EPIGENETIC MEDIATION OF PARTICULATE MATTER-ASSOCIATED CHANGES IN HEART RATE VARIABILITY AND QT INTERVAL DURATION

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ABSTRACT

Rahul Gondalia: Epigenetic Mediation of Particulate Matter-Associated Changes in Heart Rate Variability and QT Interval Duration
(Under the direction of Eric A. Whitsel)

Background. Ambient particulate matter (PM) air pollution is a modifiable exposure that has been consistently associated with higher cardiovascular disease (CVD) risk, at least in part through autonomic dysfunction and prolonged ventricular repolarization, as observed by decreases in heart rate variability (HRV) and increases QT interval duration (QT) on the electrocardiogram. However, the molecular mechanisms underlying these associations are not well understood.

Methods. PM associations with leukocyte count, proportions, and DNA methylation were estimated using linear mixed, covariate-adjusted models using multiply-imputed, multi-center, longitudinal data in racially, ethnically and environmentally diverse populations of U.S. women and men. Then, PM-associated changes in HRV and QT – and epigenetic mediation of those associations – were estimated.

Results. Monthly to yearly mean $PM_{2.5}$ concentrations were associated with = higher leukocyte counts, higher granulocyte proportions, and lower CD8+ T cell proportions. Methylome-wide association analyses identified three significant CpG sites (cg19004594, cg24102420, and cg12124767) annotated to *MATN4*, *ARPP21* and *CFTR* at which higher monthly mean PM_{10} and $PM_{2.5-10}$ concentrations were associated with leukocyte DNAm. However, neither monthly mean PM_{10} or $PM_{2.5-10}$ nor methylation at cg19004594, cg24102420,

or cg12124767 were appreciably associated with HRV or QT, thereby yielding null PM-DNAm-HRV and PM-DNAm-QT mediation associations.

Conclusions. Findings suggest that PM is associated with leukocyte count, composition and DNAm at concentrations below U.S. Environmental Protection Agency National Ambient Air Quality Standards. However, monthly exposures to coarser particulates, while associated with DNAm, did not exert appreciable, epigenetically mediated effects on cardiac autonomic function or ventricular repolarization. Nonetheless, the methods and results described herein may inform causal and mediation methods at the junction of epigenetics, environmental and cardiovascular epidemiology, and the findings have implications for policy-relevant decision-making and standard setting by the US Environmental Protection Agency under the Clean Air Act.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	African American
AQS	United States Environmental Protection Agency Air Quality System
ARIC	Atherosclerosis Risk in Communities
AS311	Ancillary Study 311
AV	annual visit
BAA23	Broad Agency Award 23
CI	confidence interval
CpG	Cytosine-phosphate-Guanine
CT	Clinical Trial
CVD	cardiovascular disease
DNAm	deoxyribonucleic acid methylation
EA	European American
ECG	electrocardiogram
eFORGE	Functional element Overlap analysis of Regions
EMPC	Epigenetic Mechanisms of PM-Mediated CVD Risk
EPA	Environmental Protection Agency
FDR	false discovery rate
GTP	Grady Trauma Project
GWAS	genome-wide association study
HLA	Hispanic/Latino American
HRV	heart rate variability
KORA	Cooperative Health Research in the Region Augsburg study

LC	leukocyte count
LLS	Long Life Study
LMM	linear mixed models
MESA	Multi-Ethnic Study of Atherosclerosis
MICE	multiple imputation by chained equations
MWAS	methylome-wide association study
NAAQS	National Ambient Air Quality Standards
NAS	Normative Aging Study
NK	natural killer cell
NN	normal-to-normal
OR	odds ratio
OS	Observational Study
PE	prediction error
PM	particulate matter
PM_{10}	PM < 10 μm in diameter
$PM_{2.5}$	PM < 2.5 μm in diameter
$PM_{2.5-10}$	PM > 2.5 and < 10 μm in diameter
QQ	quantile-quantile
QT	QT interval duration
RMSS	root mean square standardized
RMSSD	root mean square of successive differences between RR intervals
SD	standard deviation
SDNN	standard deviation of normally conducted NN intervals

SE	standard error
SPE	standardized prediction error
WHI	Women's Health Initiative

CHAPTER 1. SPECIFIC AIMS

Ambient particulate matter (PM) air pollution is a modifiable exposure that has been consistently associated with cardiovascular disease (CVD) morbidity and mortality, partly through changes in cardiac autonomic function and ventricular repolarization as measured by heart rate and its variability (HRV) and QT interval duration (QT). Despite the ubiquity of air pollution exposure and the continued population burden of PM, the putative mechanisms underlying PM-associated CVD have not been adequately investigated.

One such mechanism involves methylation of deoxyribonucleic acids (DNAm), conventionally measured at Cytosine-phosphate-Guanine (CpG) sites in DNA. As measured, DNAm is a heritable, but dynamic epigenetic modification that can influence gene expression without altering the genome and may be central to mediation of PM-associated CVD risk. Indeed, PM exposure has been implicated in DNAm near candidate genes involved in inflammation, oxidative stress, and coagulation, abnormalities of which have established associations with CVD.

However, few studies have agnostically evaluated PM and leukocyte DNAm associations on a methylome-wide scale. They also were conducted in geographically and socio-demographically homogenous populations. Moreover, its analyses neither characterized the inflammatory effects of PM – a driver of leukocyte DNAm values – nor attempted to elucidate putative epigenetic mechanisms linking PM with increased CVD risk.

To carefully address limitations of the extant research in this area using data from large, multi-ethnic and geographically diverse US populations enrolled in the Women's Health Initiative (WHI) and Atherosclerosis Risk in Communities study (ARIC), I therefore propose to:

(1) Estimate associations between PM and leukocyte traits (count; proportions),

(2) Estimate methylome-wide associations between PM and leukocyte DNAm, using models informed by (1), and

(3) Assess mediation of PM-HRV and PM-QT associations by DNAm at PM-sensitive CpG sites

Collectively addressing the three aims will provide insight into epigenetic mechanisms underlying environmentally induced cardiac autonomic dysfunction and prolonged ventricular repolarization, the existence of which may help substantiate the biological plausibility and causality of PM-CVD associations being considered by US Environmental Protection Agency as it sets National Ambient Air Quality Standards for PM under the Clean Air Act.

CHAPTER 2. BACKGROUND AND SIGNIFICANCE

A. Cardiovascular disease burden

Cardiovascular disease (CVD) carries a substantial healthcare burden in the United States (US), where it accounts for approximately $396 billion in direct medical costs and is projected to increase to over $900 billion by 2030. Although CVD incidence and mortality in the US has been declining since the 1970s, CVD still accounts for nearly 31% of all deaths.[1] Consequently, lifestyle and therapeutic interventions have been directed at high-risk groups in an attempt to reduce its prevalence and incidence. However, implementing population-level interventions on ubiquitous risk factors, such as exposure to ambient particulate matter (PM) air pollution, could also decrease the overall burden of CVD.

B. Particulate matter

B1. Background

The relationship between ambient air pollution exposure and disease led the US to make substantial air quality improvements beginning with the 1970 establishment of the US Environmental Protection Agency and amendment of the Clean Air Act.[2] Accordingly, ambient air pollution decreased over time and related public health benefits have been observed.[3,4] However, the ubiquity of exposure and its potential threat to public health even at today's lower concentrations remain concerning. Indeed, a Global Burden of Diseases Study estimates that around 4.2 million deaths annually are attributable to PM exposure, which is the fifth leading cause of global mortality[5], with significant contributions to CVD-related morbidity and mortality.[2,5-7]

B2. Particulate matter composition, sources, and size

PM is a complex, aerosolized mixture of solid and liquid matter that can consist of inorganic ions (nitrates, sulfates, ammonium, chlorides, hydrogen ions), carbonaceous aerosols (organic and black carbon), metals (trace and crustal elements), and other organic matter (e.g. bacteria, viruses, pollen, mold, fungal spores).[8-10] The size and composition of PM varies spatiotemporally and by source, with the majority of PM stemming from human activity (i.e. anthropogenic) and including combustion-related products of power generation, industry, and transportation.[2,6]

PM is measured as a concentration in micrograms per meter cubed ($\mu g/m^3$) and is classified by its aerodynamic diameter, which affects where it typically deposits in the human airway. Specifically, PM $\leq$ 2.5, 2.5-10, and $\leq$ 10 micrometers (μm) in diameter ($PM_{2.5}$, $PM_{2.5-10}$, and PM_{10},) refer to fine, coarse, and thoracic particulates that often deposit in alveoli/small airways, bronchi/trachea, and both regions of the respiratory tract. Notably, PM_{10} includes particulates from the $PM_{2.5}$ and $PM_{2.5-10}$ size fractions.[6,7]

B3. Policy

The 1970 Clean Air Act requires the US Environmental Protection Agency (EPA) to set and enforce National Ambient Air Quality Standards (NAAQS) for six "criteria" air pollutants (PM, carbon monoxide, lead, nitrogen dioxide, ozone, and sulfur dioxide; Table 2-1) to protect health, including that of "sensitive" populations (e.g. children, elderly, persons with asthma). As of 2013, 24-hour and annual averages of $PM_{2.5}$ may not exceed 12 $\mu g/m^3$ and 35 $\mu g/m^3$, while 24-hour averages for PM_{10} may not exceed 150 $\mu g/m^3$. Although prior standards for annual PM_{10} (at 50 $\mu g/m^3$) were once in place, they were revoked in 2006 due to purported lack of a scientific basis linking long-duration PM_{10} exposure to poor health.[11] No standards are currently in place for $PM_{2.5-10}$.

Table 2-1. National Ambient Air Quality Standards

Pollutant		Averaging duration	Standard	Form
Particle matter (PM)	$PM_{2.5}$	Annual	15 $\mu g/m^3$	Annual mean, averaged over 3 years
		24-hour	35 $\mu g/m^3$	98th percentile, averaged over 3 years
	PM_{10}	24-hour	150 $\mu g/m^3$	Not to be exceeded more than once per year on average over 3 years

B4. Cardiovascular disease and mortality associations

Short- (< 1 month) and long- (≥ 1 month) duration exposures to ambient $PM_{2.5}$, PM_{10}, and $PM_{2.5-10}$ have been associated with CVD and mortality in epidemiologic studies.[6,7] Short-duration increases in $PM_{2.5}$ and PM_{10} were associated with a slight but consistent increase in daily mortality risk, ranging from 0.4% to 1.0% for a 10 $\mu g/m^3$ increase in $PM_{2.5,}$ PM_{10} or $PM_{2.5-10}$.[7] Relative to short-duration exposure, long-duration exposure to PM was more prominently associated with mortality. Three notable studies observed elevated mortality risk with an increase of long-duration $PM_{2.5}$ by 10 $\mu g/m^3$: the Harvard Six Cities study observed increases in all-cause and CVD mortality risk by 14% (95% confidence interval [CI]: 1.07, 1.22) and 26% (95% CI: 1.14, 1.40)[3]; the Women's Health Initiative (WHI) Observational Study (OS) of postmenopausal women in the US observed a substantial, 76% (95% CI: 1.25, 2.47) increase in CVD mortality risk;[12] and a recent study in over sixty million US Medicare beneficiaries found a 7.3% (95% CI: 7.1, 7.5) increase in all-cause mortality risk.[13] In addition, the Nurses Health Study found a similar increase in all-cause (1.11, 95% CI: 1.01, 1.23) and CVD (1.35, 95% CI: 1.03, 1.77) mortality risk with a 10 $\mu g/m^3$ increase in PM_{10} concentrations among women living in the north eastern region of the US.[14]

Ambient PM exposure was also associated with nonfatal CVD, particularly with coronary heart disease (CHD). The European Study of Cohorts for Air Pollution Effects (ESCAPE) of

100,000 participants across eleven European cohorts found 13% (95% CI: 0.98, 1.30) and 12% (95% CI: 1.01, 1.25) increases in CHD risk with a 5 $\mu g/m^3$ increase of long-duration $PM_{2.5}$ and a 10 $\mu g/m^3$ increase of long-duration PM_{10} exposures.[15] The WHI OS study mentioned earlier similarly observed elevated CHD and myocardial infarction (MI) risk with long-duration $PM_{2.5}$ exposure.[12] Short-duration PM exposure was also associated with incident MI in several case-crossover studies,[16-20] and a study in US Medicare beneficiaries estimated a reduction of 1,523 (95% CI: 19, 2,976) CHD-related hospitalizations per year with a 10 $\mu g/m^3$ decrease of short-duration $PM_{2.5}$ exposure.[21]

Finally, PM exposure was associated with other nonfatal forms of CVD, including heart failure and cardiac arrhythmias. A meta-analysis of thirty-five studies found that a 10 $\mu g/m^3$ increase in $PM_{2.5}$ or PM_{10} was associated with an approximate 2% increase in heart failure risk.[22] Associations with cardiac arrhythmias were also observed, but largely in case-crossover studies of patients with implantable cardioverter defibrillators[23-31], with mixed results[32-40] possibly due to variations in study design, socio-demographic characteristics, and exposure duration and composition.[7,34] Two epidemiologic analyses in the Reasons for Geographic And Racial Differences in Stroke study (REGARDS), a bi-racial study of men and women in the US, found increases in risk of premature ventricular and atrial contractions with $PM_{2.5}$ exposure.[41,42] However, associations between PM and physician-confirmed ventricular ectopy in WHI were only observed in smokers.[36]

C. Particulate matter and leukocyte traits

C1. Leukocyte background

Leukocytes, also known as white blood cells, originate from primitive stem cells in bone marrow and constitute a major portion of the immune system. As such, they represent a nonspecific indicator of immune response and inflammation related to acute or chronic infection

or exposure to toxicant stimuli. Leukocytes are classified into three types, approximately 65%, 30% and 5% of which are granulocytes, lymphocytes, and monocytes. Granulocytes are further sub-classified as neutrophils (~95%), eosinophils (~4%), and basophils (~1%), while lymphocytes are sub-classified as B (~13%), T (~75%), and natural killer (NK; ~12%) cells.[43,44] Leukocyte composition is determined by the proportions of leukocyte cell types present in peripheral blood, the so-called "differential" of clinical hematology.

