genetic polymorphism in human populations (26). 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 (27,28). The human pancreas predominantly expresses NAT1 (29) 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 the year 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 USA 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-min 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 >100 cigarettes in their lifetime were defined as ever smokers. Smokers who have quit smoking for >1 year before recruitment were defined as former smokers. Those who consumed four 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 standards of the US Department of Agriculture. The 75th percentile value of weekly alcohol intake (grams) 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 (i) failure to donate a blood sample, (ii) failure to complete the risk questionnaire, (iii) having a prior history of cancer (except for non-melanoma skin cancer) and (iv) 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 (20). 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 six SNPs of the NAT2 gene (G282T, T341C, C481T, G590A, A803G and 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 (30) and NAT2 (31) using Tagman-based methods at the University of Virginia. 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 overmapped in both batches of DNA samples. About 10% of the samples were also analyzed using a PCR–RFLP method (32,33).

Statistical analysis

χ2-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 χ2-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 were 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, *12B, *12C and *13 are rapid acetylator NAT2 alleles and all others are slow acetylator NAT2 alleles (25,28). 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 (ORa), smokers with non-at-risk genotype (ORb), 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 \( (S \text{ index}) \) \((34,35)\). 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 \( OR_{11} - 1/\left(OR_{10} + OR_{01} \right) \). All statistical analyses were performed using STATA and SPSS software. \( P \)-values < 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 non-Hispanic whites \( (P = 0.31) \). The distributions of sex and race were non-Hispanic whites \( (P = 0.91) \). Hispanics and African Americans were 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 \( (319 \text{ cases and } 335 \text{ controls}) \). The mean \( \pm SD \) age of cases and controls was 62.3 \( \pm 10.4 \) and 60.4 \( \pm 11.1 \) years, respectively \( (P = 0.01) \). As shown in Table I, controls were overrepresented with individuals younger than 52 years of age. A total of 52% of the cases and 58% of the controls were from Texas, and the remaining were from 39 other USA states. Family history of cancer \( (\text{in the first degree relatives}) \) was not associated with the risk for pancreatic cancer \( (\text{data not shown}) \), 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 \( (>3 \text{ versus } \geq 3 \text{ years}) \). 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 four 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 versus 86 \( g \) in cases and controls, respectively \( (P = 0.002 \text{, Mann–Whitney’s test}) \). However, heavy alcohol consumption \( (>263 \text{ g/week}) \) did not translate into increased risk of pancreatic cancer, and light drinking \( (\leq 263 \text{ g/week}) \) 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 \( (63\% \text{ of controls and } 65\% \text{ of cases}) \) than females \( (40\% \text{ of controls and } 58\% \text{ of cases}) \), but female smokers had a greater risk of developing pancreatic cancer \( (AOR: 2.0, 95\% \text{ CI: } 1.2–3.5) \) than male smokers \( (AOR: 1.0, 95\% \text{ CI: } 0.6–1.5) \). Overall, ever smokers had a 30% increased risk for pancreatic cancer \( (95\% \text{ CI: } 0.9–1.9) \). Former smokers had a 2.2-fold increased risk for pancreatic cancer among females, but not among males. A dose–response relationship was observed between the intensity \( (\text{cigarettes smoked per day}) \) and duration \( (\text{years smoked}) \) of smoking, as well as the product of intensity and duration \( (\text{pack years}) \) 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 with 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 \( (\text{all } P\text{-values } < 0.05) \) 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 trends as Hispanics, with the corresponding allele/genotype frequencies of 0.42, 0.50, 56 and 22% to 22% respectively. Because of

### Table I. Risk factors for pancreatic cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case</th>
<th>Control</th>
<th>OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n = 319 )</td>
<td>( n = 335 )</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \leq 52 )</td>
<td>56 (17.6)</td>
<td>83 (24.8)</td>
<td>1.0</td>
</tr>
<tr>
<td>53–62</td>
<td>97 (30.4)</td>
<td>90 (26.9)</td>
<td>1.60 (1.02–2.49)</td>
</tr>
<tr>
<td>63–69</td>
<td>76 (23.8)</td>
<td>81 (24.2)</td>
<td>1.39 (0.88–2.21)</td>
</tr>
<tr>
<td>( \geq 70 )</td>
<td>90 (28.2)</td>
<td>81 (24.2)</td>
<td>1.65 (1.05–2.59)</td>
</tr>
<tr>
<td>Family history of pancreatic cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>301 (94.4)</td>
<td>322 (97.0)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18 (5.6)</td>
<td>10 (3.0)</td>
<td>1.93 (0.87–4.24)</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>254 (80.6)</td>
<td>294 (91.9)</td>
<td>1.0</td>
</tr>
<tr>
<td>&lt;3 years</td>
<td>36 (11.4)</td>
<td>14 (4.4)</td>
<td>2.98 (1.57–5.64)</td>
</tr>
<tr>
<td>( \geq 3 ) years</td>
<td>25 (7.9)</td>
<td>12 (3.8)</td>
<td>2.41 (1.19–4.90)</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>291 (91.2)</td>
<td>333 (99.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>&lt;3 years</td>
<td>22 (6.9)</td>
<td>0 (0)</td>
<td>3.43 (0.69–17.1)</td>
</tr>
<tr>
<td>( \geq 3 ) years</td>
<td>6 (1.9)</td>
<td>2 (0.6)</td>
<td>0.84 (0.62–1.15)</td>
</tr>
<tr>
<td>Alcohol use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>148 (46.5)</td>
<td>136 (43.4)</td>
<td>1.0</td>
</tr>
<tr>
<td>Ever</td>
<td>170 (53.5)</td>
<td>185 (56.6)</td>
<td>0.84 (0.62–1.15)</td>
</tr>
<tr>
<td>Ethanol/week (g)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>148 (48.4)</td>
<td>136 (43.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>( \leq 263 ) g/wk</td>
<td>96 (31.4)</td>
<td>135 (42.7)</td>
<td>0.65 (0.46–0.93)</td>
</tr>
<tr>
<td>( &gt;263 ) g/wk</td>
<td>62 (20.3)</td>
<td>45 (14.2)</td>
<td>1.27 (0.81–1.98)</td>
</tr>
</tbody>
</table>

*Estimated by unconditional logistic regression.

