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Proposal Title

Associations of Pipe and Cigar Smoking with DNA methylation at the Aryl Hydrocarbon Receptor

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Background

Tobacco smoking, including the combustion of cigars, pipes, and cigarettes, is the single leading cause of preventable death in the United States.¹ While cigarette smoking remains the most common form of tobacco consumption, 17.9 million US adults identify as current or former cigar smokers, and 2.6 million adults report smoking over 50 pipes in their lifetime.² Though cigarette smoking declined between 2000 and 2015, cigar consumption increased by 85.2% and pipe smoking by 556.4%.³ Despite the growing prevalence of cigar and pipe smoking, few prior studies have examined the contribution of pipe and cigar smoking to incident lung disease, and fewer studies have examined the biological mechanisms through which pipe and cigar smoking may generate lung function impairment.

Smoking-induced changes in DNA methylation represent one potential mechanism through which smoking increases long-term risk of incident lung disease. Cigarette smoke alters DNA methylation at thousands of cytosine-phosphate-guanine (CpG) sites in nucleated blood cells,⁴⁻⁶ some of which localize to genes associated with inflammatory pathways and chronic obstructive pulmonary disease (COPD).^{7,8} In particular, cigarette smoking reduces DNA methylation levels

at the aryl hydrocarbon receptor repressor (AHRR) gene in both blood and lung tissue.⁹⁻¹¹ Smoking-induced reductions in AHRR methylation inhibit expression of detoxifying enzymes that remove harmful environmental chemicals including hydrocarbons contained in tobacco smoke.¹² Thus, smoking-induced AHRR demethylation may represent a mechanism of increased lung disease in smokers.¹³

While prior research has established the impact of cigarette smoking on AHRR methylation, no prior studies have examined the impact of pipe and cigar smoking on AHRR methylation levels. In addition, it remains unknown whether AHRR methylation levels among pipe and cigar smokers are associated with incident obstructive lung disease. We therefore propose to define associations of pipe and cigar exposure with AHRR methylation levels and to determine whether AHRR methylation levels in pipe and cigar smokers are associated with incident airflow limitation.

Specific Aims:

Aim 1. Evaluate whether pipe and cigar smoking are associated with decreased levels of AHRR methylation

Hypothesis: Increased smoking pipe-years and cigar-years will be associated with lower methylation M-values at the AHRR cg05575921 site (**Figure 1**).

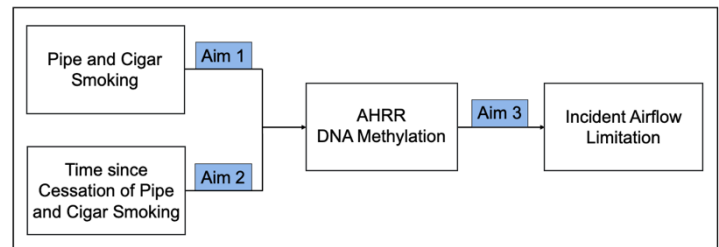


Figure 1. Conceptual model of the project

Aim 2. Evaluate whether pipe and cigar smoking cessation is associated with increased levels of AHRR (cg05575921) methylation

Hypothesis: Increased time since quitting pipe and cigar smoking will be associated with increased methylation M-values at the AHRR cg05575921 site.

Aim 3. Evaluate whether AHRR methylation levels among pipe and cigar smokers are associated with risk of incident airflow limitation

Hypothesis: Lower AHRR cg05575921 methylation levels will be associated with increased risk of incident airflow limitation among current and former pipe and cigar smokers

Methods

Population: We will include study participants from six prospective cohort studies

- Atherosclerosis Risk in Communities (ARIC)
- Coronary Artery Risk Development in Young Adults (CARDIA)
- Cardiovascular Health Study (CHS)
- Framingham Heart Study-Offspring (FHS-O)
- Multi-ethnic Study of Atherosclerosis (MESA)
- Strong Heart Study (SHS)

Exposures:

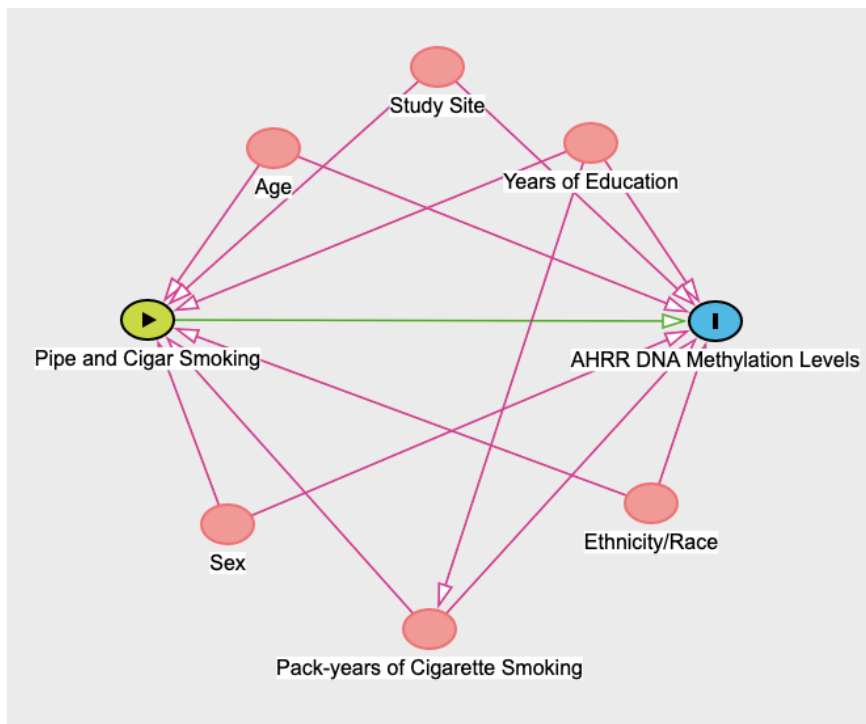
- Aim 1: Cumulative pipe and cigar smoke exposure
 - Pipe-years (defined as years from self-reported age of starting to quitting [or current age among current pipe smokers] x pipe-bowls smoked per day)

- Cigar-years (defined as years from self-reported age of starting to quitting [or current age among current cigar smokers] x cigars smoked per day)
- Aim 2: Time in years since quitting pipe and cigar smoking
- Aim 3: DNA methylation M-values of AHRR cg05575921

Outcomes:

- Aims 1 and 2: DNA methylation M-values of AHRR cg05575921
- Aim 3: Incident airflow limitation (defined as the forced expiratory volume in 1 second/forced vital capacity [FEV1/FVC] <0.7)

Covariates:



- Aims 1 and 3: Model covariates will include age, sex, ethnicity/race, cigarette smoking pack-years, educational attainment and study site
- Aim 2: Models will be additionally adjusted for pipe/cigar-years in order to increase precision

Analysis Plan:

- Aim 1: Among all participants with available DNA methylation data, linear regression models will be used to evaluate associations of smoking pipe- and cigar-years with methylation M-values at the AHRR cg05575921 site. Models will be adjusted for age, sex, ethnicity/race, cigarette smoking pack-years, educational attainment, and study site. In a sensitivity analysis, associations of pipe- and cigar-years with methylation M-values at the AHRR site will be modeled using linear regression among participants who never smoked cigarettes.

- Aim 2: Among former pipe and cigar smokers, linear regression models will be used to evaluate associations of years since smoking cessation with methylation M-values at the AHRR cg05575921 site. Models will be adjusted for age, sex, ethnicity/race, cigarette smoking pack-years, pipe/cigar-years, educational attainment, and study site. In a sensitivity analysis, associations of years since pipe- and cigar-smoking cessation with methylation M-values at the AHRR site will be modeled using linear regression among participants who never smoked cigarettes.
- Aim 3: Among current and former pipe and cigar smokers who did not have airflow limitation ($FEV1/FVC \geq 0.7$) at the time of DNA methylation measurements, Cox proportional hazards models will be used to evaluate the association of methylation M-values at the AHRR cg05575921 site with risk of incident airflow limitation. Models will be adjusted for age, sex, race/ethnicity, cigarette smoking pack-years, educational attainment and study site. In a sensitivity analysis, associations of methylation M-values at the AHRR site with incident airflow limitation will be modeled using Cox proportional hazards models among participants who never smoked cigarettes.

Proposed Timeline

	August	September	October	November	December	January
Data Acquisition						
Data Analysis						
Manuscript Preparation						
Manuscript Submission						

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