Leukocytes have been implicated in endothelial injury, atherosclerotic disease progression, and subsequent increases in CVD risk. Several factors that promote endothelial dysfunction are well known, such as cigarette smoking, hypertension, hypercholesterolemia, and hyperglycemia. Resultant microvascular injury stimulates adhesion and coagulation molecules, thereby recruiting monocytes into atherosclerotic lesions. As inflammation progresses, the recruitment of monocytes and lymphocytes from peripheral blood increases leukocyte content within the atherosclerotic plaque, making it more vulnerable to rupture.[45-47] Due to the pathogenicity inflammation plays in atherogenesis, the effects of leukocyte counts and proportions on cardiovascular health have been studied.

C2. Leukocyte epidemiology

The biological underpinnings linking systemic inflammation with CVD risk have been well supported in studies of leukocyte counts. In patients with prevalent CVD, higher leukocyte counts were associated with increased risks of recurrent cardiovascular events[48,49] and mortality.[50-55] Associations for CVD and all-cause mortality risk were also observed in community-based studies in the US[56-63] and abroad.[64-68] In fact, a meta-analysis of nineteen prospective studies found a 40% (95% CI: 1.3-1.5) increase in CHD risk with a $2.8x10^9$/liter increase in leukocyte count.[69] Associations were also observed with other nonfatal forms of CVD

(e.g. heart failure,[70] atrial fibrillation,[71] hypertension,[72,73] ischemic stroke,[60] diabetes,[74,75] chronic kidney disease[76,77]) and behavioral risk factors (e.g. smoking[78] and physical inactivity).[79]

Studies of leukocyte cell types are fewer, but yielded consistent results primarily driven by neutrophils. In a systematic review of patients with acute coronary syndromes, neutrophil count was a strong and independent predictor of cardiovascular outcomes.[80] A meta-analysis of seven prospective cohort studies (n = 30,374) also found that participants with neutrophil counts in the upper tertile had a 33% (95% CI: 1.17, 1.50) increase in CHD risk relative to the lower tertile.[81] Finally, community-based studies observed elevated CVD and all-cause mortality risk with increases in neutrophil and monocyte counts.[60,82,83]

C3. Particulate matter and leukocyte count associations

Exposure to ambient PM concentrations can stimulate immune response and enhance the release and redistribution of leukocytes in peripheral blood.[47,84] However, PM-leukocyte associations in previous studies were generally mixed. In panel and other small-scale studies of specialized populations, short-duration exposure to PM were sometimes associated with leukocyte counts, though results were variable,[47,85-97] likely attributable to differing study designs, participant characteristics, PM mass fractions, and exposure durations. In larger, community- or population-based studies, short-duration PM_{10} exposure was largely not associated with leukocyte counts,[98-100] with the exception of associations observed in NHANES.[101]

Long-duration exposure to ambient PM was more consistently associated with leukocyte and neutrophil counts. Cross-sectional NHANES data linked long-duration PM_{10} exposure to leukocyte counts.[102] Comparable findings with $PM_{2.5}$ and PM_{10} were also observed using longitudinal data from the German Heinz Nixdorf Recall Study[103] and cross-sectional data from Social Environment and Biomarkers of Ageing Study (SEBAS) in Taiwan.[104]

C4. Summary and limitations

Inflammation plays a major role in atherogenesis, plaque formation and rupture, and subsequent downstream cardiovascular consequences. Indeed, studies observed that increases in systemic inflammation, as measured by leukocytes, are associated with CVD incidence, CVD mortality, and all-cause mortality.

As such, it has been hypothesized that systemic inflammation evoked by PM inhalation may be a mechanism by which PM increases CVD risk. Epidemiologic evidence has generally supported this hypothesis; however, results have been mixed and the quality of evidence in the US has been subpar. Specifically, all three community-based longitudinal studies of this topic were conducted abroad,[98,99,103] which limits the generalizability to US populations due to differing socio-demographic and exposure characteristics (e.g. PM composition and concentrations). Also, there has not been an exhaustive evaluation of associations between leukocyte count, cell type proportions, and PM size fractions over short to long exposure durations. Finally, estimating associations between PM exposure and leukocyte DNA methylation (DNAm) is an emerging area of study that can additionally enhance the understanding of the inflammatory consequences of PM inhalation.

D. Particulate matter and DNA methylation

D1. DNA methylation background

Epigenetics is the study of heritable, but dynamic, changes in gene expression (transcription) due to factors other than changes in the DNA sequence itself.[105,106] Epigenetic modification of DNA can take several forms, one of which is methylation. DNAm is an enzymatic reaction catalyzed by specific DNA methyltransferases. In this reaction, methyl groups (CH_3) from S-adenosyl-L-methionine donors are bound to 5’ cytosine nucleotides linked to 3’ guanine nucleotides by phosphate bridges, i.e. Cytosine-phosphate-Guanine (CpG) sites.

The products of the reaction are 5-methyl-cytosine (5-mC) residues on the DNA sequence.[106] CpG methylation thereby changes the physical structure around a DNA sequence without actually altering the order of its nucleotides. Two major ways CpG methylation can in turn influence, and oftentimes suppress gene expression are by: 1) physically interfering with binding of transcription factors to gene promoters, thereby inhibiting transcription, and 2) binding to methyl-CpG-domain binding proteins, thereby recruiting chromatin remodeling proteins, modifying histones, and forming inactive heterochromatin.[106,107]

D2. Cardiovascular disease epidemiology of DNA methylation

Given the influential role of DNAm in gene expression, studying it may plausibly elucidate underlying mechanisms of disease. Research in human heart tissues and animal models found links between DNAm and biological origins of CVD[108,109] in the form of atherosclerosis,[110,111] aortic fatty streaks,[112] cardiomyopathy,[113] and inflammation.[114]

Although heart and other (e.g. nervous) tissues are appropriate for studying the role of DNAm in cardiovascular disease, their collection is highly invasive and not practical, especially in large populations.[108,115] As such, leukocytes extracted from peripheral blood have been widely used surrogate tissues[108] in part given demonstrated consistency of DNAm patterns across relevant tissues types.[116-118] In peripheral blood leukocytes, small-scale human studies related inflammation,[119] hypertension,[120] and mortality[119] with global and candidate-gene DNAm. Larger and more generalizable studies leveraged existing peripheral blood samples to examine DNAm mechanisms and observed associations with prevalent and incident CHD,[121-123] prevalent and incident stroke,[121,123] and CVD and all-cause mortality.[121] DNAm also was found to differ across populations by age,[124-129] sex,[124,130,131] and race/ethnicity[130,132], and by behavioral CVD risk factors such as diet,[133-139] smoking,[140-151] and exercise,[152] all of which suggest that epigenetic processes play a role in cardiovascular health.

D3. Particulate matter and DNA methylation associations

DNAm has been associated with air pollution exposure in occupational, panel, and community-based studies. Several small-scale, occupational or panel studies in adults suggested inverse relationships between exposure to PM and global DNAm,[153-158] while other results were mixed.[159-161] They also detected inverse relationships with DNAm at candidate genes involved in oxidative stress response,[155,156,162,163] coagulation,[154] and vasoconstriction.[154,158,163] Parallel associations were also observed at candidate genes involved in inflammation,[154,155,164] but the literature is less consistent.[165]

Research in the Normative Aging Study (NAS) – a prospective cohort study of white, elderly male veterans living in the greater Boston area[166] – has provided well-powered, high-quality epidemiologic evidence suggestive of PM influences on DNAm. By leveraging repeated PM and DNAm data from up to four study visits, results suggested inverse associations between ambient concentrations of $PM_{2.5}$ over short-durations[167] with global DNAm of long interspersed nucleotide element-1 [*LINE-1*]. However, associations were not observed with *LINE-1* methylation at long-duration exposure[168] or with methylation of short interspersed nucleotide Alu repetitive elements (*Alu*).[167,168] In candidate gene studies, short-duration exposure to $PM_{2.5}$ was associated with decreasing methylation in genes related to inflammation (i.e. *GCR* and *ICAM-1*),[169,170] oxidative stress (i.e. *iNOS*),[169] and coagulation (i.e. *F3*).[164] However, exposure was also suggestively associated with increasing methylation (i.e. *IL-6*)[164] and no change in methylation (i.e. *IFN-γ* and *TLR-2*)[164,170] in other inflammation-related genes.

The Multi-Ethnic Study of Atherosclerosis (MESA) – a diverse, community-based cohort study of men and women in the US – investigated exposure to long-duration concentrations of $PM_{2.5}$ with monocyte DNAm at 2,713 CpG sites linked with mRNA expression and global methylation at *LINE-1* and *Alu*. $PM_{2.5}$ was statistically associated with methylation at five CpG

sites (increases at one and decreases at four sites), with a false discovery rate < 0.05, and was non-significantly associated with decreases in *LINE-1* and *Alu* methylation.[171]

Few studies investigated the relationship between exposure to ambient concentrations of PM and DNAm on a methylome-wide scale (i.e. at over 450,000 CpG sites), although in demographically and geographically homogeneous populations[172-174]. Only one, a collaborative assessment of $PM_{2.5}$-DNAm associations in NAS and Cooperative Health Research in the Region of Augsburg study (KORA) – a community-based study of European ancestry men and women living in Augsburg, Germany – identified statistically significant CpG sites (one for 2-, one for 7-, and ten for 28-day $PM_{2.5}$ averages) with Bonferroni-corrected p-values < 7.5×10^{-8}, of which three were associated with an increase and nine with a decrease in methylation.[173]

D4. Summary and limitations

The complex interplay between epigenetic and environmental risk factors may reveal underlying mechanisms for increased CVD risk, but they have not been thoroughly evaluated. Nevertheless, recent research has described associations between exposure to ambient PM and DNAm globally, at candidate genes, and at specific CpG sites from methylome-wide association studies. While global methylation metrics can be valuable biomarkers for exposure, their links to specific disease pathways are unclear.[175] Candidate gene approaches can inform biological mechanisms with statistical efficiency, but findings may be prone to type I error, as observed in early genetic epidemiology studies.[176,177] With this caveat, evidence from candidate gene studies supports previously described mechanisms that relate PM to inflammation, oxidative stress, coagulation, and vasoconstriction.[7] To discover epigenetic associations agnostically, NAS and KORA conducted a methylome-wide association study and discovered twelve novel CpG sites associated with $PM_{2.5}$ concentrations, however associations have not yet been replicated in demographically, geographically, and environmental diverse populations.[173]

The current state of PM and methylation research relies on results from mostly panel and occupational studies, and a few community-based studies. Although panel and occupational studies of DNAm can efficiently provide biological insight, participant characteristics and PM exposure characteristics are not often representative of the broader population. Community-based studies of PM and DNAm in NAS and KORA have similarly limited generalizability in terms of demographic, behavioral, and exposure characteristics. MESA allowed for analyses in multi-ethnic US populations, however associations have not been evaluated and replicated methylome-wide.

