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Haplotype of *N*-Acetyltransferase 1 and 2 and Risk of Pancreatic Cancer

Li Jiao[1], **Mark A. Doll**[4], **David W. Hein**[4], **Melissa L. Bondy**[2], **Manal M. Hassan**[1], **James E. Hixson**[3], **James L. Abbruzzese**[1], and **Donghui Li**[1]

1*Department of Gastrointestinal Medical Oncology, The University of Texas M. D. Anderson Cancer Center*

2*Department of Epidemiology, The University of Texas M. D. Anderson Cancer Center*

3*Human Genetics Center, The University of Texas School of Public Health, Houston, Texas*

4*Department of Pharmacology and Toxicology and Brown Cancer Center, University of Louisville School of Medicine, Louisville, Kentucky*

Abstract

We examined the association between *N*-acetyltransferase 1 and 2 (*NAT1* and *NAT2*) haplotype and risk of pancreatic cancer by genotyping eight *NAT1* and seven *NAT2* single nucleotide polymorphisms in 532 patients and in 581 healthy controls (all non-Hispanic white) who were recruited at M. D. Anderson Cancer Center from January 2000 to December 2006. Haplotypes were reconstructed by using an expectation-maximization algorithm. Odds ratios and 95% confidence intervals were estimated by using unconditional logistic regression models. Covariates included age (continuous variable), sex, pack-year of smoking (categorical), and history of diabetes when appropriate. *NAT1* and *NAT2* genotype was mutually adjusted. The prevalence of haplotype *NAT1*10-NAT2*6A* was 4.3% versus 2.7% ($P = 0.06$) and *NAT1*11-NAT2*6A* was 1.2% versus 0.4% ($P = 0.05$) in patients and controls, respectively. The diplotype *NAT1*10/*10* or *NAT1*10/*11* and *NAT2*6A*/any slow allele was associated with a higher risk of pancreatic cancer compared with other diplotypes (multivariate odds ratio, 4.15; 95% confidence interval, 1.15-15.00; $P = 0.03$). *NAT2* slow genotype were associated with increased risk of pancreatic cancer among heavy smokers and among individuals with a history of diabetes. We for the first time found that rare *NAT1*10* or *NAT1*11-NAT2*6A* diplotype may be an “at-risk” genetic variant for pancreatic cancer. The *NAT2*6A*/any slow acetylation genotype may be a predisposing factor for pancreatic cancer among diabetics with smoking exposure. Our observations must be confirmed in larger independent studies.

Introduction

Pancreatic cancer is considered an “environmental” cancer in that cigarette smoking (1) and red meat intake (2) have been linked with its etiology. Aromatic amines are putative carcinogens in cigarette and heterocyclic amines in meat cooked at high temperature (3,4). Aromatic amine and heterocyclic amine carcinogens require metabolic activation to induce DNA damage. In humans, aromatic amine and heterocyclic amine are bioactivated in a two-step process that includes oxidation by cytochrome P_{450} enzymes and subsequent acetylation by the expression products of either of the arylamine *N*-acetyltransferase (*NAT*) genes (EC 2.3.1.5), *NAT1* and *NAT2* (5,6). This conjugation reaction can result in either detoxification

Requests for reprints: Donghui Li, Department of Gastrointestinal Medical Oncology, The University of Texas M. D. Anderson Cancer Center, Unit 426, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-834-6690; Fax: 713-834-6153. E-mail: dli@mdanderson.org.

(*N*-acetylation) or activation (*O*-acetylation) of these metabolites. The *N*-acetoxy metabolites are unstable and react with DNA to form covalent adducts, which is believed to be the first event in aromatic amine - induced carcinogenesis. Several studies have suggested that acetylation polymorphism is a factor in genetic predisposition to cancers related to aromatic amine and/or heterocyclic amine exposure (7). If NAT plays a role in metabolizing aromatic amine/heterocyclic amine, we would expect genetic variants of *NAT* modify individual susceptibility to pancreatic cancer.

In humans, *NAT1*, *NAT2*, and a pseudogene, *NATP*, are located at chromosome 8p21.3-23.1 (8), which is an unstable region of the human genome that is often deleted and sometimes overrepresented in tumors (9). Human *NAT1* and *NAT2* loci are highly polymorphic. As of May 2007, 26 *NAT1* and 36 *NAT2* alleles (or haplotypes) have been identified in human populations (10,11).[5] Most of the *NAT1* and all of the *NAT2* nucleotide polymorphisms are present in the coding region. These nucleotide substitutions result in alterations of substrate affinity, catalytic activity, protein degradation, or stability of the recombinant *NAT* allozymes (12-14). Good concordance between *NAT2* genotype and acetylator phenotype has been established (15). Both NAT1 and NAT2 are expressed in the human pancreas, but NAT1 expression is more predominant in this organ (16).

Our previous study showed that *NAT1* genotype alone modified risk of pancreatic cancer and both *NAT1* and *NAT2* genotype modified risk of pancreatic cancer among smokers, especially among women. A possible interaction between *NAT1* and *NAT2* genotype was also observed (17). In the current study, we examined *NAT1* and *NAT2* haplotypes and risk of pancreatic cancer in a larger study population. We hypothesized that the combination of rapid *NAT1* allele (increased capacity to activate aromatic amine carcinogen via *O*-acetylation) and slow *NAT2* allele (reduced capacity to detoxify aromatic amine carcinogens via *N*-acetylation) increases the risk of developing pancreatic cancer. We also did hypothesis-generating analysis to explore the interaction between *NAT* genotype, history of diabetes and pancreatitis, and family history of cancer in addition to cigarette smoking.

