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Relationship Between *CYP2A6* and *CHRNA5-CHRNA3-CHRNB4* Variation and Smoking Behaviors and Lung Cancer Risk

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Genetic variations in the *CYP2A6* nicotine metabolic gene and the *CHRNA5-CHRNA3-CHRNB4* (*CHRNA5-A3-B4*) nicotinic gene cluster have been independently associated with lung cancer. With genotype data from ever-smokers of European ancestry (417 lung cancer patients and 443 control subjects), we investigated the relative and combined associations of polymorphisms in these two genes with smoking behavior and lung cancer risk. Kruskal–Wallis tests were used to compare smoking variables among the different genotype groups, and odds ratios (ORs) for cancer risk were estimated using logistic regression analysis. All statistical tests were two-sided. Cigarette consumption ($P < .001$) and nicotine dependence ($P = .036$) were the highest in the combined *CYP2A6* normal metabolizers and *CHRNA5-A3-B4* AA (tag single-nucleotide polymorphism rs1051730 G>A) risk group. The combined risk group also exhibited the greatest lung cancer risk (OR = 2.03; 95% confidence interval [CI] = 1.21 to 3.40), which was even higher among those who smoked 20 or fewer cigarettes per day (OR = 3.03; 95% CI = 1.38 to 6.66). Variation in *CYP2A6* and *CHRNA5-A3-B4* was independently and additively associated with increased cigarette consumption, nicotine dependence, and lung cancer risk. *CYP2A6* and *CHRNA5-A3-B4* appear to be more strongly associated with smoking behaviors and lung cancer risk, respectively.

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Genetic variation in *CYP2A6* and in the nicotinic receptor subunit gene cluster, *CHRNA5-CHRNA3-CHRNB4* (*CHRNA5-A3-B4*), has been modestly associated with lung cancer susceptibility in independent studies (1–5). *CYP2A6* and *CHRNA5-A3-B4* are both involved in the pharmacology of nicotine and nitrosamines, which are lung cancer procarcinogens (6). CYP2A6 inactivates nicotine and also activates tobacco-specific nitrosamines (1,7), whereas nicotinic receptors mediate nicotine-induced reward (8) and nitrosamine-induced carcinogenic signaling (9). Thus, genetic variations in *CYP2A6* and *CHRNA5-A3-B4* have the potential to influence lung cancer risk directly and indirectly through alteration of smoking behavior (10–13).

We investigated the associations between *CYP2A6* and *CHRNA5-A3-B4* polymorphisms, alone and in combination, with smoking behaviors and lung cancer risk. Ever-smoking non–small cell lung cancer patients (n = 417) and control subjects (n = 443) of European ancestry matched by age, sex, and smoking variables were selected from our previously published genome-wide association discovery set (4) (Supplementary Table 1, available online). Participants were genotyped for *CYP2A6*2*, **4*, **9*, and **12* (14,15), reduced enzymatic function alleles common in those of European ancestry, and grouped by predicted metabolic activity. *CYP2A6* carriers were defined as either *CYP2A6* normal metabolizers (participants who lacked variant alleles) or *CYP2A6* reduced metabolizers (participants who carried at least one variant allele) (14,16).

Participants were also grouped according to genetic variation in the *CHRNA5-A3-B4* cluster, represented by the tag single-nucleotide polymorphism rs1051730 G>A (4). The AA genotype is the established risk genotype for smoking intensity and lung cancer risk