Finally, all prior studies relied on methylation data from peripheral blood with varying proportions of leukocyte cell types, each type of which possesses a distinct methylation pattern. Consequently, peripheral blood DNAm is partially driven by leukocyte composition. A common practice[178,179] is therefore to restrict assay of DNAm to a single type of leukocyte.[171] More commonly, DNAm exposure-outcome models are statistically adjusted for leukocyte proportions determined via cytometry as part of a complete blood count / differential, or in its absence, by constraining the sum of estimated CD8+ T cells, CD4+ T cells, NK cells, B cells, monocytes, and granulocytes in whole blood to 100% and regressing them on DNAm data.[173] However, PM may plausibly influence DNAm by affecting leukocyte proportions (see section C3), so without its adjustment it may yield spurious associations of PM with DNAm through its inflammatory rather than epigenetic mechanisms. Additionally, current mediation analyses of DNAm may be biased if leukocyte proportions confound the DNAm-outcome association (Figure 2-1).[180,181]

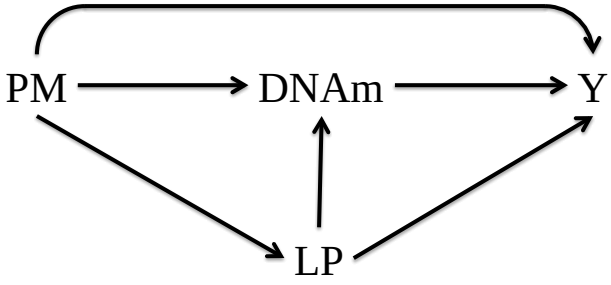

Figure 2-1. Diagram of the association between particulate matter (PM) and outcome Y, partly mediated though DNA methylation (DNAm) and leukocyte proportions (LP). Adjustment for LP would 1) block the path from PM to Y through LP, thereby attenuating the total effect of PM on Y, and 2) block the path from PM to Y through LP and DNAm, biasing estimates for DNAm mediation.

E. Particulate matter and cardiac autonomic function

E1. Cardiac autonomic function

Intrinsic heart rate and rhythm are modulated by the sinoatrial (SA) node (Figure 2-2) which is largely regulated by vasomotor centers in the hypothalamus, brainstem, and spinal cord, via autonomic (parasympathetic and sympathetic) efferent innervation of the heart.

Parasympathetic neurons in the midbrain, pons, and medulla oblongata of the brain stem decrease SA node activity via tenth cranial (vagus) nerve-mediated release of acetylcholine, muscarinic cholinergic receptor activation, and hyperpolarization. More specifically, vagal activity increases transmembrane potassium current and decreases the rate of SA node depolarization during Phase 4 of the SA node action potential (Figure 2-3). Conversely, sympathetic nerves originating from the thoracic segments of the spinal cord increase SA node activity by releasing norepinephrine; activating beta-adrenergic receptors; increasing transmembrane calcium, sodium, and potassium currents; and thereby increasing the rate of SA node depolarization during Phase 4 (Figure 2-3).[182-187]

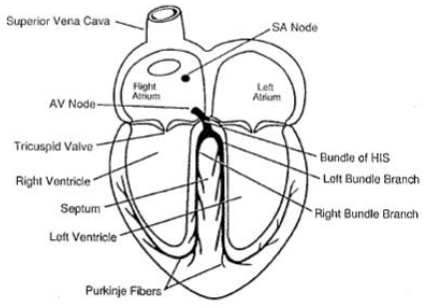

Figure 2-2. Structure of the heart (figure reproduced from Sperelakis 2001[188])

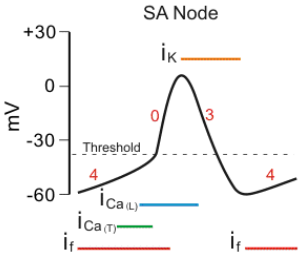

Figure 2-3. Sinoatrial (SA) node action potential. Abbreviations: mV, millivolts, i_K, potassium current; $i_{Ca(L)}$, L-type calcium channel current, $i_{Ca(T)}$, T-type calcium channel current; i_f, funny current (figure reproduced from Klabunde 2017[189])

Autonomic nervous system control of heart rate and rhythm appear to involve underlying genetic influences in multi-ethnic populations.[190-192] For example, a recent genome-wide association study (n = 53,174) implicated seventeen single nucleotide polymorphisms across eight genes associated with heart rate variability (HRV), including *HCN4* (hyperpolarization activated cyclic nucleotide gated potassium channel 4) *GNG11* (G protein subunit gamma), and *RGS6* (regulator of G-protein signaling). *HCN4*'s putative role involves the enhanced HCN channel permeability and increased rate of SA node depolarization characteristic of sympathetic activity while those of *GNG11* and *RGS6* involve the blunting of G protein-gated inwardly rectifying potassium (GIRK) channel activity and decreased rate of SA node depolarization characteristic of parasympathetic activity.[190]

Parasympathetic and sympathetic activity dynamically influences the beat-to-beat sinus rhythm resulting in fluctuations of the heart rate around the mean. Consequently, an increase in sympathetic activity reduces HRV, while an increase in parasympathetic activity increases HRV. Although both systems are continuously reacting to exogenous demands, their imbalance may signal lack of cardiovascular adaptability and resilience. Such autonomic dysfunction is typically

manifested as an overactive sympathetic and underactive parasympathetic system, resulting in decreases in HRV on electrocardiograms (ECGs).[183,185]

E2. Heart rate variability

Autonomic function can be quantified non-invasively in the frequency and time domains using HRV measures derived from ECGs. Frequency domain measures in Hertz (Hz) are obtained from power spectral analysis that decomposes the heart rate (beats/min) or its unit-corrected inverse, RR interval (RR, ms), over a given time period into sinusoidal functions of fluctuating amplitudes and frequencies.[183,193,194] Two commonly used frequency domain measures are low and high frequency spectral powers (LF and HF).[185] LF (0.04 – 0.15 Hz) reflects both sympathetic and parasympathetic activity, while HF (0.15 – 0.40 Hz) typically represents only parasympathetic activity.[185] Frequency domain measures can be computed from short (i.e. 0.5 – 5 minute) and long (i.e. 24-hour) duration ECGs.[183]

Time domain measures of autonomic function estimate HRV in milliseconds (ms) using mathematical functions of successive RR intervals between normally conducted beats, i.e. normal-to-normal (NN) intervals. Two common measures are the standard deviation of successive NN intervals (SDNN) given by,

$$SDNN = \sqrt{\frac{\sum_{i=1}^{n}(NN_{mean} - NN_i)^2}{n-1}}$$

and the root mean square of successive differences between NN intervals (RMSSD), given by

$$RMSSD = \sqrt{\frac{\sum_{i=1}^{n-1}(NN_{i+1} - NN_i)^2}{n}}$$

where n is the total number of NN intervals over the ECG duration. When measured using long duration ECGs, SDNN is a marker of total HRV (sympathetic + parasympathetic activity), but it becomes more representative of parasympathetic activity as recording duration decreases,[182] while RMSSD is a marker of parasympathetic activity for short and long duration ECGs.[182,183,185]

Although long-duration ECGs are ideal for quantifying HRV, ultra-short duration, i.e. 10-second ECGs can be more conveniently and consistently recorded in various settings.[195-197] In the epidemiologic context, time domain measures of SDNN and RMSSD from resting, standard twelve-lead ECGs are reliable and valid [195,198,199] measures of parasympathetic activity.[200]

E3. Epidemiology of heart rate variability

Epidemiologic evidence suggests that autonomic dysfunction (i.e. hyperactive sympathetic and hypoactive parasympathetic system), as characterized by decreases in HRV, is a common indicator of declining cardiovascular health.[185] Specifically, studies demonstrated that HRV is inversely associated CVD [201-203] and all-cause mortality,[202-205] with similar mortality susceptibility in patients with CHD,[206-212] diabetes,[203,213] hypertension,[203] and heart failure.[214-216] Additionally, decreases in HRV were predictive of increasing serum glucose[217-220], insulin[217,219], and cholesterol[221-223], incident hypertension,[220,223-225] and CVD[202,220,226-228]; and associated with risk factors for CVD, including male sex,[229-231] increasing age,[230,231] physical inactivity,[229,230,232-234] and smoking.[235-238]

E4. Particulate matter and heart rate variability associations

One hypothesized pathway linking the association of air pollution exposure to CVD is through changes in cardiac autonomic function. Indeed, many occupational, panel, and community-based studies observed decreases in HRV associated with increasing PM exposure. A meta-analysis of 29 studies (total n = 18,667) found that SDNN, RMSSD, HF, and LF decreased -0.1%, -2.2%, -2.4%, and -1.7% per 10 $\mu g/m^3$ increase in ambient, short-duration

$PM_{2.5}$ concentrations.[239] These associations were consistent with studies of short-duration exposure to PM_{10}[240-242] and $PM_{2.5-10}$ size fractions.[243-247] Importantly, a separate meta-analysis observed magnitudes of short-duration PM_{10}-HRV associations further from the null when substituting imputed concentrations of personal PM_{10} exposure for ambient concentrations, suggesting that the effects of ambient PM exposure on HRV are likely underestimates of the true association.[248]

Longer-duration exposures to $PM_{2.5}$ and $PM_{2.5-10}$ in MESA yielded similar, but attenuated, associations.[200,249] However in a Swiss study, long-duration PM_{10} was associated with decreases in HRV only among participants taking ACE inhibitors, suggesting that underlying health conditions may confer susceptibility.[250] Indeed, such susceptibility was observed among the elderly[89,251-257] and in those with asthma,[244,246] dyslipidemia,[200] hypertension,[200,242,252,253,258] glucose dysregulation,[241] diabetes,[200,253] metabolic syndrome,[200] and coronary heart disease.[245,253,259-261]

E5. Particulate matter and heart rate variability mechanisms

The biological mechanisms underlying PM-associated decreases in HRV are not fully established. It has been hypothesized that inhaled PM induces a reflexive, sympathetic stress response by directly activating pulmonary chemoreceptors. PM also may be translocated through nasal epithelium, the first cranial (olfactory) nerve, and / or alveolar epithelium to the blood, vasomotor centers of the brain, and / or heart where it may act directly on receptors or indirectly by stimulating release of macrophages and pro-inflammatory cytokines resulting in downstream sympathetic activation, parasympathetic withdrawal, increased heart rate, and decreased HRV.[239,262,263]

Oxidative stress-response mechanisms were also implicated from gene-environment interaction studies within the NAS cohort.[264-268] In these studies, the $PM_{2.5}$-HRV association was

modified by *GSTM1* (glutathione-S-transferase M1),[265,267] *HMOX-1* (heme oxygenase-1),[267] and *HFE* (a hemochromatosis gene),[264] all genes directly or indirectly responsible for reactive oxygen species metabolism. This hypothesis is further supported by the attenuation of PM-HRV associations with statin use,[265] methyl nutrients,[266] and omega-3 fatty acids.[269]

Lastly, PM-induced changes in DNAm at CpG sites proximal to genes[190-192] associated with cardiac autonomic function (see section E1), such as *HCN4*, *GNG11*, and *RGS6*, offer other pathways by which particulate exposure may indirectly decrease HRV.

E6. Summary and limitations

HRV measures the cardiac response to the autonomic nervous system, which consists of sympathetic and parasympathetic nerves that modulate beat-to-beat variation in heart rate. Increases in heart rate and decreases in HRV are characterized by sympathetic activation and parasympathetic withdrawal and have been associated with CVD and mortality.[185]

Ambient PM air pollution has been consistently associated with decreases in HRV, suggesting autonomic dysfunction as a pathophysiological pathway linking PM with cardiovascular morbidity and mortality. However, the state of evidence largely relies on small-scale panel and occupational studies which do not accurately describe the demographic, clinical, and exposure characteristics of the general population, thereby limiting generalizability. Indeed, of the twenty-nine studies included in a recent meta-analysis of PM and HRV,[239] only five were community-based studies,[200,241,242,253,270] of which one leveraged longitudinal data.[241] Still, all studies yielded mostly consistent results,[239] and true magnitudes of associations are plausibly larger than what was observed in studies of ambient PM exposure.[248]

Finally, the biological mechanisms linking PM and autonomic dysfunction remain unclear, but likely involve reflexive pulmonary responses to PM or its translocation to the blood, brain, or heart where it may act directly or indirectly though inflammatory, oxidative, or

epigenetic pathways that potentiate sympathetic activity. However, these mechanisms are uncertain and the degree to which they contribute to disease is unknown.