Materials and Methods

Study Subjects

The study design, patient recruitment, and data collection methods have been described previously (17). Written informed consent was obtained from each participant for an in-person interview and donation of biological specimens. The research protocol was approved by The University of Texas M. D. Anderson institutional review board.

All patients were recruited from the Gastrointestinal Cancer Clinic at M. D. Anderson Cancer Center. The eligibility criteria were having pathologically confirmed primary pancreatic ductal adenocarcinoma (International Classification of Diseases for Oncology code C25.3, WHO, 2000) and U.S. residency. Controls were recruited from healthy individuals (spouses, friends, or other family members) who were escorting cancer patients during their visits to M. D. Anderson and were not genetically related to their respective patients. A total of 1,716 participants (938 patients and 778 controls) were recruited from January 2000 to December 2006, with a response rate (recruited/approached) of 78.6% for patients and 80.0% for controls. Among them, 760 (81.0%) cases and 654 (84.1%) controls donated biospecimens for genotyping (including mouth wash samples from 18 patients and 121 controls). Sixty-nine patients and one control were excluded because of prior history of cancer (except for nonmelanoma cancer of the skin), yielding 691 patients and 653 controls. Fifteen controls were

[5] http://www.louisville.edu/medschool/pharmacology/NAT.html

excluded because of incomplete questionnaire data. Because of the striking ethnic variation in *NAT1* and *NAT2* allele frequencies and the small number of participants in minorities, we did risk association analyses in non-Hispanic whites only, including 597 patients and 582 controls (accounting for 86.4% of the patients and 91.2% of the controls).

Exposure Data Collection

At the time of recruitment, trained interviewers conducted in-person interviews with patients and controls to collect information on demographics and smoking exposure with the use of a structured questionnaire. No proxy respondent or inducement was used. Definitions of the smoking exposure variables have been described previously (17). Briefly, subjects were classified as "ever smokers" or "never smokers" according to whether they had smoked >100 cigarettes in their lifetime. Pack-year of smoking was calculated from the average number of cigarette smoked daily and the number of years of smoking. The median pack-year of controls (22 pack-years) was used as the cutoff to define "light" and "heavy" smokers. Information on family history of cancer, history of cancer, and history of pancreatitis and diabetes was also collected. Body mass index (BMI; kg/m^2) was calculated using self-reported height and weight at age of 14 to 19, 20, 30, 40, 50, 60, and 70 years in 682 participants (322 patients and 360 controls) who were recruited after 2005. We used BMI at age of 40 years as the usual adult BMI in the further analysis.

Laboratory Analysis

Methods used for DNA extraction and *NAT* genotyping have been described previously (17). Eight single nucleotide polymorphisms (SNP; from 5′ to 3′) of the *NAT1* gene (C97T, C190T, G445A, G559A, G560A, A752T, T1088A, and C1095A) and seven SNPs of the *NAT2* gene (G191A, C282T, T341C, C481T, G590A, A803G, and G857A) were determined by using a Taqman allele-specific assay at the University of Louisville (18,19). Ten percent of samples were duplicated for each polymorphic site, and the genotype assignments were found to be 99.4% concordant. Any discrepancies were resolved by additional genotyping. Because the aim of the current study was to examine the association between risk of pancreatic cancer and *NAT1/2* haplotype, we further excluded 65 patients and one control from the final analysis because of incomplete genotyping data caused by inadequate quality or quantity of DNA. The final sample size was 532 patients and 581 controls. *NAT1* genotype data were available for 532 patients and 581 controls, and *NAT2* genotype data were available for 526 patients and 564 controls. A total of 512 patients and 559 controls had complete data for all 15 SNPs. Demographic characteristics (age, sex, and smoking status) of patients included in the analyses were comparable with those being excluded because of incomplete genotyping data. The call rate for single allele varied from 95% to 100% for adequate DNA samples (A260/A280 >1.70 and <1.85 with adequate quantity).

Statistical Analysis

SNPAlyze software (Dynacom Co. Ltd.) was used to test the Hardy-Weinberg equilibrium of each genotype and to test the linkage disequilibrium of each two polymorphic loci of *NAT1* and *NAT2* genes. The pairwise linkage disequilibrium was measured by variables *D*' and r^2. *NAT1* and *NAT2* haplotypes were inferred based on the subjects with complete genotyping data by using an expectation-maximization algorithm (SNPAlyze software) and a Bayesian algorithm (PHASE program, version 2.0; ref. 20). Both algorithms generated the same haplotype inference, which was also shown by a previous study (21). When reconstructing the *NAT1-NAT2* combined haplotype, we excluded five SNPs (*NAT1*: C97T, C190T, G559A, and A752T; *NAT2*: G190A) because of little or no variation (less than five in count) in our study subjects. Haplotypes for the *NAT1* or *NAT2* genes were represented as a string of "0" indicating the common alleles and "2" indicating the minor allele for polymorphism from 5′ to 3′. The