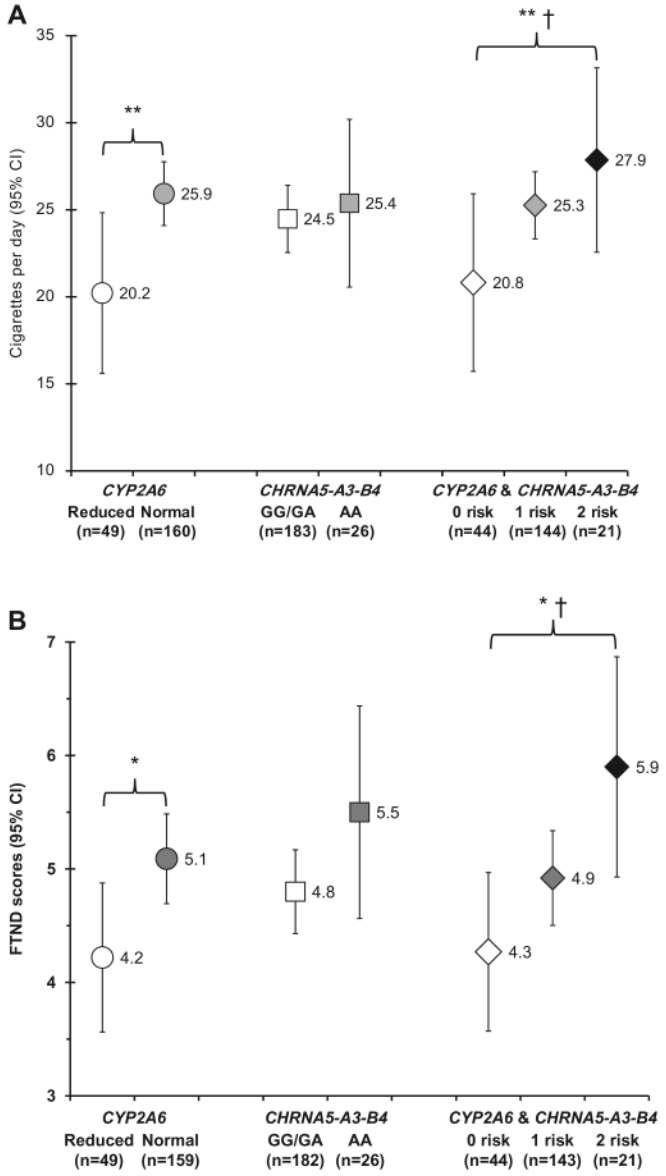

Figure 1. Association of *CYP2A6* and *CHRNA5-A3-B4* genotype with smoking behaviors. **A**) Self-reported cigarettes smoked per day and **B**) Fagerström Test for Nicotine Dependence (FTND) scores are displayed for control subjects who were current smokers and are shown as the mean with 95% confidence intervals (CIs) by genotype group. The genotypes analyzed include the *CYP2A6* genotype alone, the *CHRNA5-A3-B4* genotype alone, and the combined *CYP2A6* and *CHRNA5-A3-B4* genotype groups according to the number of risk genotypes. The low-risk group (0 risk) included *CYP2A6* reduced metabolizers with the *CHRNA5-A3-B4* GG and GA genotypes. The intermediate-risk group (one risk) included participants with either the *CYP2A6* normal metabolizer genotypes or the *CHRNA5-A3-B4* AA genotype, and the high-risk group (two risk) included *CYP2A6* normal metabolizers with the *CHRNA5-A3-B4* AA genotype. *P* values were calculated by Kruskal–Wallis tests. $*P < .05$, $**P < .001$. P_{trend} was calculated by a generalized linear model. $\dagger P_{trend} < .05$. All statistical tests were two-sided.

(4,5,17), hence rs1051730 GG and GA individuals were grouped together. Individual allele and combined group genotype results are available in Supplementary Table 2 (available online). We used the Kruskal–Wallis test to compare smoking variables among genotype groups for all participants and separately in control subjects who were current smokers. Lung cancer odds ratios (ORs) were estimated using logistic regression analysis. This study was approved by Review Boards at the M.D. Anderson Cancer Center (Houston, TX), the Institute for Cancer Research Foundation (London, UK), and the University of Toronto (Toronto, ON, Canada).