F. Particulate matter and ventricular repolarization

F1. Ventricular repolarization

The cardiac cycle consists of diastolic (relaxation) and systolic (contraction) periods that depend on cardiac depolarization initiated in the SA node then reaching the ventricles through the atrioventricular (AV) node and His-Purkinje system.[271] Action potentials within the ventricles rely on coordinated sequences of transmembrane depolarizing and mostly repolarizing cation fluxes in ventricular myocytes.[272] The action potentials have five phases. Phase 0 represents rapid depolarization caused by an influx of Na^+ followed by initial repolarization through a transient efflux of K^+ in Phase 1. Repolarization is subsequently delayed in Phase 2 (the plateau phase) by a steady influx of Ca^{2+} through the voltage-gated L-type Ca^{2+} channel and an efflux of K^+ primarily through rapid delayed rectifier channels. In Phase 3, myocytes undergo rapid repolarization caused by the inactivation of the L-type Ca^{2+} channel and K^+ efflux through rapid and slow delayed rectifier channels and K^+ influx through inward rectifier channels. Finally, delayed K^+ rectifier current ends while inward K+ rectifier current continues when resting potential is reached in Phase 4 (Figure 2-4).[273]

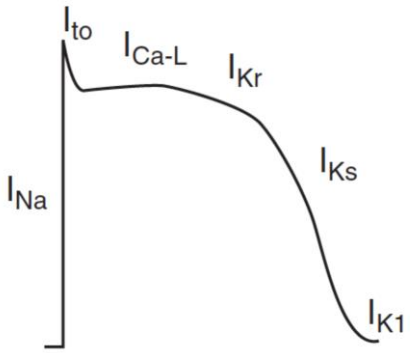

Figure 2-4. Ventricular action potential currents. Abbreviations. I_{Na}, sodium current; I_{Ca-L}, L-type Ca^{2+} current; I_{to}, transient outward K^+ current; I_{Kr}, rapid delayed rectifier K^+ current; I_{Ks}, slow delayed rectifier K^+ current; I_{K1}, inward rectifier K^+ current (reproduced from Cutler et al. 2011[273])

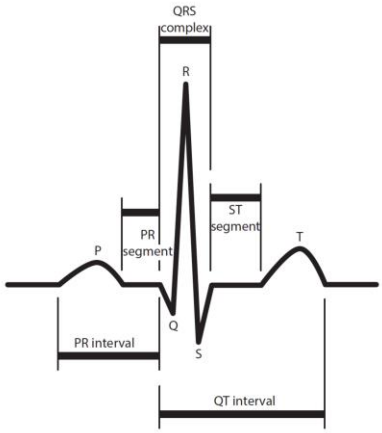

Figure 2-5. Waves and intervals on the ECG (reproduced from Houghton and Roebuck 2015[274]

As with cardiac autonomic function, genetic underpinnings of ventricular repolarization have been described in multi-ethnic populations.[275-277] A recent genome-wide association study in 76,198 individuals of European ancestry with replication in up to 103,331 individuals identified sixty-eight single nucleotide polymorphisms across thirty-five genes associated with QT interval duration, a electrocardiographic measure of ventricular repolarization (Figure 2-5, see section F2). Implicated genes highlighted the role of calcium signaling and included *ATP2A2* (ATPase sarcoplasmic/endoplasmic reticulum Ca^{2+} transporting 2), *PLN* (phospholamban), *PRKCA* (protein kinase C alpha), *SRL* (sarcalumenin), and *SLC8A1* (solute carrier family 8 member A1). *ATP2A2* encodes the SERCA2a cardiac sarcoplasmic reticulum calcium pump that is responsible for Ca^{2+} sequestration. *ATP2A2* is negatively regulated by *PLN*, which itself is negatively regulated by *PRKCA*. *SRL* encodes sarcalumenin and *SLC8A1* encodes a Na^+/Ca^{2+} exchanger, both of which regulate Ca^{2+} flux in cardiac myocytes.

Because ventricular repolarization depends on cation gradients in cardiac myocytes – particularly those of Ca^{+2} and K^{+}– genetic, pharmacologic and / or environmental factors that lengthen Ca^{2+} influx or shorten K^{+} efflux during Phase 2 lead to an intracellular excess of cations. The excess causes a depolarizing flux and prolongs ventricular repolarization, resulting in a prolonged QT interval as quantified noninvasively using ECGs.[272,275,278]

F2. QT interval duration

Ventricular repolarization as measured electrocardiographically from the onset of the QRS complex to the end of the T wave (Figure 2-5) is called the QT interval (QT, ms). As defined, QT is composed of the QRS complex and the subsequent JT interval, which itself consists of the ST segment and T wave.[272] The QRS complex represents the initial period of ventricular repolarization during Phase 1 of the ventricular action potential that directly follows rapid depolarization occurring in Phase 0. The JT interval represents Phase 2, the plateau phase with little repolarization, (i.e. the ST segment) followed by rapid repolarization in Phase 3 (i.e. the T wave).

QT varies inversely as a function of heart rate, and therefore increases with increasing RR. As such, analyses often rely on the heart rate-corrected QT (QTc) or statistical adjustment for RR when modeling QT-exposure associations. The most common form of heart rate correction involves Bazett's formula, in which QTc is estimated by dividing QT by the square root of RR. However, it produces biased measures at high and low of heart rates[279,280] so various alternatives have been proposed.[280-285]

While variability in QT can be explained by biological and environmental factors, it is additionally subject to technical variation, such as lead placement, that can artificially increase QT dispersion.[286] That said, QT as measured on ECGs is a valid and reliable time domain estimate of ventricular repolarization over the short- and long-term.[287,288]

F3. Epidemiology of QT interval duration

Epidemiologic studies have demonstrated that prolonged ventricular repolarization manifest on ECGs as prolonged QT is a risk factor for ventricular arrhythmias (e.g. Torsades de pointes)[289,290], coronary heart disease[291,292], congestive heart failure[293], stroke[294], cardiovascular mortality (particularly sudden cardiac death) [295-298], and all-cause mortality[293,295-297] in the general population. Moreover, QT prolongation has been associated with female sex[299-301] and CVD risk factors that include increasing age[300], subclinical atherosclerosis[302,303], diabetes[304-311], increasing obesity[312,313], physical inactivity[311], and smoking.[311] Other known causes of prolonged QT are Mendelian conditions that affect Na^+, K^+ and Ca^{2+} ion channels (i.e. congenital long QT syndrome [LQTS])[271], use of medications known to induce long QT (types of e.g. antiarrythmics, antimicrobials, and tricyclic antidepressants)[290,299,314,315], and electrolyte imbalances (e.g. hypokalemia and hypocalcemia).[300,316,317]

F4. Particulate matter and QT interval duration associations

PM's association with ventricular arrhythmias [23-25,27,28,30,31,37] may depend on prolonged ventricular repolarization. Several community-based and panel studies of short- and long-duration exposures to $PM_{2.5}$ and QT have been conducted. While short-duration exposures to $PM_{2.5}$ were not associated with QT in NAS[318,319], they were associated with a minor 0.23% increase in QT with a 7 $\mu g/m^3$ increase in 5-day mean $PM_{2.5}$ in a North Carolina study of patients who underwent cardiac catheterization (CATHGEN).[320] Additionally, in a subset of WHI and ARIC participants, higher ($\geq 90^{th}$ percentile) exposures to 2-day mean PM_{10} were associated with higher QT in those with the TT allele of rs1619661, a single nucleotide polymorphism proximate to *CXCL12*, suggesting possible genetic susceptibility to PM-associated increases in QT. In panel studies, positive associations with QT also were detected with short-duration $PM_{2.5}$ in adults that were healthy and nonsmoking[321], with coronary heart disease[322,323], and with diabetes.[324]

Only two community-based studies evaluated associations between QT and longer duration exposures to PM, but they similarly yielded positive associations. In NAS, $PM_{2.5}$ averaged over 28 and 365 days was associated with a 7.0 (95% CI: 2.3, 12.0) and a 6.3 (1.8, 11.0) ms increase in QT per interquartile range (3.4 and 1.9 $\mu g/m^3$) increase in $PM_{2.5}$.[319] In MESA, a 10 $\mu g/m^3$ increase in 365-day mean $PM_{2.5}$ was associated with an increase in odds of QT prolongation (odds ratio [95% CI]: 1.6 [1.0, 2.6]).[325]

F5. Particulate matter and QT interval mechanisms

While the biological pathways underlying PM-associated increases in QT are not well understood, the mechanisms of prolonged ventricular repolarization may include particulate-induced sympathetic stress responses (see section E5) resulting in decreased HRV and increased heart rate and QT. However, PM-QT associations are robust to heart rate correction and adjustment for HRV[321], suggesting that mechanisms independent of autonomic dysfunction also play a role. Such mechanisms include pulmonary oxidative stress, hematogenous translocation of PM to the heart, induction of reactive oxygen species, and production of pro-inflammatory cytokines[326] that directly or indirectly modulate[327-331] ventricular cation (i.e. Na^+, Ca^{2+}, K^+) gradients[332-335] and increase QT duration.[336-338]

In fact, such mechanisms have been highlighted in gene-environment interaction studies of QT. Specifically, *NFE2L2* (nuclear factor, erythroid 2 like 2) modified PM-QT associations in a panel of participants with previous myocardial infarction. Also, *CXCL12* (C-X-C motif chemokine ligand 12), which encodes a stromal cell-derived factor (SDF1) that modulates calcium influx through the L-type Ca2+ channel in cardiomyocytes, also was implicated in a genome-wide, gene-environment study of multi-ethnic populations within the WHI and ARIC.[339]

Furthermore, PM has been inversely associated with DNAm at *iNOS* (inducible oxide synthase), a regulator of pro-inflammatory cytokine responses and oxidative stress[340], and other

inflammation-related candidate genes[163] in epidemiologic studies.[156,163,169] PM-DNAm associations at other CpG sites proximal to genes implicated in recent ventricular repolarization genome-wide association studies[275-277] (see section F1) also may reveal other potential mechanisms.

F6. Summary and limitations

QT is a temporal measure of ventricular repolarization that depends heavily on transmembrane cation gradients in ventricular myocytes during Phase 2 of the ventricular action potential.[273] QT prolongation is associated with ventricular arrhythmias[290] and sudden cardiac death, as well as other cardiovascular diseases and all-cause mortality.[296]

Ambient PM has been associated with increases in QT, suggesting that changes in ventricular repolarization mediate PM-associated CVD. However, evidence of short-duration $PM_{2.5}$ associations is based on small-scale panel or larger community-based studies (i.e. in NAS[318] and CATHGEN[320]) that lack the demographic, clinical, and exposure characteristics that would allow generalization of the results to broader populations. Evidence of longer-duration $PM_{2.5}$ exposures also relied on NAS[319] in addition to MESA[325], a more diverse study population. However, MESA results were based on cross-sectional data and a dichotomous (versus an interval-scale) indicator for QT prolongation, which would not capture modest, population-level, PM-associated increases in QT. Only one study investigated PM_{10}-QT associations, however it was in a subset of WHI and ARIC participants with genomic data.[339] The aggregate evidence nonetheless suggests that PM exposure is associated with increases in QT.

While the mechanisms that underlie PM-associated increases in QT are unknown, they are hypothesized in include autonomic, oxidative stress, pro-inflammatory, or epigenetic responses to PM inhalation and hematogenous translocation to the heart. However, supporting evidence, is scant.

CHAPTER 3. RESEARCH PLAN

A. Overview

This work will be conducted in three parts with data from participants in the Women's Health Initiative (WHI) and the Atherosclerosis Risk in Communities (ARIC) study. In Specific Aim 1, the association between ambient particulate matter (PM) air pollution and leukocyte traits will be estimated to inform the causal framework and modeling strategies for Specific Aims 2 and 3. In Specific Aim 2, the methylome-wide association between PM and leukocyte DNA methylation (DNAm) at Cytosine-phosphate-Guanine (CpG) sites will be evaluated. Specific Aim 2 will provide a set of PM-sensitive CpG sites that will be used to assess DNAm mediation of the PM-heart rate variability (HRV) and QT interval duration (QT) associations in Specific Aim 3.

B. Study populations

B1. Women's Health Initiative

The Women's Health Initiative[197,341] (WHI) is a multicenter prospective study of risk factors for cardiovascular disease (CVD), cancer, osteoporotic fractures, and other causes of morbidity and mortality among postmenopausal women. Between 1993 and 1998, 68132 and 93676 women aged 50-79 years from forty WHI clinical centers throughout the United States (Figure 3-1) were enrolled in the Clinical Trials (CT) or Observational Study (OS). Of eligible women during 2004-2005, 77% consented to be followed through 2010 for WHI Extension Study I and 87% again consented in 2010 to be followed through 2015 for WHI Extension Study II.[342]

The WHI CT[197,341] investigated the effects of hormone therapy (i.e. estrogen with or without progestin), calcium and vitamin D supplementation, and dietary modification on risk of breast and colorectal cancer, CVD, and osteoporotic fractures. The WHI OS[197,341] participants were recruited if they were interested in the diet modification or hormone therapy arms of the WHI CT, but were otherwise ineligible, unwilling, or unresponsive to a direct invitation.