combined haplotype for the *NAT1* and *NAT2* gene was represented in the same manner. Pearson's χ^2 test was used to test the difference in the distribution of the haplotypes between the patients and controls. Cornfield odds ratio (OR) and 95% confidence interval (95% CI) were calculated. Multivariate ORs and 95% CIs were estimated to measure the strength of the association between each genotype, diplotype, and pancreatic cancer risk by using unconditional logistic regression models. For *NAT1*, **10* and **11* alleles were defined as the "rapid" acetylator allele (22), and all others were combined as the reference group. The presence of 445A and 1095A variants defined the *NAT1*11* allele. For *NAT2*, subjects with two slow acetylator alleles (**5*, **6*, **7*, and **14B*) were considered "slow acetylators"; those with two rapid acetylator alleles (**4*, **12A*, and **13*) were "rapid acetylators"; and those having a combination of rapid and slow acetylator alleles were "intermediate acetylators" (12,23). Because *NAT2*6A* allele was a putative "at-risk" allele revealed by the haplotype analysis, any "slow acetylator" containing **6A* was considered the at-risk group and all other alleles were pooled as the reference group. Potential confounding factor was adjusted in the multivariate model when its removal from the multivariate model caused the β estimate to change by >10%. Consequently, age (as a continuous variable), sex, pack-year of smoking (categorical), and history of diabetes were included as covariates in the multiple logistic regression models when appropriate. *NAT1* and *NAT2* genotype was mutually adjusted in the model because of the potential interaction between the two genes. For 682 participants, BMI at age of 40 years was available and was evaluated as a potential confounder for the interaction of *NAT* polymorphism and diabetes in modifying pancreatic cancer risk. We found that the addition of BMI (categorical using WHO standard) in the model changed the risk estimated by 4.4%. Therefore, we did not consider BMI as a significant confounder in the current analysis. Interaction between *NAT* genotype and smoking status (ever versus never), pack-year of smoking (never, light, and heavy), diabetes, pancreatitis, and family history of cancer in modulating risk of pancreatic cancer was evaluated in a multiplicative scale using a Wald χ^2 test by including an interaction term in the unconditional logistic regression models. The likelihood ratio test was used to test the significance of the interaction term. All statistical analyses were done by using the Stata software program (version 7.0; StataCorp). All tests were two sided with an α level of 0.05. We estimated the false-positive report probability (FPRP) for the observed statistically significant association using the methods described by Wacholder et al. (24). The FPRP depends on the power of the study, the observed *P* value, and the prior probability that the SNP or gene under investigation is involved in the disease. We considered that a prior probability of 25% might be appropriate when there is biological plausibility and availability of previous epidemiologic evidence for such an association and that a prior probability of 0.1% might be appropriate when lack of both biological knowledge and epidemiologic data. The FPRP value for noteworthiness was set as 0.5 for new findings or 0.2 for repeating previously observed association.

Results

Risk Factors

The distributions of age, sex, and smoking status of 532 patients and 581 healthy controls were similar to those described previously (17). Briefly, the age distribution (in 10-year intervals) of patients and controls was equivalent ($P = 0.28$, χ^2 test). Men contributed 64.0% of the controls and 60.0% of the patients ($P = 0.12$, χ^2 test). Cigarette smoking had a significant positive association with the risk of pancreatic cancer (OR, 1.45; 95% CI, 1.13-1.85; $P = 0.002$). Heavy smokers (>22 pack-years of smoking) were at significantly higher risk compared with never smokers (OR, 1.68; 95% CI, 1.28-2.21; $P < 0.001$). The family history of cancer-related OR was 1.64 (95% CI, 1.25-2.16; $P = 0.003$) and family history of pancreatic cancer-related OR was 2.39 (95% CI, 1.06-5.74; $P = 0.02$). Significantly more patients than controls reported ever being diagnosed with diabetes (20.3% versus 8.9%; OR, 2.59; 95% CI, 1.80-3.77; $P <$

0.001) and pancreatitis (8.1% versus 0.9%; OR, 10.1; 95% CI, 3.95-32.8; $P < 0.001$). In 682 participants, BMI at age of 40 years was used in risk estimate. Compared with individuals with BMI ≤25, those with BMI >30 were at a significantly higher risk of developing diabetes in controls (OR, 7.90; 95% CI, 2.59-24.2) and pancreatic cancer (OR, 1.61; 95% CI, 1.01-2.55) after adjustment for age, sex, and pack-years of smoking.

Single Nucleotide Polymorphism

Table 1 presents *NAT1* and *NAT2* SNP information and variables of linkage disequilibrium between alleles. Of the eight *NAT1* SNPs investigated, C97T was monomorphic and three others (190T, 559A, and 752T) were very rare (less than five in count) in our study subjects. Of the seven *NAT2* SNPs investigated, 191A was found in only one person. These SNPs are not listed in Table 1. All observed genotype frequencies were in good agreement with the expected frequencies deduced by the Hardy-Weinberg law in controls. For *NAT1*, the 1088A and 1095A SNPs were the most prevalent and were in complete linkage disequilibrium ($D' = 1.0$). For *NAT2*, frequencies of all SNPs except 857A were more than 25%, and considerable linkage disequilibrium existed, with all D' values ≥0.95. Moderate linkage disequilibrium was present between the two major *NAT1* SNPs and all *NAT2* SNPs except for G857A.

Genotype

Table 2 presents the distribution of the *NAT1* and *NAT2* genotypes in patients and controls. *NAT1* rapid acetylation genotype (presence of **10/*10* and **10/*11*) was associated with significantly increased risk of pancreatic cancer. The adjusted OR was 1.97 (95% CI, 1.02-3.82; $P = 0.04$) compared with the *NAT1*4/*4* genotype and 1.96 (95% CI, 1.03-3.73; $P = 0.04$) compared with all other *NAT1* genotypes in combination. We estimated the FPRP of this observation to be 0.078 to 0.20 given a prior probability of 10% to 25% because data on both epidemiologic association (17) and functional significance of *NAT1* allele (25,26) were available. The FPRP below threshold of 0.2 indicated noteworthiness. The distribution of *NAT2* genotype was comparable between patients and controls.