Daily cigarette consumption was statistically significantly associated with the *CYP2A6* and *CHRNA5-A3-B4* genotypes, alone and in combination, in the overall study population (Supplementary Table 3, available online). Here, we present the association in control subjects who were current smokers (n = 209) (Figure 1, A),

CONTEXT AND CAVEATS

Prior knowledge

CYP2A6 and the nicotinic receptor subunit gene cluster *CHRNA5-CHRNA3-CHRNB4* (*CHRNA5-A3-B4*) are involved in nicotine and tobacco-specific nitrosamine metabolism and signaling, respectively, and have been independently associated with cigarette consumption and lung cancer risk in previous studies, leading to the hypothesis that genetic variation in both *CYP2A6* and *CHRNA5-A3-B4* may influence lung cancer risk in smokers directly, as well as indirectly through altered cigarette exposure.

Study design

Ever-smokers of European ancestry selected from a published genome-wide association study (417 non-small cell lung cancer patients and 443 control subjects) were grouped by predicted *CYP2A6* metabolic activity and according to genetic variation in the *CHRNA5-A3-B4* cluster. Smoking variables among the genotype groups were compared, and the relationship between the genotype groups and lung cancer risk was examined.

Contribution

Combined genetic variation in *CYP2A6* and *CHRNA5-A3-B4* was associated with increased cigarette consumption and nicotine dependence. Variation in these genes was independently associated with an increased risk of lung cancer with an even higher relative increase in risk from these genes among the lighter-smoking stratum.

Implications

In addition to mediating lung cancer risk through smoking behavior, genetic variation in *CYP2A6* and *CHRNA5-A3-B4* may mediate carcinogenesis directly. Whereas variation in *CYP2A6* may play a larger relative role in smoking behaviors, variation in *CHRNA5-A3-B4* may play a larger relative role in lung cancer risk.

Limitations

The study population included ever-smokers of European ancestry and the application of the findings to other races/ethnicities is unclear. Also, cigarette consumption among the study participants was self-reported.

From the Editors

because cancer diagnosis and recall bias among former smokers are minimized as confounders in this analysis. In control subjects who were current smokers, *CYP2A6* normal metabolizers smoked statistically significantly more cigarettes per day (CPD) (mean = 25.9 CPD, 95% CI = 24.1 to 27.8 CPD) compared with reduced metabolizers (mean = 20.2 CPD, 95% CI = 15.6 to 24.8 CPD) ($P < .001$). The *CHRNA5-A3-B4* AA genotype was associated with a statistically significant increase in CPD compared with the *CHRNA5-A3-B4* GG and GA genotypes in the overall study population (Supplementary Table 3, available online) but did not reach statistical significance among control subjects who were current smokers ($P = .416$).

To assess the association of *CYP2A6* and *CHRNA5-A3-B4* genotypes in combination with smoking behavior, we separated participants into three groups as follows: *CYP2A6* reduced metabolizers with the *CHRNA5-A3-B4* GG and GA genotypes were identified as the low-risk group, *CYP2A6* normal metabolizers with the *CHRNA5-A3-B4* AA genotype were identified as the high-risk group, and control subjects with the either the *CYP2A6* normal metabolizer genotypes or the *CHRNA5-A3-B4* AA genotype were identified as the intermediate-risk group. Among control subjects who were current smokers, cigarette consumption was statistically significantly different ($P < .001$) between the low-risk group (mean = 20.8 CPD, 95% CI = 15.7 to 25.9 CPD), intermediate-risk group (mean = 25.3 CPD, 95% CI = 23.3 to 27.2 CPD), and high-risk group (mean = 27.9 CPD, 95% CI = 22.6 to 33.2 CPD) and increased linearly across these three genotype groups ($P_{trend} = .042$). Similar results were observed for the overall study population (Supplementary Table 3, available online).