The WHI CT and OS participants were asked to complete a screening visit (SV), at which fasting blood was drawn and other demographic, socioeconomic, behavioral, and medical information was collected, and annual mailed, self-administered questionnaires following the SV. WHI CT participants were also asked to participant in detailed examinations at one, three, six, and nine years after randomization / enrollment (AV1, AV3, AV6, AV9), while WHI OS participants were asked to participate in only one detailed follow-up examination at AV3.

B1.2. WHI LLS

The WHI Long Life Study[343] (LLS) consisted of 7,875 consenting, non-institutionalized women from the WHI Medical Records Cohort, a group of 1) Hormone Trial participants and 2) African Americans or Hispanic/Latinos enrolled in WHI Extension Study II with genetic and CVD biomarker data at baseline. At this one-time, in-person visit between 2011 and 2012, a blood draw, clinical assessment, and a functional status assessment were conducted.

B1.3. WHI-MIMS

The WHI – Myocardial Ischemia and Migraine Study[344] (WHI-MIMS) study was a ten-center ancillary study in a sample of WHI OS participants that consisted of 3,369 women recruited between 1997 and 2000 between the screening to the third annual follow-up visit (SV or AV3). The objective of WHI-MIMS was to investigate the relationships among migraine headache, myocardial ischemia as measured by a 24-hour ambulatory ECG Holter monitor, and panic symptoms.

B1.4. WHI-EMPC

WHI – Epigenetic Mechanisms of PM-Mediated CVD[345] (WHI-EMPC) is a WHI ancillary study of epigenetic mechanisms underlying associations between ambient PM air pollution and CVD in the WHI CT cohort. WHI-EMPC focused on an exam site- and race/ethnicity-stratified, randomly selected 6% minority oversample of WHI CT participants who had repeated, fasting blood draws and resting, standard, twelve-lead ECGs beginning at baseline. From this population, WHI-EMPC randomly selected 2,200 participants at SV, AV3, or AV6 that had 1) an available aliquot of DNA between 1993 and 2001 for peripheral blood leukocyte methylation assay, 2) core analyte data, 3) an address in the contiguous forty-eight US, 4) no conditions that affect the availability or accuracy of DNA methylation or ECG measures, and 5) estimated concentrations of ambient particulate matter air pollution, but were not taking anti-arrhythmic medications at the time. In 200 participants, DNAm was measured at a second visit (AV3 or AV6) and in 43 participants, it was measured at a third visit (LLS) yielding 2,443 total observations.

B1.5. WHI-BAA23

WHI – Broad Agency Award 23 (WHI-BAA23)[346], also known as Integrative Genomics and Risk of CHD and Related Phenotypes in the Women's Health Initiative, is a case-control study of coronary heart disease (CHD) among women who were enrolled in the WHI CT (n=1,546) or OS (n=442). Women who had previously undergone genome-wide genotyping and profiling of seven CVD-related biomarkers (total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, C-reactive protein, creatinine, insulin, and glucose) were selected from two WHI ancillary studies. The first was the WHI Single Nucleotide Polymorphism (SNP) Health Association Resource (SHARe) cohort of over 8,000 African American women and over 3,500 Hispanic American women.[347,348] The second consisted of

European Americans from the Hormone Therapy trials.[349,350] Participants were sampled in two sets—one including 637 CHD cases and 631 non-cases identified as of Sept 30, 2010 and another including 432 CHD cases and 472 non-cases identified as of September 17, 2012. For each participant, DNAm was measured in blood collected at the SV.

B1.6. WHI-AS311

WHI – Ancillary Study 311[351] (WHI-AS311), also known as the Bladder Cancer and Leukocyte Methylation study, is a case-control study of bladder cancer among women nested within the WHI CT (n = 405) and OS (n = 455). Bladder cancer cases were identified during cohort follow-up through annual medical questionnaires as of September 2012 and adjudicated by blinded and trained physicians using pathology, cytology, operative reports, and hospital discharge information. Cases were selected if diagnosed with urothelial carcinoma, the most common subtype of transitional cell carcinoma that originates in urothelial cells lining the inner bladder. Controls were matched to cases on age (+/- 2 years), year of enrollment, follow-up time (≥ their matched case), and DNAm extraction method. After excluding cases lacking a matched control, cases with a self-reported history of any cancer, and participants without an available aliquot of high-quality DNA at baseline, 441 cases and 442 controls were eligible for the study and DNAm was measured in blood collected at the SV.

B2. Atherosclerosis Risk in Communities study

The Atherosclerosis Risk in Communities[196] study (ARIC) was a community-based prospective cohort study of atherosclerosis and its clinical outcomes (e.g. CHD, heart failure, stroke) conducted in four communities in the US: Washington County, Maryland; Forsyth County, North Carolina; selected suburbs of Minneapolis, Minnesota; Jackson, Mississippi (Figure 3-2). During enrollment in 1987-1989, ARIC participants were selected as a community-stratified probability sample of 15,792 African American and European American men and

women aged 45-64. The cohort participated in up to four subsequent examinations (Visit 2-5 [V2-V5]) following the baseline clinical examination (Visit 1 [V1]), at which demographic, socioeconomic, behavioral, and medical data were collected.

The ARIC DNA Methylation Working Group selected 2,796 African Americans (ARIC- from Forsyth County or Jackson (ARIC-AA) and 1,139 European Americans from Forsyth County or Minneapolis enrolled in the BrainMRI/Omics study and had cerebral magnetic resonance imaging data[352] (ARIC-EA) who were consenting participants from V2 (1990-1992) or V3 (1993-1995), had an available aliquot of DNA for peripheral blood leukocyte methylation assay, and had no conditions that affect the availability or accuracy of DNA methylation. ARIC-EA participants.

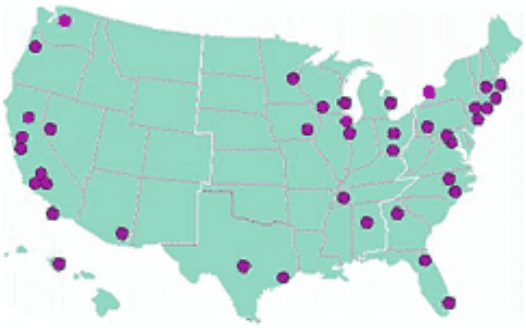

Figure 3-1. Forty WHI clinical centers

Figure 3-2. Four ARIC centers

C. Covariate assessment

C1. Particulate matter

The proposed study will focus on three ambient particulate matter (PM) air pollutants ($\mu g/m^3$), two of which ($PM_{2.5}$; PM_{10}) are regulated under the Clean Air Act by the US Environmental Protection Agency (EPA) according to its National Ambient Air Quality Standards (NAAQS).

PM exposures have been estimated at all geocoded[353,354] WHI and ARIC participant addresses in the contiguous US since the baseline examinations using US EPA Air Quality System (AQS) monitoring data for PM_{10} (since 1987) and $PM_{2.5}$ (since 1999).[355] Estimation of daily mean concentrations involved a spherical model for spatial interpolations and national-scale, log-normal ordinary kriging.[356-358] Validity of the estimation was assessed using standard cross-validation statistics: average prediction error (PE), standardized prediction error (SPE), root mean square standardized (RMSS), and the standard error (SE). Observed values of PE and SPE near zero, RMSS near one, and RMS near SE have provided evidence of model validity.[355]

Because daily mean concentrations of ambient $PM_{2.5}$ were not available until 1999 when EPA AQS monitoring data for $PM_{2.5}$ became more widely available, monthly mean concentrations between 1987 and 1999 were instead spatiotemporally estimated using generalized additive mixed models, the log-transformed ratio of $PM_{2.5}$ to predicted PM_{10}, and geographic information system-based predictors. Monthly mean concentrations of PM_{10} were also estimated in this way. A five- to ten-fold, out-of-sample cross-validation of the estimates in which the squared Pearson correlation between excluded monthly observations and model predictions (R^2 = 0.68-0.77) suggested that estimation models performed well.[359]

Daily mean concentrations of PM will be averaged over 2, 7, 28, and 365 days before (and including) examination days. Monthly mean concentrations of $PM_{2.5}$ and PM_{10} also will be averaged over twelve months before (and including) examination months to estimate 12-month exposures. Finally, $PM_{2.5-10}$ concentrations for each averaging duration will be calculated as differences between PM_{10} and $PM_{2.5}$ concentrations.

C2. DNA methylation

Peripheral blood leukocytes were isolated from visit-specific fasting blood draws for WHI-EPMC, WHI-BAA23, WHI-AS311, ARIC-AA and ARIC-EA study participants (Table 3-

1). Samples were then processed and stored at -70°C according to WHI and ARIC protocols. DNA was extracted from peripheral blood leukocyte samples and then DNAm was measured on a methylome-wide scale using the Illumina 450K Infinium Methylation BeadChip (Illumina Inc.; San Diego, CA, USA). Specifically, DNAm was measured at 485,577 potentially relevant Cytosine-phosphate-Guanine (CpG) sites including CpG shores / islands, miRNA promoter regions, and disease-associated regions. Methylation was quantitatively represented by beta, the proportion of methylated cytosines over the sum of methylated and unmethylated cytosines.

To adjust for probe bias, DNAm data were normalized using Beta Mixture Quantile (BMIQ).[360] To control for variation due to batch effects, information on assay plate, chip, and row were collected. Leukocyte proportions for CD8+ T cells, CD4+ T cells, B cells, NK cells, monocytes, and granulocytes were estimated using Houseman methods to adjust for leukocyte composition.[179] Study-specific quality control filters were applied, yielding DNAm beta values from 461,014 to 463,916 CpG sites in 8,983 participants (Table 3-1).

Table 3-1. Methylome-wide DNAm data exclusions in WHI and ARIC

	Sample Exclusions		Probe Exclusions				
Study	N after exclusions[1]	Detection p-value[4]	n CpGs after exclusions[2]	Detection p-value[5]	Y Chr	Bead Count[5]	Non-CpG CH_3
WHI-EMPC [3]	2,200	> 0.01 in > 1%	463,916	> 0.01 in > 10%	Yes	No	No
WHI-BAA23	1,988	No	461,014	> 0.01 in > 10%	Yes	No	Yes
WHI-AS311	860	No	461,136	> 0.01 in > 1%	Yes	< 3 in > 10%	Yes
ARIC-AA	2,796	> 0.01 in > 1%	463,431	> 0.01 in > 1%	No	< 3 in > 5%	No
ARIC-EA	1,209	> 0.01 in > 1%	462,543	> 0.01 in > 5%	No	< 3 in >5%	No

[1]Additional study-specific sample exclusions: gender mismatch or SNP discordance with previous genotyping, and / or outliers in principal component analysis

[2]Additional probe exclusion: CpG sites with multi-modal DNAm distributions in ≥ 1 study

[3]200 participants had a second and 43 had a third DNAm measure at a subsequent visit (n observations = 2,443)

[4]Of probes

[5]Of samples

C3. Leukocyte count and proportions

Analysis of fasting blood samples collected from all WHI CT and OS participants at SV by certified staff at each of WHI Clinical Center included measurement of leukocyte count on automated hematology cell counters at local laboratories following standard quality-assurance procedures. Measurement of leukocyte count was repeated among subsets of WHI OS participants at the AV3 (Table 3-2).

Fasting blood samples from ARIC participants at V1 and V2 were collected[361] and within 24 hours, leukocyte count was measured in local hospital-based hematology laboratories using automated cell counters (Coulter Diagnostics, Hialeah, Florida). Measurement of leukocyte count was repeated among subsets of Washington County participants at V3-V5, Forsyth Country participants at V4-V5, and Jackson / Minneapolis participants at V5 (Table 3-3).