Haplotype

Table 3 shows the 12, 11, and 17 haplotypes that were inferred for the *NAT1*, *NAT2*, and *NAT1-NAT2* genes, respectively. For *NAT1*, haplotype 1 (wild-type **4* allele defined as no SNP) was present in three quarters of the chromosomes tested. Haplotypes 1 and 2 (1088A/1095A, *NAT1*10* allele) accounted for 93.0% of the *NAT1* haplotypes in the controls. For *NAT2*, 341C-481T-803G (**5B* allele) was the most common haplotype followed by 282T-590A (**6A* allele) and the reference **4* (defined as no SNP). Among the *NAT1-NAT2* haplotypes, five were present at frequencies ≥5%, including *NAT1*4-NAT2*5B*, *NAT1*4-NAT2*6A*, *NAT1*4-NAT2*4*, *NAT1*10-NAT2*4*, and *NAT1*10-NAT2*5B*.

The haplotype frequency was comparable between patients and controls for both *NAT1* and *NAT2*. However, we observed a higher frequency of *NAT1*10-NAT2*6A* ($P = 0.06$) and *NAT1*11-NAT2*6A* ($P = 0.05$) in patients than in controls, although both are at borderline significance. Compared with *NAT1*4-NAT2*4*, the Cornfield ORs were 1.61 (95% CI, 0.93-2.82) for the *NAT1*10-NAT2*6A* and 2.94 (95% CI, 0.88-12.6) for the *NAT1*11-NAT2*6A* haplotype.

Diplotype

Table 4 shows diplotype (haplotype pair) analysis for combined *NAT1-NAT2* genes. The comparison group consisted of 15 people carrying the *NAT1* homozygous haplotype **10/*10* or **10/*11* (rapid/rapid) and *NAT2*6*/any slow allele. All other diplotypes were pooled as the reference group. Twelve of the 15 study subjects (1.3% of total) carrying the at-risk diplotype

were patients, giving an OR (adjusted for age, sex, diabetes, and smoking pack-year) of 4.15 (95% CI, 1.15-15.00; $P = 0.03$). The FPRP for this association was 0.36 if we set FPRP value for noteworthiness as 0.50. A prior probability of 10% is appropriate because the association is biologically plausible but there was no previous epidemiologic evidence.

Joint Effect of *NAT2* Genotype and Smoking

NAT2 acetylator genotype interacted with smoking pack-years in modulating the risk of pancreatic cancer (*P* value for likelihood ratio test = 0.04). Compared with never smokers who carried the *NAT2* rapid, the multivariate OR (95% CI) was 0.75 (0.53-1.06) for never smokers who carried slow genotype, 1.34 (0.89-2.20) for heavy smokers who carried the *NAT2* rapid, and 1.54 (1.05-2.26) for heavy smokers who carried the *NAT2* slow acetylator genotype. For this observation, we considered FPRP below threshold 0.2 to be noteworthy. We estimated the FPRP to be 0.15 given a prior probability of 25% because data on both epidemiologic association (17) and functional significance of *NAT2* allele (6,27) were available.

We did not observe significant interaction effect between *NAT1* genotype and *NAT2* genotype ($P_{interaction} = 0.23$), smoking ($P_{interaction} = 0.44$), diabetes ($P_{interaction} = 0.22$), pancreatitis, or family history of pancreatic cancer in modulating risk of pancreatic cancer.

Joint Effect of *NAT2* Genotype and Diabetes

Table 5 shows a joint effect of *NAT2*6A*/any slow allele and diabetes in modulating risk of pancreatic cancer. Compared with nondiabetics who had all other *NAT2* genotype, diabetics with the *NAT2*6A*/any slow allele had a multivariate OR of 3.51 (95% CI, 1.90-6.50; $P < 0.001$). Compared with nondiabetics with the *NAT2* rapid/intermediate allele, diabetics with the *NAT2* slow allele had a multivariate OR of 3.10 (95% CI, 1.90-5.05; $P < 0.001$). However, this effect was present among smokers (OR, 4.65; 95% CI, 2.29-9.42) but absent among nonsmokers (OR, 2.07; 95% CI, 1.03-3.99). In the stratified analysis, the association between *NAT2*6A*/any slow genotype and increased risk of pancreatic cancer was seen among diabetics (multivariate OR, 2.28; 95% CI, 1.02-5.10) but not among nondiabetics (multivariate OR, 0.86; 95% CI, 0.65-1.14). Because recent onset of diabetes could be a manifestation of pancreatic cancer, we did these analyses after excluding participants who had recent diagnosis of diabetes (less than a year before recruitment). Compared with nondiabetics with all other *NAT2* genotype, diabetics with all other *NAT2* genotype had a multivariate OR of 1.23 (95% CI, 0.69-2.19) and diabetics with *NAT2*6A*/any slow allele had a multivariate OR of 2.49 (95% CI, 1.21-5.12; $P = 0.01$).

Discussion

In this hospital-based case-control study, we found that *NAT1*10-NAT2*6* and *NAT1*11-NAT2*6*, with a prevalence of 3% in our study population, may be the at-risk haplotype in the development of pancreatic cancer. Further, people carrying the diplotype *NAT1*10/*10* or *NAT1*10/*11-NAT2*6A*/any allele were more likely to have pancreatic cancer than those carrying other diplotypes. We found carriers of the *NAT2*6A*/any slow allele to be a predisposing factor for pancreatic cancer among smokers with a history of diabetes. Meanwhile, we confirmed our previous observation that *NAT1* rapid (**10* and **11*) and *NAT2* slow acetylator alleles were associated with a significantly increased risk of pancreatic cancer among the entire study population and among heavy smokers, respectively (17). These replicated associations are likely to be true based on the FPRP calculation. Our study provides further evidence supporting an important role of *N*-acetylation polymorphisms in the etiology of pancreatic cancer.

