**Prior CYP2A6 Associations with NMR, Abstinence, CPD and Lung Cancer Risk.**

In the twin-based laboratory-based study used in our Stage I analysis, variation at *CYP2A6* was previously associated with the rate of nicotine metabolism, and additive genetic influence remained after accounting for six *CYP2A6* variants [[1](#_ENREF_1), [2](#_ENREF_2)]. In analyses of 148 *ad libitum* smokers, levels of nicotine and of *trans*-3’-hydroxycotinine, and multiple ratios of nicotine metabolites, including the NMR, are significantly correlated with *CYP2A6* genotypes [[3](#_ENREF_3)], grouped by predicted CYP2A6 activity [[1](#_ENREF_1), [4-12](#_ENREF_4)]. The “CYP2A6 activity model” modeled the association between seven *CYP2A6* variants, sex and smoking status with several metabolic ratios in a laboratory study of nicotine metabolism [[13](#_ENREF_13)] in European American ancestry individuals [[14](#_ENREF_14)]. The CYP2A6 activity model accounted for 47.6% of the variance of the laboratory study-based NMR 240 minutes after labeled compound administration [[13](#_ENREF_13)].

In retrospective analyses of therapy efficacy in four clinical trials [[15-17](#_ENREF_15)], European American or African American ancestry treatment-seeking smokers with a NMR in the lowest quartile [[18-20](#_ENREF_18)], or with *CYP2A6* variants associated with low enzyme activity [[21](#_ENREF_21), [22](#_ENREF_22)], and randomized to nicotine replacement therapy or to placebo pharmacotherapy, were significantly more likely to remain abstinent than those in the upper three quartiles of the NMR or with *CYP2A6* variants associated with normal activity. In retrospective analysis of clinical trial participants [[23](#_ENREF_23)], and stratifying individuals by the 25th percentile of the CYP2A6 activity model, three hypotheses of association of CYP2A6 activity and relapse risk were confirmed: a) slow-metabolizer status decreases risk; b) slow-metabolizer status and randomization to active pharmacotherapy each decrease risk; and c) slow-metabolizer status and randomization to nicotine replacement therapy (NRT) each decrease risk [[24](#_ENREF_24)]. In a prospective NMR-stratified smoking cessation trial including both African American and European American ancestry participants randomized to nicotine patch, varenicline and placebo therapies, the NMR was significantly associated with abstinence at end of treatment and at six months in interaction with treatment, with normal metabolizers more likely to quit than slow metabolizers when randomized to varenicline compared to nicotine patch [[25](#_ENREF_25)].

In three samples of smokers of two different ancestries drawn from two cohort studies totaling 2,401 individuals [[14](#_ENREF_14), [26](#_ENREF_26)], European American ancestry smokers with loss of function *CYP2A6* haplotypes exhibited significantly reduced CPD [[27](#_ENREF_27)]; and, among smokers with FTND/FTCD scores ≥4, the CYP2A6 activity model was significantly associated with continuous CPD (*P*=0.005 European American ancestry, *P*=0.027 African American ancestry) [[27](#_ENREF_27)]. In 860 European smokers included in a lung cancer case:control study, *CYP2A6* normal metabolizers smoked significantly more CPD than reduced metabolizers (27.7 vs 24.7 CPD, *P*=0.01) [[28](#_ENREF_28)]. In meta-GWAS analyses of European smokers, *CYP2A6* SNPs rs1801272 (c.479T>A/p.L160H) and rs4105144 (-2729A>G) are significantly associated with CPD [effect sizes of 0.68 CPD (N=66,380, *P*=1.1E-4) and 0.39 CPD (N=83,317, *P*=2.2E-12)] [[29](#_ENREF_29)]. Bloom *et al* estimate that rs1137115 is in strong linkage disequilibrium (*D*’≥0.95) with rs4105144 [[27](#_ENREF_27)].

In an Italian case:control study (N=4,221) [[30](#_ENREF_30)], rs1801272 was significantly associated with lung cancer (*P*=0.026) [[31](#_ENREF_31)]. In the lighter-smoking stratum from a lung cancer case:control study of European American ancestry indivdiuals, *CYP2A6* metabolizer status, defined by haplotype, was significantly associated with lung cancer (*P*=0.036) [[32](#_ENREF_32)]. In a meta-GWAS analysis (N=42,289), rs4105144 was significantly associated with lung cancer (*P*=0.04) [[29](#_ENREF_29)]. In 845 European current smokers in a lung cancer case:control study [[28](#_ENREF_28)], rs4105144 is associated with increased serum cotinine (*P*trend=0.0001) [[28](#_ENREF_28)], a function of decreased cotinine clearance [[33](#_ENREF_33)], and with decreased lung cancer risk (*P*=0.01, adjusted for cotinine) [[28](#_ENREF_28)].

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