Table 3-2. Leukocyte counts in WHI by study & visit

Study	Participants w/ leukocyte counts	
	SV	AV3
WHI	160,116	75,677
WHI CT	68,084	--
WHI OS	92,032	75,677

Table 3-3. Leukocyte counts in ARIC by center & visit

Center	Participants w/ leukocyte counts				
	V1	V2	V3	V4	V5
ARIC	15,546	14,213	3,404	6,003	6,303
Forsyth County	3,991	3,629	--	2,825	1,393
Jackson, MS	3,540	3,088	--	--	1,296
Minneapolis, MN	4,006	3,811	--	--	1,889
Washington County	4,009	3,685	3,404	3,178	1,725

Leukocyte proportions were estimated for CD8+ T cells, CD4+ T cells, NK cells, B cells, monocytes, and granulocytes using Houseman methods[179] in WHI-EMPC, WHI-BAA23, WHI-AS311, ARIC-AA, and ARIC-EA (Table 3-1). Briefly, Houseman and others developed

validated[179,362] statistical methods that leveraged differentially methylated regions among leukocyte cell types i.e. CpG sites with stable DNAm within, but differing DNAm among types, to impute proportions from peripheral blood leukocyte samples. Estimated proportions using Houseman methods were deemed valid with a median root-mean-square-error (rMSE) of 8.2% (range: 5.4-11.6%)[179] for CD8+ T cell, CD4+ T cell, NK cell, B cell, monocyte, and granulocyte proportions and an rMSE of 5% and 6% for monocytes and aggregated lymphocyte proportions.[362]

C4. Heart rate variability and QT interval duration

Heart rate variability (HRV) and QT interval duration (QT) was assessed at participant examinations and examination sites in WHI and ARIC (Tables 3-4 and 3-5) using three reliably[195,287] estimated HRV measures (mean RR interval duration [RR, ms], i.e. the unit-corrected inverse of mean heart rate; the standard deviation of normally conducted RR intervals [SDNN, ms]; and the square root of mean squared differences in successive, normally conducted RR intervals [RMSSD, ms] and median QT (QT, ms) from orthogonal XYZ leads. In the WHI CT and ARIC, the estimates were based on ten-second, resting, supine, standard twelve-lead ECGs[363,364] recorded by MAC PCs (MAC PC, GE Marquette Electronics Inc., Milwaukee, WI), then transmitted to a central laboratory (Epidemiological Cardiology Research Center, Wake Forest School of Medicine, Winston-Salem, NC) for visual inspection, identification of technical errors / inadequate quality, and analysis using the 2001 version of the GE Marquette 12-SL program (GE Marquette, Milwaukee, WI). In WHI-MIMS, the estimates were based on 24-hour, ambulatory three-lead ECGs recorded by a Holter monitor and a Zymed Model 3100–001 digital recorder.

Table 3-4. ECG data in WHI by study & visit

Study	SV	AV3	AV6	AV9
WHI CT	62,915	54,350	50,019	10,843
WHI-MIMS*	3,372	--	--	--

*24-hour electrocardiograms

Table 3-5. ECG data in ARIC by visit

Study	V1	V2	V3	V4	V5
ARIC	15,697	14,303	12,778	11,594	5,475

C5. Other variables

Other variables will be considered in analyses as statistical adjustments:

Socio-demographic variables: age (years), race/ethnicity (European, African, Hispanic/Latino, Native American, Asian/Pacific, and other), sex (male, female), individual-level education (high school education or lower, more than high school), and neighborhood socioeconomic status[365];

Health behaviors: smoking status (current, former, never), alcohol use (current, former, never), physical activity (metabolic equivalent of task [MET-hours/week]), and body mass index (BMI, kg/m^2);

Clinical outcomes: hypertension (anti-hypertensive medication use, history, systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg), hyperlipidemia (anti-hyperlipidemic medication use; history; or in ARIC, total cholesterol > 240 mg/dL), diabetes (anti-diabetic medication use; history; or in ARIC, fasting glucose ≥ 126 mg/dL), chronic lung disease (history of asthma, emphysema, or lung cancer), coronary heart disease (anti-anginal medication use; history of angina, myocardial infarction, or coronary artery revascularization; or interim CHD presentation, based on physician review of medical records, incident event classification, and adjudication), and heart failure (HF; cardiac glycoside and loop or potassium-

sparing diuretic use; history of HF; or interim HF presentation, based on physician review of medical records, incident event classification, and adjudication);

Relevant meteorological and temporal variables: mean temperature (°C), dew point (°C), barometric pressure (kPa), season (using sine/cosine functions)[366], and interval-scale measures for calendar time;

Methylation-related variables: ten principal components (PCs) for genetic ancestry, estimated leukocyte proportions (CD8+ T cells, CD4+ T cells, B cells, NK cells, monocytes, and granulocytes), technical covariates (assay plate, chip, and row).

All analyses will consider socio-demographic (including sex in ARIC) and behavioral variables, study center, randomly assigned treatment group (in WHI CT), case-control status (in WHI-AS311 and WHI-BAA23), and other sampling-related variables in WHI-AS311 (enrollment year, age at enrollment, follow-up time, DNAm extraction method). Additionally, Specific Aim 1 will consider interval-scale measures for calendar time and meteorological variables. Specific Aim 2 will consider meteorological and methylation-related variables. Specific Aim 3.1 will consider meteorological and clinical variables, Specific Aim 3.2 will consider clinical and methylation-related variables, and Specific Aim 3.3 will consider meteorological, clinical, and methylation-related variables.

D. Data availability

Table 3-6. Number of participants with available data for each Specific Aim and study population

	n SA 1.1 (PM-LC)	n SA 1.2 (PM-LP)	n SA 2 (PM-DNAm)	n SA 3.1 (PM-ECG)	n SA 3.2 (DNAm-ECG)	n SA 3.3 (PM-DNAm-ECG)
WHI CT	68,084	--	--	62,915	--	--
WHI OS	92,032	--	--	--	--	--
WHI-EMPC	--	2,200	2,200	--	2,200	2,200
WHI-BAA23	--	1,988	1,988	--	1,546	1,546
WHI-MIMS	--	--	--	3,372	--	--
WHI-AS311	--	860	860	--	405	405
ARIC	15,546	--	--	15,697	--	--
ARIC-AA	--	2,796	2,796	--	2,796	2,796
ARIC-EA	--	1,139	1,139	--	1,139	1,139
Total	175,662	8,983	8,983	81,984	8,086	8,086

Abbreviations: AA, African American; ARIC, Atherosclerosis Risk in Communities; AS311, Ancillary Study 311; BAA23OS, Broad Agency Award 23; CT, clinical trial; DNAm, DNA methylation; EA, European American; EMPC, Epigenetic Mechanisms of PM-Mediated CVD; ECG, electrocardiographic traits (i.e. heart rate variability and QT interval duration); LC, leukocyte count; LP, leukocyte proportions; MIMS, Myocardial Ischemia and Migraine Study; OS observational study; PM, particulate matter

E. Meta-analysis

For each Specific Aim and sub-aim, subpopulation-specific (i.e. study- and /or study- and race/ethnicity-specific) association estimates will be forest plotted to visualize consistency and assessed for heterogeneity using Cochran's Q test statistics,[367] a test of homogeneity where Q is a χ^2 test statistic equal to the weighted sum of squared deviations between study-specific and combined estimates, with degrees of freedom equal to the number of contributing subpopulations minus one.[368] If there is not enough evidence to reject the null hypothesis of homogeneity ($P_{Cochran's\ Q} < 0.10$), then fixed-effects, inverse variance-weighted meta-analysis will be used to

combine estimates; otherwise estimates will be reported separately and combined using random-effects meta-analytic methods.[369]

F. Specific Aim 1

F1. Overview

To assess the association between PM and leukocyte traits, PM-leukocyte count associations will be estimated in WHI CT, WHI OS, and ARIC then PM-leukocyte proportion[179] associations will be estimated in WHI-EMPC, WHI-BAA23, WHI-AS311, ARIC-AA, and ARIC-EA (Table 3-6).

F2. Exclusions

Observations in WHI centers outside of the contiguous 48 states, on study visit dates for which PM was not estimable, among participants with leukocytosis, leukopenia, and common conditions associated with established abnormalities of leukocyte count and/or proportions, including hematological malignancy or use of an oral/parenteral glucocorticosteroid, granulocyte/macrophage colony stimulating factor, lithium, or antibiotic (as a proxy for infection) will be excluded.

F3. Covariates

Covariates that will be considered for analysis are socio-demographic, behavioral, meteorological variables, interval-scale variables for calendar date, and randomly assigned treatment group in WHI CT.

F4. Multiple imputation

To address potential selection bias in complete-data analyses when data are missing at random[370], multivariate imputation by chained equations (MICE)[371,372] will be used to impute ten datasets to address missingness in $PM_{2.5}$, PM_{10}, and $PM_{2.5-10}$ exposures (for all averaging durations), leukocyte counts, leukocyte proportions, and other model covariates. Briefly, MICE

will cycle through covariates with the lowest to highest number of missing values using a series of conditional regression models for robust imputation based on predicted values of each covariate regardless of their scale (e.g. interval, binary, count, and categorical).[371,372] MICE will be implemented for participants present at visits within centers where leukocyte counts (for Specific Aim 1.1; Tables 3-2 and 3-3) and proportions (for Specific Aim 1.2; Table 3-1) are expected to be available.

F5. Specific Aim 1.1

F5.1. Attrition weights

To address potential bias due to non-random attrition over time in WHI and ARIC (Tables 3-2 and 3-3), stabilized inverse probability of attrition weights for each participant will be calculated at each examination using logistic regressions, where the numerator will be the marginal probability of the participant not being lost to follow-up at an examination and the denominator will be the probability of the participant not being lost to follow-up at an examination conditional on their covariate patterns at the prior examination.[373]

F5.2. Statistical analyses

Study- and center-stratified, attrition-weighted and covariate-adjusted, two-level, linear mixed-effects longitudinal models will leverage repeated measures to estimate associations between PM and leukocyte count. The models will have a random intercept for examination at the participant, as given by

$$(1) \qquad LC_{ij} = \beta_0 + \beta_1 PM_{ij} + \beta_2 Z_{ij} + b_{0j}^{P} + \varepsilon_{ij}^{E},$$

where i and j denote the i^{th} examination (level 1) of the j^{th} participant (level 2), LC is the leukocyte count, β_0 is the intercept, PM is 2-, 7-, 28-, or 365-day or 1- or 12-month means of $PM_{2.5}$, PM_{10}, and $PM_{2.5\text{-}10}$, and Z is a vector of covariates. The terms $(b_0^P) \sim N(O, G)$ is a random

intercept for examination at the participant level and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level. The study- and center-stratified measures of association (β_1) and their 95% confidence intervals (CIs) using $\beta_1 \pm 1.96 * standard\ error\ (SE)$ will be reported for a 10 μg/m³ increase in PM, forest plotted, and pooled in random-effects meta-analyses [369] after testing homogeneity of associations among strata ($P_{Cochran's\ Q} < 0.10$) [374].

F6. Specific Aim 1.2

For analyses of estimated leukocyte proportions, multivariate, compositional data analysis methods by Aitchison[375] and Egozcue[376] will be implemented. Briefly, compositional data comprise a set of positive, mutually exclusive components, such as proportions, that represent parts constituting a whole. Therefore, a composition is defined as a set of components that are multi-collinear and collectively sum to 1. As such, the components exist in a constrained space called a simplex (which conforms to Atchison geometry). Standard multivariate approaches, however, assume that compositional data are unconstrained, thereby existing in a real space (which conforms to Euclidean geometry), thus erroneously imposing that the components vary independently. To appropriately allow for standard multivariate analyses of compositional data, Atchison and others have defined log-ratio transformations that transfer compositional data from the simplex to real space.[377] Although several log-ratio transformations exist, isometric log-ratio (ilr) transformations[376] allow for the relative positions between *d* components in the simplex space to be retained when transferred,[377,378] resulting in *d*-1 orthogonal coordinates (i.e. that are *not* multi-collinear). The ilr is also relatively advantageous in standard multivariate analyses because transformed values can be back-transformed into component proportions from multivariate results.[379]

F6.1. Statistical analyses

In each subpopulation, covariate-adjusted, cross-sectional models will estimate multivariate associations between PM and leukocyte composition determined by estimated leukocyte proportions. Proportions will be isometrically log-ratio transformed (ilr) in preparation for compositional data analysis models, as given by

$$(2) \qquad ilr(LP) = \beta_0 + \beta_1 PM + \beta_3 Z + \varepsilon$$

where $ilr(LP)$ denotes the isometrically log-ratio transformed estimated leukocyte proportions, β_0 is the intercept, PM is 2-, 7-, 28-, or 365-day or 1- or 12-month mean of $PM_{2.5}$, PM_{10}, and $PM_{2.5\text{-}10}$, and Z is a vector of covariates, and $\varepsilon \sim (O, \sigma^2)$ is the random error term. The vector of association measures (β_1) denotes the five orthogonal coordinates, the back-transformation of which represents the corresponding change in each of the six leukocyte proportions per 10 μg/m³ increase in PM. Because the standard errors of β_1 cannot be back-transformed, the standard errors of back-transformed leukocyte proportion associations will be estimated using 1,000 bootstrap samples. Subpopulation-specific measures of association will be reported as absolute percentage changes (%) and pooled in random effects meta-analyses [369] after testing homogeneity of associations among strata ($P_{Cochran's\ Q} < 0.10$) [374].