The genotype analysis found a significantly increased risk for pancreatic cancer in *NAT1*10* or *NAT1*11* carriers. The genotype-phenotype relationship for *NAT1*10* and *NAT1*11* allele has not been well elucidated (25,26,28) as for *NAT2* gene (15). The *NAT1*10* allele may be a rapid acetylator allele because it has been associated with elevated NAT1 activity in bladder and colon tissues (28) and higher levels of DNA adducts in urinary bladder mucosa (29) as well as an increased risk of colorectal (28), bladder (30), and breast cancer (31). The *NAT1*11* allele may also be a rapid allele. One study found that the missense G445A ($Val^{149}Ile$) substitution yielded recombinant NAT1 proteins that were twice as active as the wild-type protein in *N*-hydroxy-aromatic amine acetylation (32). The *NAT1*11* allele has been linked to an elevated risk of breast cancer associated with smoking or the consumption of well-done meat (22). The study on phenotype of *NAT2* alleles was done in a caffeine test; the acetylation activity of **5/*6A* was significantly lower than that of **5/*5* and the **6A/*6A* genotype was even less active (33) The current study confirmed our previous observation that *NAT2* slow allele was associated with increased risk of pancreatic cancer among heavy smokers.

To our knowledge, no haplotype analyses have been conducted on *NAT1* and *NAT2* SNPs in relation to pancreatic cancer. In the current study, we found the following combined *NAT1-NAT2* haplotypes (in descending order of frequency): **4-*5B*, **4-*6A*, **4-*4*, **10-*4*, **10-*5B*, **10-*6A*, **4-*5A*, **4-*7B*, and **11-*6A*. We found that the rapid acetylator *NAT1*10* or *NAT1*11* alleles in combination with the slow acetylator *NAT2*6A* allele conferred a borderline significant higher risk of developing pancreatic cancer, and the risk was more than 3-fold higher among participants who carried the *NAT1*10* or *NAT1*11-NAT2*6A* diplotype. However, the low frequency of this diplotype limited the study power with regard to drawing conclusions. Notably, we found that 12 of 15 *NAT1*10/*11-NAT2*6A* carriers were smokers and 10 of the 12 smokers and 2 of the 3 nonsmokers were pancreatic cancer patients. Our observations support the notion that haplotype structure rather than individual allozyme can be the principal determinant of phenotypic consequences (21).

NAT1 is highly expressed in the human pancreas (16). Our observations may be explained by an insufficient deactivation of carcinogens (*N*-acetylation) mediated by slow acetylator of *NAT2* in the liver and higher-level *NAT1*-catalyzed activation (*O*-acetylation) within the pancreas. It has been proposed that tissue-specific NAT-catalyzed *O*-acetylation of the *N*-hydroxy-aromatic amines is an important determinant of DNA adduct formation (34). Previous studies on *NAT1* genotype and DNA adducts in the human pancreas have been limited (35, 36). The regulatory mechanisms of NAT1 in the human pancreas deserve further investigation.

We observed a possible effect of *NAT2*6A*/any slow acetylation genotype on risk of pancreatic cancer among individuals with a history of diabetes and this effect seems to be present in smokers but absent in nonsmokers. Interestingly, *NAT2* slow allele (especially **6A*, 282T-590A) was found to confer a 5-fold increased risk of type II diabetes in a Turkish study of 79 cases and 104 controls (37). Among nondiabetic Canadian people, those with the *NAT2* 282T SNP had significantly higher plasma fasting glucose levels than those with the 282C SNP (38). In fact, C282T is a silent allele but it is in strong linkage with the G590A allele that has been shown to reduce thermostability and level of soluble NAT2 protein (39). However, the *NAT2* slow acetylator genotype was not shown to be associated with diabetes either in the same study (38) or in a Japanese study (40). Whether acetylation polymorphisms affect the biotransformation of endogenous or exogenous substrates associated with diabetes is unknown. Because smoking is associated with increased risk of diabetes and NAT is involved in the metabolism of tobacco carcinogens, the observed effect of *NAT2* genotype on diabetes-associated risk of pancreatic cancer in the current study most likely is a confounding effect of cigarette smoking. Because diabetes is such a common disease and ~35% of Caucasians are **6A*/slow *NAT2* acetylators, our finding deserves further replication in other studies.

By genotyping several SNPs with a validated assay, our study minimized the bias produced by misclassification of the *NAT1* and *NAT2* genotype (41,42). The *NAT1* and *NAT2* allele, haplotype, and genotype frequencies were similar to or within the ranges previously reported for Caucasians (15,33,43). The current study also had several limitations. First, because different racial groups show large variations in slow and rapid acetylator distribution, our findings may not be generalizable to other ethnicities. Second, selection bias is a potential concern because patients and controls voluntarily participated in the study; however, it is unlikely that genotype frequencies vary by willingness of participation (44). Third, this study was underpowered in examining relationship between haplotype and pancreatic cancer risk and the observed associations were largely borderline significant. For example, the difference between *NAT1-NAT2* haplotype frequencies is not statistically significant if multiple comparisons are adjusted. Although FPRP report suggested our findings were noteworthy, it only indicated that these observations deserve confirmation in larger independent studies. Fourth, because obesity is involved in the etiology of diabetes and pancreatic cancer, its confounding effect on *NAT2* polymorphism and diabetes interaction on risk of pancreatic cancer should be fully evaluated in future studies.