**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, 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 with 25 and 48 among cases, respectively.**
The frequencies of major \textit{NAT1} genotypes among non-Hispanic white controls was \(4^*4:0.60, \text{ }4^*10:0.27, \text{ }10^*10:0.03, \text{ }4^*3:0.03\) and \(4^*11:0.02. \text{ }\text{NAT1} \text{ alleles were further classified into rapid (}10^* \text{ and } 11^* \text{) and reference (}3^* \text{ and } 4^* \text{) 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 \textit{NAT1} rapid alleles between cases and controls was observed among non-Hispanic whites (\(P = 0.06\)). Logistic regression analysis demonstrated that the \textit{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 \textit{NAT2} allele frequencies detected in the non-Hispanic white control subjects of the current study are comparable with those reported in a large pool of Caucasian controls (36). The observed (versus previously reported) frequencies of the most common \textit{NAT2} genotypes were \(4^*4:0.23, \text{ }5^*/6^*:0.22 \text{ }(0.22), \text{ }4^*/4^*:0.17 \text{ }(0.21), \text{ }4^*/6^*:0.11 \text{ }(0.13), \text{ }4^*/4^*:0.05 \text{ }(0.07) \text{ and } 6^*/6^*:0.08 \text{ }(0.07). \text{ The distribution of the rapid, intermediate and slow acetylator \textit{NAT2} genotypes in cases and controls are shown in Table III. There was no significant difference in the distribution of \textit{NAT2} genotypes between cases and controls within the non-Hispanic white group. The distribution of the \textit{NAT1} rapid genotype was higher among individuals with the \textit{NAT2} rapid than those with the \textit{NAT2} slow genotypes. The frequency of \textit{NAT1} rapid was 41.5% in \textit{NAT2} rapid and 25.3% in \textit{NAT2} slow controls, 51.8% in \textit{NAT2} rapid and 32.5% in \textit{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 \textit{CYP1A2} \textit{IF} allele or \textit{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 with 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 with women carrying the non-at-risk genotypes and who never smoked. There was a significant additive interaction between the presence of \textit{CYP1A2} \textit{IF} 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, \textit{CYP1A2}^1F allele (AOR: 1.2, 95% CI: 0.8–2.0) or \textit{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 \textit{CYP1A2}^1D, \textit{CYP1A2}^1F, \textit{NAT1} or \textit{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 with women who never smoked and carrying the non-at-risk genotypes, women who smoked <20 pack years and carrying the \textit{CYP1A2}^1D \textit{deIT} allele, \textit{CYP1A2}^1F C allele, \textit{NAT1} rapid or \textit{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 \textit{CYP1A2}^1D and \textit{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 \textit{CYP1A2}^1F C allele and \textit{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 \textit{NAT1} rapid and \textit{CYP1A2}^1F C alleles compared with individuals carrying the \textit{NAT1} reference and \textit{CYP1A2}^1F AA/AC genotypes. This effect was more prominent in never smokers (AOR: 0.29, 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 \textit{NAT1} and \textit{NAT2}. This effect was predominantly caused by \textit{NAT1} genotype because individuals with rapid \textit{NAT1} and slow \textit{NAT2} had similar AORs as those with both rapid \textit{NAT1} and \textit{NAT2}.

### Discussion

To our knowledge, the current study is the first to report a significant effect of the \textit{NAT1} gene and interactions of \textit{NAT1} and \textit{CYP1A2} genotypes with smoking on the risk for pancreatic cancer. We have shown that the \textit{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 \textit{CYP1A2}^1F C allele and \textit{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 \textit{CYP1A2} and \textit{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 \textit{CYP1A2}^1F polymorphism in several different populations has been reported. Among populations in Britain (21), Germany (22), Denmark (37), Egypt (38) and China (39), the C allele frequency was ~0.31–0.34. The frequency was relatively higher among Japanese (0.39) (available at www.imm.ki.se/CYPalleles/cyp1a2.htm) and Ethiopians (0.40) (40). In the USA, the only reported study was conducted among Hawaiian women, and the frequency was 0.30 (41). The C allele frequency among non-Hispanic white controls in

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*Obtained from a logistical regression model with adjustment for age, diabetes, pancreatitis and ethanol intake (all as categorical variables).
Neither the study of 49 colon cancer cases and 65 controls (22) nor the study of 164 ovarian cancer cases and 194 controls (41) 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 (19,42,43). 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 (44). However, the functional significance of the CYP1A2*IF allele is not clear at present. One study found a higher enzyme activity associated with the A allele among smokers (21), another study found no difference in the enzyme activity and inducibility between the A and C alleles (40). Our study was 0.31, which is quite comparable with the reported frequencies.

The association between the CYP1A2*IF polymorphism and risk of cancer has previously been investigated in two studies.
of the short distance between the two genes on the same chromosome has been previously reported in several studies (56–58). Because the current study observed a differential distribution of NAT1 rapid genotypes 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 rapid allele in NAT2 rapid acetylators has been previously reported in several studies (56–58). 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 among men (6). In addition, epidemiologic studies have observed higher smoking-related relative risks of pancreatic cancer among women than among men (59,60). 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 as 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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References


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