G. Specific Aim 2

G1. Overview

Analyses of study- and race/ethnicity-stratified (i.e. European-, African-, and Hispanic/Latino-American) PM-DNAm associations will be conducted at each CpG methylation site on the Illumina 450K Infinium Methylation BeadChip. The association analyses will be based on DNAm data from seven studies: WHI-EMPC, WHI-BAA23 CT & OS, WHI-AS311 CT & OS and ARIC- AA and ARIC-EA (n = 8,983; Table 3-6). Association estimates for each

CpG site will be meta-analytically combined within and among races/ethnicities (see section F), then ranked according to statistical significance. Functional characterization of CpG sites will be conducted using publicly accessible genomic databases to assess their putative function and biological plausibility and replication will be attempted in subpopulations within the Cooperative Health Research in the Region Augsburg (KORA) study.

G2. Covariates

Covariates that will be considered for analysis are socio-demographic, behavioral, meteorological, and methylation-related variables, as well as study-specific covariates, including randomly assigned treatment group (CT subpopulations of WHI-AS311, WHI-BAA23, WHI-EMPC); case-control status (WHI-AS311, WHI-BAA23); and control matching criteria (WHI-AS311).

G3. Multiple imputation

MICE[370-372] (see section F4) will be used to impute ten datasets to address missingness in $PM_{2.5}$, PM_{10}, and $PM_{2.5-10}$ exposures (for all averaging durations) and other model covariates for all participants who underwent DNAm profiling (Table 3-1), but will not involve imputation of DNAm and methylation-related variables.

G4. Statistical analyses

In each subpopulation, covariate-adjusted, multi-level, linear mixed-effects models will estimate PM-DNAm associations:

In WHI-EMPC, three-level longitudinal models will have a random intercept for examination at the participant level, a random intercept and slope and for PM at the WHI center level, and a random intercept for chip (ComBat will be used to adjust for plate; see Table 3-1), as given by

$$(3) \qquad DNAm_{ijk} = \beta_0 + \beta_1 PM_{ijk} + \beta_2 Z_{ijk} + b_{0k}^{C} + b_{1k}^{C} PM_{ijk} + b_{0jk}^{P} + b_{0ijk}^{E} + \varepsilon_{ijk}^{E}.$$

In WHI-BAA23 CT & OS, and WHI-AS311 CT & OS, two-level cross-sectional models will have a random intercept and slope for PM at the WHI center level and a random intercept for plate and chip, as given by

$$(4) \qquad DNAm_{ik} = \beta_0 + \beta_1 PM_{ik} + \beta_2 Z_{ik} + b_{0k}^{C} + b_{1k}^{C} PM_{ik} + b_{0ik}^{E} + \varepsilon_{ik}^{E}.$$

In ARIC-AA and ARIC-EA, one-level cross-sectional models will have a random intercept for plate and chip, as given by

$$(5) \qquad DNAm_{i} = \beta_0 + \beta_1 PM_{i} + \beta_2 Z_{i} + b_{0i}^{E} + \varepsilon_{i}^{E}.$$

where i, j and k denote the i^{th} examination of the j^{th} participant in the k^{th} center, $DNAm$ is the beta value at a given CpG site, β_0 is the intercept, PM is 2-, 7-, 28-, or 365-day or 1- or 12-month means of $PM_{2.5}$, PM_{10}, and $PM_{2.5\text{-}10}$, and Z is a vector of covariates. The terms $(b_0^C, b_1^C) \sim N(O, G)$ are a random intercept and a random slope for PM at center level, $(b_0^P) \sim N(O, G)$ is a random intercept for examination at the participant level, and $(b_0^E) \sim N(O, G)$ represents random intercepts for technical covariates and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level. Measures of association (β_1) and their 95% confidence intervals ($\beta_1 \pm 1.96\ x\ standard\ error$) will be reported as an absolute percentage change in DNAm per 10 µg/m³ increase in PM.

G5. Meta-analysis

For each PM size fraction and exposure averaging duration in association analyses, subpopulation-specific estimates will be meta-analytically combined within and among race/ethnicities (n = 8,983). Established protocols will be followed for subpopulation-specific and meta-analyzed results, including review of results by graphing the observed -$\log_{10}$-

transformed *P* values for each CpG site against the expected values from a theoretical χ^2 distribution in quantile-quantile (QQ) plots; and estimating the genomic inflation factor (λ), where λ is defined as the ratio of the median observed to median expected $-\log_{10}P$ value.[380,381] In the proposed analyses, genomic inflation is expected due to residual confounding by batch effects, leukocyte heterogeneity, and unmeasured biological factors. Inflation is also likely because DNAm across many CpG sites is plausibly correlated and / or associated with PM exposure.

G6. Technical validation

In a random subset of 200 WHI-EMPC participants, bisulfite pyrosequencing will be used to validate the Illumina 450K measures of DNAm at ten PM-sensitive CpG sites ($P < 1 \times 10^{-5}$). CpG sites with poor next generation sequencing data or situated in CpG-rich, repetitive element, or low sequence complexity regions of the genome will be excluded as candidates for pyrosequencing. Site-specific comparisons of DNAm measures will be based on mean Illumina 450K minus bisulfite pyrosequencing differences (**Δ**), Pearson correlation coefficients (r), and Deming regression estimates of their intercepts (α) and slopes (β).[382] When the two measures are nearly identical, **Δ**, r, α, and β approach values of 0, 1, 0, and 1, respectively.

G7. Functional annotation

Statistically significant ($P < 1 \times 10^{-7}$) CpG sites will be functionally characterized using publicly accessible genomic databases, including National Human Genome Research Institute (NHGRI) Genome-Wide Association Study Catalog[383], Genotype-Tissue Expression (GTEx) database[384], associations between DNAm and gene expression in human blood cells were obtained from a study of approximately 400,000 CpG sites and > 13,000 transcripts in the *Multi-Ethnic Study of Atherosclerosis* (MESA) and *Grady Trauma Project* (GTP) cohorts[385], and experimentally derived Functional element Overlap analysis of ReGions from EWAS (eFORGE)

v2.0[386] with data from the Encyclopedia of DNA elements (ENCODE) [387], Roadmap Epigenomics Project [388], and BLUEPRINT.[389] Overlap of CpG site-specific PM sensitivity, histone modification, and DNase I hypersensitivity will be evaluated in eFORGE with a false discovery rate (FDR) threshold of 0.05.

G8. Replication

The Cooperative Health Research in the Region of Augsburg (KORA) study is a population-based cohort from the region of Augsburg, Southern Germany. Replication will involve up to 2,176 participants from two studies of the population-based KORA cohort: F3 (n = 464) and F4 (n = 1,712). KORA F3 (2004-2005) and F4 (2006-2008) are follow-up studies of the KORA S3 and S4 cohort participants, including German nationals aged 25-74 years from Augsburg, Germany [390,391].

Significant CpG sites that are not heterogeneous across subpopulations ($P < 1.0x10^{-7}$; $P_{Cochran's\ Q} > 0.10$) will undergo replication and meta-analyses in KORA F3 and F4. Pollutant- and averaging duration-specific replication thresholds were Bonferroni-corrected by dividing the conventional alpha level (0.05) by the number of CpG sites carried into replication.

H. Specific Aim 3

H1. Overview

Mediation of the PM-ECG (i.e. PM-HRV and PM-QT) associations by DNAm will be assessed in populations within WHI and ARIC (Table 3-6). Mediation typically requires associations between the exposure and outcome, between the exposure and mediator, and between the mediator and the outcome.[180] The association between PM (i.e. the exposure) and HRV and QT (i.e. the outcomes) will be evaluated in this aim using longitudinal data from 10-second ECGs in the WHI CT and ARIC, and 24-hour ECGs in WHI MIMS (total n=81,924; Table 3-6). The association between PM and DNAm (i.e. the mediator) will have been evaluated

in Specific Aim 2. Then, associations of CpG sites identified as being sensitive to PM ($P < 1.0 \times 10^{-7}$) with HRV and QT will be estimated in studies with both DNAm and ECG data, i.e. WHI-EMPC, WHI-BAA23 CT, WHI-AS311 CT, ARIC-AA, and ARIC-EA (total n = 8,086; Table 3-6). Then, DNAm associations at those CpG sites will be estimated with HRV and QT in the same studies. Finally, causal mediation analyses will determine the degree to which DNAm mediates the PM-HRV and PM-QT associations.[180,392,393]

H2. Exclusions

Observations in WHI centers outside of the contiguous 48 states, on study visit dates for which PM was not estimable, and among participants with conditions that affect the availability or quality of HRV or QT interval duration measures including electronic pacers; poor quality grades; Wolff Parkinson White syndrome; atrial fibrillation; atrial flutter; atrioventricular block; antiarrhythmic medication will be excluded. HRV analyses will also exclude observations made on participants with ventricular or supraventricular tachycardia, supraventricular rhythm, pauses, < 5 or 50% normal-to-normal RR intervals, or ventricular ectopy. QT analyses will exclude observations made on participants with heart failure or QRS interval > 120 ms.

H3. Covariates

Covariates that will be considered for analyses are socio-demographic, meteorological (for Specific Aims 3.1 and 3.3), behavioral, clinical, and methylation-related variables and study-specific covariates (for Specific Aims 3.2 and 3.3) including randomly assigned treatment group (in WHI), case-control status (in WHI-AS311 and WHI-BAA23), and other sampling-related variables in WHI-AS311 (enrollment year, age at enrollment, follow-up time, DNAm extraction method).

H4. Multiple imputation

MICE[370-372] (see section F4) will be used to impute ten datasets to address missingness in $PM_{2.5}$, PM_{10}, and $PM_{2.5-10}$ exposure (for all averaging durations), HRV measures (i.e. RR, RMSSD, and SDNN), QT interval duration, and other model covariates. For Specific Aim 3.1, MICE will be performed for all participants present at each visit (Tables 3-4 and 3-5). For Specific Aims 3.2 and 3.3, MICE will be performed for all participants who underwent methylation profiling (Table 3-1), but will not involve imputation of DNAm and methylation-related variables.

H5. Specific Aim 3.1

H5.1. PM-HRV and PM-QT association

The PM-HRV and PM-QT associations will be estimated for mean concentrations of ambient $PM_{2.5}$, PM_{10}, and $PM_{2.5-10}$ that are associated with DNAm ($P < 1 \times 10^{-7}$). The right-skewed HRV measures from WHI CT, WHI-MIMS, and ARIC (Table 3-4 and 3-5) will be log-transformed for use in study-specific analyses. MICE will be used to impute missing and attrition weights will be calculated to control for bias related to loss to follow-up (see section F5.1).

H5.2. Statistical analyses

In each subpopulation, covariate-adjusted, linear mixed-effects models will estimate PM-HRV and PM-QT associations:

In WHI CT, attrition-weighted and covariate-adjusted, three-level longitudinal models will contain a random intercept for examination at the participant level and a random intercept and slope for PM at the study center level, as given by

$$(6) \qquad ECG_{ijk} = \beta_0 + \beta_1 PM_{ijk} + \beta_3 Z_{ijk} + b_{0k}^{C} + b_{1k}^{C} PM_{ijk} + b_{0jk}^{P} + \varepsilon_{ijk}^{E}$$

In ARIC, attrition-weighted and covariate-adjusted, two-level longitudinal models will adjust for clinical center as a fixed effect and had a random intercept for examination at the participant level, as given by

$$(7) \qquad ECG_{ij} = \beta_0 + \beta_1 PM_{ij} + \beta_3 Z_{ij} + b_{0j}^{P} + \varepsilon_{ij}^{E}$$

In WHI MIMS, covariate-adjusted, two-level cross-sectional models will contain a random intercept and slope for PM at the study center level, as given by

$$(8) \qquad ECG_{ik} = \beta_0 + \beta_1 PM_{ik} + \beta_3 Z_{ik} + b_{0k}^{C} + b_{1k}^{C} PM_{ik} + \varepsilon_{ik}^{E}$$

where i, j, and k denote the i^{th} examination of the j^{th} participant in the k^{th} center, ECG is the QT interval or the log-transformed measure of RR, RMSSD, or SDNN, β_0 is the intercept, PM is 2-, 7-, 28-, or 365-day or 1- or 12-month means of $PM_{2.5}$, PM_{10}, and $PM_{2.5\text{-}10}$, and Z is a vector of covariates. The terms $(b_0^C, b_1^C) \sim N(O, G)$ are a random intercept and a random slope for PM at the center level, $(b_0^P) \sim N(O, G)$ is a random intercept for examination at the participant level, and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level.