In summary, our findings provide further evidence that acetylation polymorphisms modify susceptibility to pancreatic cancer especially among smokers and diabetics. These observations need further replication.

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Table 1

Characteristics of *NAT1* and *NAT2* SNPs and the linkage disequilibrium coefficients between 10 SNPs

Gene	Reference SNP ID	Amino acid change	MAF	*P* value HWE	Pairwise linkage disequilibrium coefficients (*D'* above diagonal, r^2 below diagonal)									
					NAT1				*NAT2*					
					G445A	G560A	T1088A	C1095A	C282T	T341C	C481T	G590A	A803G	G857A
NAT1	G445A	V149I	0.017	0.20				**1.00**						**0.07**
	G560A	R187Q	0.024	0.50			**1.00**	**1.00**		**-0.60**	-0.59		**-0.58**	
	T1088A	None	0.196	0.37		**0.10**		**1.00**	**-0.55**	**-0.27**	**-0.34**	**-0.50**	**-0.23**	
	C1095A	None	0.242	0.90	**0.05**	**0.08**	**0.76**		**-0.50**	**-0.23**	**-0.29**	**-0.47**	**-0.18**	
NAT2	C282T	None	0.292	0.15			**0.03**	**0.03**		**-1.00**	**-1.00**	**0.99**	**-0.99**	**1.00**
	T341C	I114T	0.474	0.52		**0.007**	**0.02**	**0.01**	**0.37**		**1.00**	**-1.00**	**0.99**	**-1.00**
	C481T	None	0.464	0.62		**0.007**	**0.03**	**0.02**	**0.36**	**0.96**		**-1.00**	**0.95**	**-1.00**
	G590A	R197Q	0.275	0.27			**0.02**	**0.03**	**0.90**	**0.34**	**0.32**		**-0.99**	
	A803G	K268R	0.458	0.64		**0.007**	**0.01**	**0.009**	**0.34**	**0.92**	**0.87**	**0.31**		**-1.00**
	G857A	G286E	0.016	0.15	**0.005**				**0.04**	**0.01**	**0.01**		**0.01**	

NOTE: Blank indicates that SNPs were not in significant linkage disequilibrium.

Abbreviations: MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.

Table 2

Association between *NAT1* and *NAT2* genotypes and risk of pancreatic cancer

Genotype	Patients, *n* (%)	Controls, *n* (%)	OR (95% CI)*	Acetylator phenotype
*NAT1*4/*4*	291 (55.8)	337 (58.4)	1.00	Reference
*NAT1*4/*other†	13 (2.5)	16 (2.8)	1.04 (0.45-2.43)	
*NAT1*10/*other†	142 (27.2)	154 (26.7)	1.05 (0.76-1.46)	
*NAT1*11/*other†	24 (4.6)	18 (3.1)	1.19 (0.56-2.54)	
*NAT1*10/*10 + NAT1*10/*11*	31 (5.9)	23 (4.0)	1.97 (1.02-3.82)	Rapid
*NAT1*4/*3*	21 (4.0)	29 (5.0)	0.79 (0.39-1.59)	Reference
NAT1 other	487 (94.0)	553 (96.0)	1.00	
*NAT1*10/*10 + NAT1*10/*11*	31 (6.0)	23 (4.0)	1.96 (1.03-3.73)	Rapid
*NAT2*4/*4*	31 (5.9)	27 (4.8)	1.00	Rapid
*NAT2*4/*5B*	111 (21.1)	125 (22.2)	0.59 (0.30-1.16)	Intermediate
*NAT2*4/*6A*	65 (12.4)	71 (12.6)	0.61 (0.30-1.27)	Intermediate
*NAT2*4/*5*‡	9 (1.7)	5 (0.9)	1.75 (0.36-8.43)	Intermediate
Other *NAT2* intermediate acetylator genotype‡	10 (1.9)	10 (1.8)	0.85 (0.27-2.69)	Intermediate
*NAT2*5B/*6A*	118 (22.4)	124 (22.0)	0.72 (0.36-1.42)	Slow
*NAT2*5B/*5B*	83 (15.8)	104 (18.4)	0.67 (0.33-1.34)	Slow
*NAT2*6A/*6A*	37 (7.0)	45 (8.0)	0.64 (0.29-1.40)	Slow
*NAT2*5A/*5B*	18 (3.4)	14 (2.5)	1.07 (0.39-3.00)	Slow
*NAT2*5B/*7B*	12 (2.3)	11 (1.9)	0.77 (0.25-2.39)	Slow
*NAT2*6A/*7B*	12 (2.3)	9 (1.6)	0.80 (0.24-2.67)	Slow
*NAT2*5/*5* and *NAT2*5/*6*‡	8 (1.7)	9 (1.6)	0.87 (0.27-2.81)	Slow
*NAT2*5A/*6A*‡	6 (1.1)	9 (1.6)	0.69 (0.19-2.57)	Slow
Other *NAT2* slow acetylator genotype‡	5 (0.9)	1 (0.2)	3.91 (0.39-38.9)	Slow
NAT2 other	349 (66.4)	371 (65.8)	1.00	
*NAT2*6A*/any*	177 (33.6)	193 (34.2)	0.97 (0.75-1.26)	Slow

* OR adjusted for age, sex, pack-year of smoking (categorical), history of diabetes, and *NAT2* genotype (slow versus other, for the *NAT1* analysis) or *NAT1* genotype (rapid versus other, for the *NAT2* analysis).

