Cigarette smoking remains a worldwide health epidemic. In the United States, despite the well-established risks, cigarette smoking remains the leading cause of preventable disease and death accounting for >480,000 deaths annually, with 1 in every 3 of such deaths related to cardiovascular diseases (CVD). For every patient who dies because of smoking, at least 30 people live with a serious smoking-related illness, including ischemic heart disease, stroke, and peripheral vascular disease, etc. Although cigarette smoking has declined during the past decade among US adults from 20.9% in 2005 to 17.8% in 2013, an estimated 42.1 million adults in the US remain current smokers. In lieu of these sobering statistics, it remains imperative to better understand the molecular mechanisms by which smoking drives the development of CVD. Such insights, particularly when considering smoking as a mediator of accelerated atherosclerosis, may provide both preclinical diagnostic biomarkers and drug targets for therapeutics of CVD among smokers and nonsmokers alike.

Recent studies have shown that smoking is highly associated with both genome-wide epigenetic cytosine-(phosphate)-guanine (CpG) DNA methylation alterations and downstream transcriptome changes. Among such effects, coordinated DNA methylation and gene expression alteration of the Aryl Hydrocarbon Receptor Repressor (AHRR) in various cell types is the most significant and robust change associated with smoking. Maternal smoking has been consistently shown to be associated with DNA methylation change of AHRR in newborn cord blood cells in a dose- and duration-dependent manner. Other reports have indicated decreased AHRR methylation levels among adult smokers occurring in a dose-dependent fashion, which may also be partially reversible on smoking cessation. Such findings have been seen in a variety of cell types and interestingly may represent a quantitative biomarker of accumulated exposure that integrates both smoking dose and duration. AHRR is a transcription factor that is transcriptionally induced through activation of the Ah receptor (AhR) pathway, and the expressed AHRR can act as a negative feedback mechanism to further repress AhR-dependent gene expression. Among the thousands of chemicals in tobacco smoke, many are known agonists of AhR signaling with persistent activation of the AhR signaling pathway hypothesized to contribute to atherogenesis. Although there is currently no known role for AHRR in atherogenesis, studies have demonstrated upregulation of AHRR expression in monocyte-to macrophage differentiation and suppression of anti-inflammation. Beyond its well-recognized role in AhR signal cascade, AHRR has also been indicated in regulation of cell growth and differentiation as a tumor repressor gene and can interact with other transcription factors, such as hypoxia-inducible factor-1 and estrogen receptor α, which have known roles in a broad range of pathophysiological processes including atherogenesis. Given the pleotropic effects of AHRR on several interactive cellular processes, AHRR provides a plausible biological link between smoking and atherosclerotic plaque formation.

In this issue of Circulation: Cardiovascular Genetics, Reynolds et al. directly address our knowledge gap in this area by evaluating AHRR methylation levels in CD14+ monocytes, a cell type sensitive to cigarette smoking and involved in atherogenesis. Through a series of sequential analyses evaluating the associations between smoking and CpG methylation, CpG methylation and carotid plaque scores, and CpG methylation and cis-gene expression, they create a convincing case linking AHRR methylation to atherosclerosis. The investigators begin by identifying 33 (of 542) AHRR CpG sites, whose degree of methylation was significantly associated with smoking in CD14+ monocytes from 114 current smokers and 502 never smokers, from 1264 participants of the Multi-Ethnic Study of Atherosclerosis (MESA). Among these smoking-responsive CpGs, methylation of cg05575921 within the AHRR gene body (P=6.07×10^{-13}) represented the most significantly differentially methylated CpG. Novel associations between cg05575921 methylation and carotid plaque scores (P=3.1×10^{-10}) were then identified, which remained significant in current and former smokers after adjusting for self-reported smoking habits, urinary cotinine, and well-established CVD risk factors. Functionally, cg05575921 methylation correlated with AHRR mRNA profiles (P=1.4×10^{-7}) obtained from RNA sequencing conducted on a subset (n=373) of the MESA samples. Using independent cohorts, the investigators replicated the association of cg05575921 methylation in hepatic cells (n=141) with smoking (P=0.002), and with subclinical atherosclerosis (extent of fatty streaks in the right coronary arteries and left halves of the aortas; P=0.002) measured at autopsy in biopsies from 141 young smokers, which may also be partially reversible on smoking cessation.

[The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association. From the Department of Neurology, Maryland Stroke Center, University of Maryland School of Medicine, Baltimore (J.W.C.); Department of Neurology, Baltimore VA Medical Center, Baltimore (J.W.C.); and Division of Endocrinology, Department of Medicine, University of Maryland School of Medicine, Baltimore (H.X.). Correspondence to John W. Cole, MD, MS, Department of Neurology, Maryland Stroke Center, University of Maryland School of Medicine, Bressler Research Bldg, Room 12-006, 655 West Baltimore St, Baltimore, MD 21201. E-mail jcole@som.umaryland.edu]

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men (<35 years). Reduced representation bisulphite sequencing in independent monocyte samples revealed that cg05575921 was adjacent to 7 novel smoking-associated CpGs within a predicted gene expression regulatory element (enhancer). Utilizing mediation analysis to explore whether methylation was intermediate in the association between smoking (pack-years and cigarettes per day) and higher carotid plaque score, they found cg05575921 methylation significantly mediated 37% of the total effect of self-reported pack years on carotid plaque score in current and former smokers, and 60% of the total effect of cigarettes per day on carotid plaque score in current smokers. In summary, key findings correlated smoking status (current, former, and never) with reduced methylation levels at cg05575921 upregulated AHRR mRNA expression in monocytes and carotid plaque scores; then replicating these findings in independent samples.

Reynolds et al. have added to the growing body of evidence that AHRR methylation may be functionally related to AHRR expression in monocytes and may represent a potential biomarker of subclinical atherosclerosis in smokers. Although the study results are consistent with the stated hypothesis relating AHRR methylation to cigarette smoking and atherosclerosis, it is important to note that it remains unclear whether the identified smoking-associated AHRR expression change in monocytes is causally related to atherogenesis or occurs as a bystander in parallel to the AhR activation-related effects on plaque formation. As the investigators point out, their mediation analysis results are unable to distinguish confounding from mediating effects; therefore, although the observed cg05575921 methylation significantly mediated the effect of pack-years on carotid plaque score, these results do not confirm biological mediation and should be interpreted with caution. Additional investigations into the mechanisms driving these associations are warranted. Future study with longitudinal cohort design and bench molecular biology experiments using human cell lines or model organisms would help verify the functional role of AHRR in atherogenesis. Furthermore, additional evaluations focusing on fully identifying the causative chemical agents within cigarettes are also required, with such results potentially informing on atherogenesis in nonsmokers because the responsible agents (or similar) may be ubiquitous in some nonsmoking environments, including dietary. Clinicians should continue to focus their CVD prevention efforts on the early identification and treatment of traditional modifiable vascular risk factors, including hypertension, diabetes mellitus, dyslipidemia, and obesity, while emphasizing abstinence from smoking. As any amount of smoking increases CVD risk, including exposure to secondhand smoke.

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Disclosures
None.

References


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