

Characterization of regulatory mechanisms at DNA variant, methylation and RNA levels for 57 tobacco smoking susceptibility genes in human brain

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Abstract

Although a number of susceptibility genes for smoking have been identified, the mechanistic steps between the genetic variation and smoking-related traits generally are not understood. In this study, we mapped *cis* expression and methylation quantitative trait loci (eQTL and mQTL) for 57 candidate smoking-related genes using the BrainCloud cohort ($n = 94$ African Americans [AAs] and 84 European Americans [EAs] for expression; and $n = 31$ AAs and 29 EAs for methylation). A eQTL, with a range of 75 Kb between the two eQTL variants furthest away (from rs117032961 to rs193122850), was identified that significantly affected *EGLN2* expression in the EA sample, where multiple associated low-frequency variants were previously found to be affecting smoking quantity in the aggregate. Two mQTLs with ranges of 121 Kb (from rs56159305 to rs2163018) and 35 Kb (rs10424026 to rs10425932) were detected for CpG sites in *NRXN1* and *CYP2A7*, respectively. Particularly, we found for the first time that the minor allele of SNP rs3745277, located in *CYP2A7P1* (downstream of

CYP2B6), significantly decreased methylation of *CYP2A7* at the CpG site (cg25427638; $P = 5.31 \times 10^{-7}$), reduced expression of *CYP2B6* ($P = 0.03$), and lowered the percentage of smokers (8.8% vs. 42.3%; odds ratio: 0.14; 95% confidence interval: 0.02–0.62; $P = 4.47 \times 10^{-3}$) in a dominant way for the same cohort. Conditional analysis indicated only a negligible contribution of smoking to either cg25427638 methylation or *CYP2B6* expression beyond the genetic variation effect. Our results provide the first link of genetic variation, DNA methylation and mRNA expression with smoking status among the same participants, which depicts a regulatory mechanism from mQTL to phenotypic manifestation. Additionally, we demonstrated different regulatory effects of low-frequency and common variants on mRNA expression and DNA methylation, respectively.

Keywords: eQTL, methylation, expression, smoking, brain, functional SNPs