† *NAT1*4*/other comprises **4/*14B, *4/*15, *4/*17, *4/*22, *4/*14A*, and **10/*14B*; **10*/other comprises **4/*10, *3/*10*, and **10/*14A*; **11*/other comprises **4/*11A* or **3/*11B, *4/*11B, *3/*11A*, and **11B/*22*.

‡ Intermediate comprises **4/*6B, *4/*7B, *5B/*12A, *5B/13, 5B/*14B, *6A/*12A*, and **6A/*13*; **5* comprises **5A* and **5C*; **5/*5* and **5/*6* comprise **5B/*5C, *5C/*5C, *5B/*6C*, and **5C/*6A*; other slow comprises **5A/*7B, *5B/*13, *6A/*6C*, and **7B/*7B*.

Table 3

Haplotype frequencies of *NAT1* and *NAT2* in patients and controls

Haplotype	Haplotype sequence	Allele	Acetylator phenotype	Frequency in patients	Frequency in controls	*P*	Univariate OR (95% CI)
NAT1[*]							
1	0-0-0-0-0-0-0-0	**4*	Reference	0.743	0.759	0.42	1.00
2	0-0-0-0-0-0-2-2	**10*	Rapid	0.191	0.171	0.26	1.14 (0.90-1.45)
3	0-0-2-0-0-0-0-2	**11*	Rapid	0.024	0.014	0.12	1.72 (0.85-3.64)
4	0-0-0-0-0-0-0-2	**3*	Reference	0.020	0.031	0.14	0.67 (0.36-1.23)
5	0-0-0-0-2-0-2-2	**14A*	Slow	0.012	0.020	0.12	0.55 (0.24-1.22)
6	0-0-0-0-2-0-0-0	**14B*	Slow	0.0025	0.0012	0.50	2.01 (0.10-118.9)
7	0-0-0-0-0-2-0-0	**22*	Slow	0.0010	0.0010	0.99	NE
8	0-0-0-2-0-0-2-2	**15*	Slow	0.0020	0	0.27	NE
9	0-0-2-0-0-0-0-0			0.0028	5.80E-09	0.07	NE
10	0-2-0-0-0-0-0-0			0	0.00139	0.34	NE
11	0-0-2-0-0-2-0-0			0.0010	0	0.30	NE
12	0-0-2-0-0-0-2-2			3.23E-4	0.001	0.61	NE
NAT2[†]							
1	0-0-0-0-0-0-0	**4*	Rapid	0.235	0.231	0.86	1.00
2	0-0-2-2-0-2-0	**5B*	Slow	0.411	0.433	0.33	0.93 (0.74-1.18)
3	0-2-0-0-2-0-0	**6A*	Slow	0.272	0.277	0.81	0.97 (0.75-1.25)
4	0-0-2-2-0-0-0	**5A*	Slow	0.030	0.024	0.42	1.22 (0.66-2.26)
5	0-2-0-0-0-0-2	**7B*	Slow	0.029	0.019	0.15	1.50 (0.78-2.96)
6	0-0-2-0-0-2-0	**5C*	Slow	0.014	0.008	0.25	1.63 (0.62-4.56)
7	0-0-0-0-0-2-0	**12A*	Rapid	0.0062	0.0017	0.12	2.80 (0.49-28.6)
8	0-2-0-0-0-0-0	**13*	Rapid	0.0020	0.0032	0.61	0.62 (0.05-5.48)
9	0-2-0-0-2-2-0	**6C*	Slow	1.73E-8	0.0015	0.22	NE
10	0-0-0-0-2-0-0	**6B*	Slow	9.48E-59	0.0010	0.30	NE
11	2-2-0-0-0-0-0	**14B*	Slow	9.96E-04	0	0.33	NE
NAT1-NAT2[‡]							
1	0-0-0-0-0-0-0-0-0	**4-*4*	Reference/rapid	0.125	0.122	0.83	1.00
2	0-0-0-0-0-2-2-0-2-0	**4-*5B*	Reference/slow	0.340	0.345	0.83	0.93 (0.67-1.30)
3	0-0-0-0-2-0-0-2-0-0	**4-*6A*	Reference/slow	0.212	0.245	0.09	0.88 (0.69-1.31)
4	0-0-2-2-0-0-0-0-0-0	**10-*4*	Rapid/rapid	0.084	0.076	0.50	1.12 (0.69-1.13)
5	0-0-2-2-0-2-2-0-2-0	**10-*5B*	Rapid/slow	0.050	0.062	0.23	0.81 (0.52-1.25)
6	0-0-2-2-2-0-0-2-0-0	**10-*6A*	Rapid/slow	0.043	0.027	0.06	1.61 (0.93-2.82)
7	0-0-0-0-0-2-2-0-0-0	**4-*5A*	Reference/slow	0.030	0.024	0.47	1.23 (0.67-2.28)
8	0-0-0-0-2-0-0-0-0-2	**4-*7B*	Reference/slow	0.023	0.017	0.37	1.35 (0.66-2.79)
9	2-0-0-2-2-0-0-2-0-0	**11-*6A*	Rapid/slow	0.012	0.004	0.05	2.94 (0.88-12.6)
10	0-2-2-2-0-0-0-0-0-0	**14A-*4*	Slow/rapid	0.011	0.015	0.47	0.77 (0.31-1.85)
11	0-0-0-2-0-0-0-0-0-0	**3-*4*	Reference/rapid	0.010	0.018	0.14	0.57 (0.23-1.36)
12	0-0-0-2-0-2-2-0-2-0	**3-*5B*	Reference/slow	0.009	0.011	0.60	0.78 (0.26-2.24)
13	0-0-2-2-0-2-0-0-2-0	**10-*5C*	Rapid/slow	0.007	0.004	0.47	1.71 (0.43-8.05)
14	2-0-0-2-0-2-2-0-2-0	**11-*5B*	Rapid/slow	0.0097	0.0087	0.81	1.10 (0.37-3.32)
15	0-0-2-2-2-0-0-0-0-2	**10-*7B*	Rapid/slow	0.0062	0.0035	0.02	NE
16	0-0-0-0-0-2-0-0-2-0	**4-*5C*	Reference/slow	0.0071	0.0024	0.13	NE
17	0-0-0-0-0-0-0-0-2-0	**4-*12A*	Reference/rapid	0.0046	0.0012	0.17	NE

