Genetic Influences on Smoking and Clinical Disease

Understanding Behavioral and Biological Pathways with Mediation Analysis

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This year is the 50th anniversary of the landmark Surgeon General's report on smoking and health. "Since the 1964 Surgeon General's report, cigarette smoking has been causally linked to diseases of nearly all organs of the body" (1). Despite the substantial success of public health policies for the prevention and cessation of smoking, leading to a reduction in the prevalence of smoking from approximately 43% in 1965 to 18% today (1), tobacco use remains the leading cause of preventable disease and mortality (1). Sustained efforts toward cigarette smoking prevention and cessation must continue both in public health policies and clinical practice.

Smoking behaviors, and thus exposure to the adverse effects of tobacco smoke, have genetic influences. Over the past few years, genomewide association studies have identified and replicated several loci involved in smoking behavior and exposure. These loci are primarily nicotinic acetylcholine receptors (e.g., *CHRNA5/A3/B4*) in the chromosome 15q25 region (2); other receptors (*CHRNA6/B3* on chromosome 8p11) and the nicotine-metabolizing genes *CYP2A6* and *CYP2B6* show more modest yet significant associations with smoking. For a recent succinct review of the genetics of smoking behaviors and the biological activity related to the genomewide association studies signals mentioned here, see Laukola and colleagues (3). These studies implicate the addictive properties of nicotine as the primary basis for the observed associations. The article by Bloom and colleagues in this issue of *AnnalsATS* (pp. 1003–1010) adds

to this growing body of literature by identifying the *CHRNA5/A3/B4* loci in a genomewide association study of exhaled carbon monoxide, a novel biomarker of acute smoking exposure (4).

Genomic regions associated with smoking behaviors and exposures are also associated with smoking-related diseases. Genomewide association studies found the *CHRNA5/A3/B4* region to be associated with lung cancer (2) and chronic obstructive pulmonary disease (COPD) (5); these findings have been replicated. Although it may be tempting to deduce that these observations indicate that the associations between this genomic region and clinical outcomes are mediated through smoking behavior, it is important to formally test these putative pathways.

Through the use of causal inference and mediation analysis, investigators have begun to formally test this path from gene to disease through smoking-related phenotypes (i.e., does the gene act on the clinical disease through smoking?). To determine whether this *CHRNA5/A3/B4* region is associated with clinical diseases such as COPD through smoking, several assumptions must be met: All confounders of the relationship between the gene, smoking, and the clinical disease are accounted for (6); the causal pathway is correctly specified, meaning that one correctly specifies that smoking causes COPD and not vice versa (6); and the mediator variable, such as exhaled CO, is accurately and precisely measured. Greater measurement variability of the potential mediator will diminish the ability to

identify pathways through that mediator. Figure 1 shows an example of a mediation model specifying how a gene acts on clinical disease through smoking behavior and exposure (7).

Smoking behaviors and exposures are difficult to measure in epidemiologic studies. Both self-reported smoking measures, such as cigarettes per day, and overall burden of smoking measures, such as pack-years of smoking, lack precision (8). Biomarkers of nicotine metabolism such as cotinine (9) or the nicotine metabolite ratio (10) provide a more objective measure of nicotine exposure and metabolism. Exhaled CO, the biomarker of smoking exposure used in the article by Bloom and colleagues (4), represents acute exposure to smoke. It is important to recognize that these measures, despite correlations between them, both have different measurement characteristics and measure different aspects of smoking behavior and exposure. All are surrogates of the actual biological triggers of the disease processes.

Several studies have examined the role of self-reported smoking as a mediator of the association between this *CHRNA5/A3/B4* region and clinical outcomes; however, to our knowledge, no studies have considered mediation of biomarkers of smoking exposure such as exhaled CO. For COPD, Siedlinski and colleagues found both direct effects of *CHRNA5/A3/B4* on COPD as well as indirect effects through cigarettes per day and pack-years of smoking (11). The results, to date, of mediation analysis of lung cancer are mixed. VanderWeele and colleagues found no evidence that this

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Figure 1 A causal model specifying how genetic variation increases the susceptibility to clinical disease through smoking and alternate pathways (7). In this example, the observed increased likelihood of lung cancer and chronic obstructive pulmonary disease (COPD) associated with the *CHRNA5/A3/B4* region is partitioned into a smoking-related pathway of smoking behaviors (e.g., nicotine dependence as measured by the Fagerström Test of Nicotine Dependence [FTND]) (16) and exposure to smoking, as measured by exhaled carbon monoxide, and an alternate unspecified pathway. The alternate pathways may be biological; that is, *CHRNA5/A3/B4* has additional effects beyond smoking or may relate to experimental variability in the smoking-related traits.

CHRNA5/A3/B4 region was associated with lung cancer through cigarettes per day (12), whereas others found that this *CHRNA5/A3/B4* region indirectly acts on lung cancer risk mediated through cigarettes per day (13). Wang and colleagues found that the genetic influences on lung cancer risk were mediated through three distinct pathways: current smoking status, COPD, and both smoking and COPD (14).

These studies can be valuable in estimating the potential genetic effects on smoking cessation clinical trials. A *post hoc* genotyping and mediation analysis of a smoking cessation clinical trial has been performed (15). The *CHRNA5/A3/B4* region was associated with successful abstinence with nicotine replacement therapy. Interestingly, this genotype effect on smoking abstinence was

mediated by nicotine dependence, as measured by the Fagerström Test of Nicotine Dependence. This demonstrates the value of mediation analysis in smoking cessation trials and the potential of personalized therapy for smoking cessation.

Cigarette smoking remains a major public health problem. Understanding the genetic influences on smoking behaviors and exposure that lead to clinical disease will provide important insights for novel prevention strategies and smoking cessation programs. The development of pharmaceutical compounds targeted at specific biological pathways that influence smoking cessation and reduced risk for disease offers the opportunity for personalized therapeutic approaches based on an individual's genetic susceptibility. The current publication by Bloom and colleagues (4) provides more evidence for a biological basis for the prevention and treatment of smoking to reduce the burden of smoking-related diseases. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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