We also investigated the relationship between nicotine dependence and *CYP2A6* and *CHRNA3-A5-B4* genotypes, as assessed by the Fagerström Test for Nicotine Dependence (FTND). FTND scores were statistically significantly associated with the *CYP2A6* and *CHRNA5-A3-B4* genotypes, alone and in combination, in the overall study population (Supplementary Table 3, available online). In control subjects who were current smokers, statistically significantly higher FTND scores ($P = .036$) were observed for *CYP2A6* normal metabolizers (mean = 5.1, 95% CI = 4.7 to 5.5) compared with reduced metabolizers (mean = 4.2, 95% CI = 3.6 to 4.9). The *CHRNA5-A3-B4* AA genotype was associated with a statistically significant increase in FTND scores compared with the *CHRNA5-A3-B4* GG and GA genotypes in the overall study population (Supplementary Table 3, available online) but did not reach statistical significance among control subjects who were current smokers ($P = .137$).

Nicotine dependence was also associated with the *CYP2A6* and *CHRNA5-A3-B4* genotypes in combination. Among control subjects who were current smokers, FTND scores were statistically significantly different ($P = .036$) between the low-risk group (mean = 4.3, 95% CI = 3.6 to 5.0), intermediate-risk group (mean = 4.9, 95% CI = 4.5 to 5.3), and high-risk group (mean = 5.9, 95% CI = 4.9 to 6.9) and increased linearly across these three genotype groups ($P_{trend} = .013$). Similar results were observed for the overall study population (Supplementary Table 3, available online).

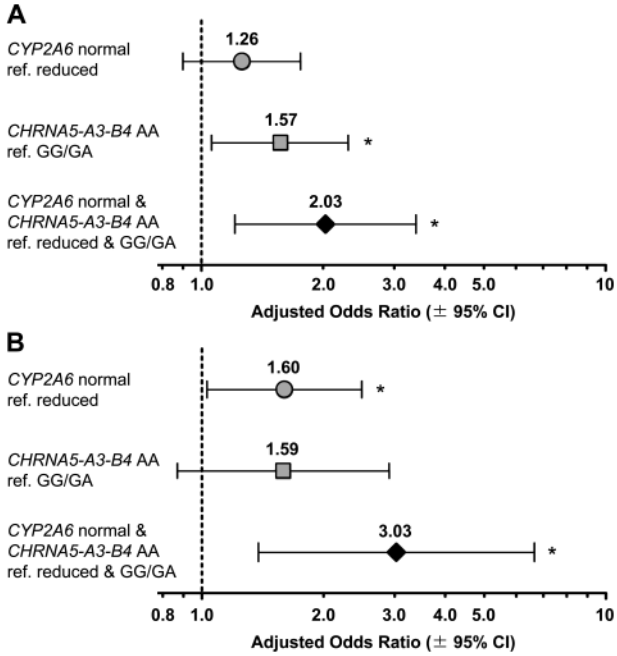

Figure 2. Lung cancer risk by *CYP2A6* and *CHRNA5-A3-B4* genotype. **A**) Overall risk of lung cancer and **B**) risk of lung cancer in the lighter-smoking stratum are shown as adjusted odds ratios with 95% confidence intervals (CIs). The lighter-smoking stratum was defined as individuals smoking 20 cigarettes or less per day on the basis of the median cigarette consumption in patients and control subjects. For each odds ratio, the lower-risk genotype group served as the reference (ref), ie, ref. CYP2A6 reduced metabolizers and/or *CHRNA5-A3-B4* GG or GA. Odds ratios were adjusted by age (continuous), sex (male or female), and log pack–years (continuous). Lung cancer odds ratios and *P* values were estimated by logistic regression analysis with $P < .05$ denoted by an asterisk. All statistical tests were two-sided.

We then investigated the impact of *CYP2A6* and *CHRNA5-A3-B4* on lung cancer susceptibility (Figure 2, A). Because both *CYP2A6* and *CHRNA5-A3-B4* were associated with cigarette consumption, we adjusted odds ratios for cigarette pack–years. *CYP2A6* normal metabolizers had a non-statistically significant increase in lung cancer risk compared with reduced metabolizers (OR = 1.26, 95% CI = 0.90 to 1.76; $P = .180$), whereas the *CHRNA5-A3-B4* AA genotype was statistically significantly associated with increased lung cancer risk (OR = 1.57, 95% CI = 1.06 to 2.31; $P = .024$). Of note, the combination of both risk genotypes, *CYP2A6* normal metabolizer and *CHRNA5-A3-B4* AA, was statistically significantly associated with increased lung cancer risk (OR = 2.03, 95% CI = 1.21 to 3.40; $P = .007$).