Abbreviation: NE, not estimated because one cell of the contingent table is 0.

[*] *NAT1* SNP (5′-3′): C97T, C190T, G445A, C559T, G560A, A752T, T1088A, and C1095A.

[†] *NAT2* SNP (5′-3′): G191A, C282T, T341C, C481T, G590A, A803G, and G857A.

[≠] *NAT1-NAT2* SNP: G445A, G560A, T1088A, C1095A, C282T, T341C, C481T, G590A, A803G, and G857A.

Table 4

Combined *NAT1* and *NAT2* diplotypes and risk of pancreatic cancer

***NAT1* diplotype**	***NAT2* diplotype**	**Patients (*n* = 524), *n* (%)**	**Controls (*n* = 578), *n* (%)**	**OR (95% CI)***
All other	All other	512 (97.7)	575 (99.5)	1.00
**10/*10 + *10/*11*	**6A/*5 + *6A/*6 + *6A/*7*	12 (2.3)	3 (0.5)	4.15 (1.15-15.00)

* OR (95% CI) adjusted for age, sex, pack-year of smoking (categorized as never smokers, light smokers, and heavy smokers), and diabetes (no versus yes).

Table 5

Joint effect of *NAT2* acetylation genotype and history of diabetes in modifying risk of pancreatic cancer

***NAT2* acetylation genotype**	**History of diabetes**	**Patients (*n* = 526)**	**Controls (*n* = 564)**	**OR (95% CI)***	**P_{trend}†**	**$P_{interaction}$‡**
		***n* (%)**	***n* (%)**			
All study participants						
All other;	No	285 (54.2)	335 (59.4)	1.00		
**6A/*5 + *6A/*6 + *6A/*7*	No	134 (25.5)	178 (31.6)	0.90 (0.68-1.19)		
All other;	Yes	64 (12.2)	36 (6.4)	2.04 (1.31-3.21)		
**6A/*5 + *6A/*6 + *6A/*7*	Yes	43 (8.1)	15 (2.6)	3.51 (1.90-6.50)	<0.001	0.12
Rapid/intermediate	No	196 (36.3)	215 (38.1)	1.00		
Slow	No	228 (43.4)	298 (52.8)	0.87 (0.67-1.14)		
Rapid/intermediate	Yes	35 (6.6)	23 (4.1)	1.53 (0.86-2.73)		
Slow	Yes	72 (13.7)	28 (5.0)	3.10 (1.90-5.05)	<0.001	0.07
Never smokers						
All others	No	114 (52.8)	161 (57.3)	1.00		
**6A/*5 + *6A/*6 + *6A/*7*	No	61 (28.2)	97 (34.5)	0.92 (0.61-1.40)		
All others	Yes	27 (12.5)	15 (5.3)	2.38 (1.19-4.75)		
**6A/*5 + *6A/*6 + *6A/*7*	Yes	14 (6.5)	8 (2.8)	2.66 (1.07-6.59)	0.01	0.88
Rapid/intermediate	No	85 (39.4)	101 (35.9)	1.00		
Slow	No	90 (41.7)	157 (55.9)	0.71 (0.48-1.06)		
Rapid/intermediate	Yes	14 (6.5)	7 (2.4)	2.14 (0.81-5.68)		
Slow	Yes	27 (12.5)	16 (5.7)	2.07 (1.03-3.99)	0.06	0.72
Ever smokers						
All others	No	171 (55.2)	171 (61.0)	1.00		
**6A/*5 + *6A/*6 + *6A/*7*	No	73 (23.6)	81 (29.0)	0.88 (0.59-1.30)		
All others	Yes	37 (11.9)	21 (7.5)	1.89 (1.04-3.42)		
**6A/*5 + *6A/*6 + *6A/*7*	Yes	29 (9.3)	7 (2.5)	4.59 (1.94-10.88)	<0.001	0.07
Rapid/intermediate	No	106 (34.2)	112 (40.0)	1.00		
Slow	No	138 (44.5)	140 (50.0)	1.05 (0.73-1.52)		
Rapid/intermediate	Yes	21 (6.8)	16 (5.7)	1.42 (0.68-2.95)		
Slow	Yes	45 (14.5)	12 (4.3)	4.65 (2.29-9.42)	<0.001	0.04

* OR (95% CI) adjusted for age, sex, and *NAT1* genotype (rapid versus others). Smoking pack-year (light versus heavy) was additionally adjusted for ever smokers. Smoking pack-year (never, light, and heavy) was additionally adjusted for all participants.

† *P* value for the trend test. Score test was used to test the linear trend by treating interaction term as the continuous variable.

‡ *P* value for the likelihood ratio test.