Adjusting the odds ratio for each genotype alone by variation in the other gene did not affect the association between genotype and lung cancer risk suggesting that variation in *CYP2A6* and *CHRNA5-A3-B4* independently affects lung cancer risk (for unadjusted, pack-year, and genotype-adjusted

odds ratios, see Supplementary Table 4, available online). A statistically significant interaction between *CYP2A6* and *CHRNA5-A3-B4* was not found in a logistic regression model of lung cancer risk, suggesting that the combined genotype effects are additive.

In subgroup analyses, the *CYP2A6* normal genotype was statistically significantly associated with lung cancer risk in the lighter-smoking stratum in which CPD was 20 or less, as defined by median CPD in patients and control subjects (OR = 1.60, 95% CI = 1.03 to 2.49; $P = .036$) (Figure 2, B). A statistically significant association with lung cancer risk was observed among those with both risk genotypes, *CYP2A6* normal and *CHRNA5-A3-B4* AA (OR = 3.03, 95% CI = 1.38 to 6.66; $P = .006$), whereas among the heavier-smoking stratum (CPD >20), no association with lung cancer risk was noted for either *CYP2A6* or *CHRNA5-A3-B4* alone or in combination (Supplementary Table 5, available online). This pattern of higher genetic risk in the lighter-smoking stratum supports the notion of a direct genotype contribution to lung carcinogenesis vs a sole contribution of genotype from altered smoking quantity and has been previously reported for the *CHRNA5-A3-B4* genotype (5). Low-exposure gene effects (18) have also been observed for other polymorphic drug-metabolizing enzymes such as *CYP1A1*, *NAT2*, and *MPO* (19–22) and merit attention. Lighter smokers, a growing segment of the smoking population (23,24), tend to have reduced concerns about the negative health effects associated with smoking. Our findings, however, suggest that the genetic risk for lung cancer may remain high among lighter smokers. Furthermore, the levels of smoking may obscure genetic signals in association studies conducted in smoking populations. We also conducted a subgroup analysis by histology (Supplementary Table 6, available online) and found evidence of a stronger association of *CYP2A6* and *CHRNA5-A3-B4* with adenocarcinoma (OR = 2.09, 95% CI = 1.08 to 4.03, $P = .029$) vs squamous cell carcinoma (OR = 1.44, 95% CI = 0.65 to 3.20, $P = .372$).

The major limitation of the current study is the use of self-reported cigarette consumption without biochemical verification, such as plasma cotinine levels or total urinary nicotine equivalents. In addition, neither the smoking behavior analyses nor our lung cancer risk models were able to incorporate potential changes in smoking patterns of study participants over time.

This study demonstrates for the first time, to our knowledge, that genetic variation in *CYP2A6* and *CHRNA5-A3-B4* combines to increase cigarette consumption and nicotine dependence and independently and additively combines to increase lung cancer risk. Our results also suggest that variation in *CYP2A6* appears to have a larger relative role in smoking behaviors, whereas variation in *CHRNA5-A3-B4* may play a larger relative role in lung cancer risk. Given that metabolic activation of procarcinogens is an early step along the pathway to cancer (6), altered *CYP2A6* nitrosamine activation may make a small contribution to lung cancer risk. The α5, α3, and β4 subunits are expressed in the respiratory tract (25) and have been implicated in the pathological effects of nitrosamines on epithelial cells (26). Thus, genetic variation in these subunits could influence nitrosamine carcinogenic signialing. These findings further our understanding of genetic risk factors for smoking and lung cancer and provide insight into mechanisms of lung cancer carcinogenesis.

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