Measures of association (β_1) and 95% confidence intervals (CI) from analyses of QT interval duration will be reported as milliseconds changes (Δ, ms) and of log-transformed HRV measures will be reported as percent changes ($\Delta, \%$) in HRV per 10 μg/m^3 increase in PM, where

$$\Delta, \% = 100\left(10^{10\beta_1} - 1\right), 95\%\ CI\text{: } 100\left(10^{10(\beta_1 \pm 1.96SE)} - 1\right).$$

H6. Specific Aim 3.2

H6.1. Selecting potential mediators

The DNAm-HRV and DNAm-QT associations will be estimated for each PM-sensitive CpG site identified in Specific Aim 2 ($P < 1.0 \times 10^{-7}$). ECG measures will include only 10-

second ECG measures from WHI-EMPC, WHI-BAA23 CT, ARIC-AA, and ARIC-EA due to the lack of available DNAm data in WHI-MIMS (Table 3-6).

H6.2. DNAm-ECG association

In each subpopulation, covariate-adjusted, linear mixed-effects models will estimate DNAm-HRV and DNAm-QT associations:

In WHI-EMPC, two-level longitudinal models will contain a random intercept for examination at the participant level and a random intercept for chip, as given by

$$(9) \qquad ECG_{ij} = \beta_0 + \beta_1 DNAm_{ij} + \beta_2 Z_{ij} + b_{0j}^{P} + b_{0ij}^{E} + \varepsilon_{ij}^{E}.$$

In WHI-BAA23 CT, WHI-AS311 CT, ARIC-AA, and ARIC-EA, one-level cross-sectional models will estimate DNAm-HRV and DNAm-QT associations with a random intercept for plate and chip, as given by

$$(10) \qquad ECG_{i} = \beta_0 + \beta_1 DNAm_{i} + \beta_2 Z_{i} + b_{0i}^{E} + \varepsilon_{i}^{E}.$$

where i and j denote the i^{th} examination of the j^{th} participant, ECG is the QT interval or the log-transformed measure of RR, RMSSD, or SDNN from a 10-second ECG, β_0 is the intercept, $DNAm$ is the beta value at a given CpG site, and Z is a vector of covariates. The term $(b_0^P) \sim N(O, G)$ is a random intercept for examination at the participant level, and $(b_0^E) \sim N(O, G)$ represents random intercepts for technical variables plate and/or chip and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level. The measures of association (β_1) and 95% CIs $(\beta_1 \pm 1.96SE)$ will be reported as millisecond changes (Δ, ms) in QT interval duration and percent changes $(\Delta, \%)$ in HRV per 10% increase in DNAm.

H7. Specific Aim 3.3

H7.1 Mediation Analysis

For each CpG site and corresponding HRV / QT measure, mediation methods[180,392,393] will be used to decompose the total effect (TE) between PM and HRV / QT into a natural direct effect (NDE) i.e. the effect of PM on HRV / QT independent of DNAm; and a natural indirect effect (NIE), i.e. the effect of PM on HRV / QT through DNAm; where the sum of NDE and NIE is the TE.

First, for each PM-sensitive CpG site identified in Specific Aim 2 ($P < 1.0 \times 10^{-7}$), PM-DNAm associations will be re-estimated as previously described (see section G.4) but in the subpopulations with HRV and QT data meeting inclusion criteria.

Next, in each subpopulation, covariate-adjusted, linear mixed-effects models will estimate PM-HRV / -QT and estimate DNAm-HRV / -QT associations and PM x DNAm interactions:

In WHI-EMPC, three-level longitudinal models will have a random intercept for examination at the participant level, a random intercept and slope and for PM at the WHI center level, and a random intercept for chip, as given by

$$(11) \qquad ECG_{ijk} = \theta_0 + \theta_1 PM_{ijk} + \theta_2 DNAm_{ijk} + \theta_3 PM_{ijk} x DNAm_{ijk} + \theta_4 Z_{ijk} + b_{0k}^{C} + b_{1k}^{C} PM_{ijk} + b_{0jk}^{P} + b_{0ijk}^{E} + \varepsilon_{ijk}^{E}.$$

In WHI-BAA23 CT, and WHI-AS311 CT, two-level cross-sectional models will have a random intercept and slope for PM at the WHI center level and a random intercept for plate and chip, as given by

$$(12) \qquad ECG_{ik} = \theta_0 + \theta_1 PM_{ik} + \theta_2 DNAm_{ik} + \theta_3 PM_{ik} x DNAm_{ik} + \theta_4 Z_{ik} + b_{0k}^{C} + b_{1k}^{C} PM_{ik} + b_{0ik}^{E} + \varepsilon_{ik}^{E}.$$

In ARIC-AA and ARIC-EA, one-level cross-sectional models will have a random intercept for plate and chip, as given by

$$(13) \qquad ECG_{i} = \theta_0 + \theta_1 PM_{i} + \theta_2 DNAm_{i} + \theta_3 PM_{i} x DNAm_{i} + \theta_4 Z_{i} + b_{0i}^{E} + \varepsilon_{ik}^{E}.$$

where i, j and k denote the i^{th} examination of the j^{th} participant in the k^{th} center, ECG is the QT interval or the log-transformed measure of RR, RMSSD, or SDNN from a 10-second ECG, β_0 is the intercept, $DNAm$ is DNAm at a relevant CpG site, PM is 2-, 7-, 28-, or 365-day or 1- or 12-month mean $PM_{2.5}$, PM_{10}, and $PM_{2.5\text{-}10}$, $PMxDNAm$ is the PM-DNAm interaction term, and Z is a vector of covariates. The terms $(b_0^C, b_1^C) \sim N(O, G)$ are a random intercept and a random slope for PM at center level, $(b_0^P) \sim N(O, G)$ is a random intercept for examination at the participant level, and $(b_0^E) \sim N(O, G)$ represents random intercepts for technical covariates plate and/or chip and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level.

The NDE and NIE will be estimated for a change in PM exposure from level a^* (i.e. 0 μg/m^3) to level a (i.e. 10 μg/m^3) using

$$(14) \qquad NDE = [\theta_1 + \theta_3(\beta_0 + \beta_1 a^* + \beta_2 Z)](a - a^*)$$

$$(15) \qquad NIE = \beta_1(\theta_2 + \theta_3)(a - a^*)$$

$$(16) \qquad TE = NDE + NIE$$

where β_0, β_1, and β_2 denote the intercept, the PM coefficient, and a vector of coefficients for covariates Z in models of relevant CpG from that were re-estimated from Specific Aim 2 (i.e. re-

estimated values from Equations 3, 4, and 5); and θ_1, θ_2, and θ_3 are coefficients for PM, $DNAm$, and the $PMxDNAm$ interaction from Equations 11, 12, add 13.

Bootstrapping over 500 samples will be implemented to estimate standard errors and 95% CIs for the NDE and NIE estimates ($NDE\ or\ NIE \pm 1.96SE$).[24-26] Finally, if the NDE and NIE are both positive or both negative (i.e. have the same signs), the proportion mediated (%) will be estimated by dividing the NIE by the TE.[180,394] When the NDE and NIE have opposite signs, or when the total effect is small, the proportion mediated can be unstable and interpretable, with values greater than one or less than zero.[394,395]

CHAPTER 4. STUDY POWER

For all Specific Aims, minimum detectable associations (MDAs) with 80% power were estimated assuming a one-visit, cross-sectional study design, a given sample size (Table 3-6), standard deviations for independent and dependent variables from a subset available data, and a type I error rate (α) of 0.05, with Bonferroni-correction for multiple testing applied when stated. MDAs were represented as a change (Δ) or percent change (Δ,%) in the outcome using the US EPA PM Integrated Science Assessment standard increment (10 $\mu g/m^3$) in concentration[10] or a 1% increment in DNAm. Cross-sectional power calculations relied on the powerMediation package in R.[396,397]

A. Specific Aim 1.1

MDAs (Δ) for leukocyte counts ($x10^3/mm^3$) were estimated per 10 $\mu g/m^3$ increase in PM in a sample size of 175,662 participants with a type I error rate of 0.05. With 80% power, MDAs ranged from 0.010 to 0.039 $x10^3$ leukocytes/mm^3 (Table 4-1), values consistent with associations detected in previous literature.[100] Although well-powered in the cross-sectional setting, analyses will also involve 89,890 measures from participants' second visit and 20,049 measures (Tables 3-2 and 3-3) from all following visits. Therefore, the tabulated MDAs are at the upper bound. Analyses over 100 simulations leveraging repeated measures data across two visits, while allowing for loss to follow-up, also suggested that power to detect the tabulated MDAs (Table 4-1) ranges from 91% to 100% (data not shown).

Table 4-1. Minimum detectable associations (Δ) for leukocyte counts per 10 μg/m^3 increase in PM with 80% power (n=175,662)

PM	Exposure Duration	SD[1]	Δ for LC LC, SD[2]: 1.8 α = 0.05
PM_{10}	2 days	12.16	0.010
	7 days	9.26	0.013
	28 days	7.17	0.016
	365 days	5.16	0.023
	1 month	5.84	0.021
	12 months	4.11	0.029
$PM_{2.5}$	2 days	7.16	0.016
	7 days	6.1	0.020
	28 days	5.19	0.023
	365 days	3.23	0.038
	1 month	4.4	0.027
	12 months	3.09	0.039
$PM_{2.5-10}$	2 days	8.82	0.014
	7 days	6.59	0.018
	28 days	5.45	0.022
	365 days	3.08	0.039
	1 month	4.19	0.029
	12 months	3.31	0.037

Abbreviations: α, type I error rate; LC, leukocyte count; n, number of participants; PM, particulate matter; SD, standard deviation.; [1]Calculated from the first available visit from WHI-EMPC, WHI-BAA23, and ARIC-AA; [2]and WHI CT, WHI OS, and ARIC

B. Specific Aim 1.2

MDAs (Δ, %) for six leukocyte cell type proportions were estimated per 10 μg/m^3 increase in PM in a sample of 8,983 participants (Table 3-6) with a Bonferroni-corrected type I error rate of 0.05/6 = 0.0083 to conservatively account for the compositional nature of the data. Although the proposed multivariate analyses involve isometric log-ratio transformation of leukocyte proportions due to their dependence, for simplicity, power calculations were based on univariate associations for each cell type proportion. The MDAs are therefore likely to be higher

than MDAs in the actual compositional data analyses. Even so, estimated MDAs were largely well below 1% with 80% power (Table 4-2).

Table 4-2. Minimum detectable associations (Δ, %) for leukocyte proportions per 10 μg/m^3 increase in PM with 80% power (n=8,983)

Exposure			Δ for leukocyte proportions (%)					
PM	Duration	SD[1]	CD8+ T, SD[1]: 0.08 α = 0.0083	CD4+ T, SD[1]: 0.07 α = 0.0083	B cell, SD[1]: 0.04 α = 0.0083	NK, SD[1]: 0.05 α = 0.0083	Monocyte, SD[1]: 0.04 α = 0.0083	Granulocyte ,SD[1]: 0.13 α = 0.0083
PM_{10}	2 days	12.16	0.2	0.2	0.2	0.2	0.1	0.4
	7 days	9.26	0.3	0.3	0.2	0.2	0.2	0.5
	28 days	7.17	0.3	0.4	0.2	0.3	0.2	0.6
	365 days	5.16	0.5	0.6	0.3	0.4	0.3	0.9
	1 month	5.84	0.5	0.5	0.3	0.3	0.3	0.8
	12 months	4.11	0.6	0.7	0.4	0.5	0.3	1.1
$PM_{2.5}$	2 days	7.16	0.3	0.4	0.2	0.3	0.2	0.6
	7 days	6.1	0.5	0.5	0.3	0.3	0.2	0.7
	28 days	5.19	0.5	0.6	0.3	0.4	0.3	0.9
	365 days	3.23	0.8	0.8	0.5	0.6	0.5	1.4
	1 month	4.4	0.6	0.6	0.3	0.5	0.3	1.1
	12 months	3.09	0.8	0.9	0.5	0.6	0.5	1.5
$PM_{2.5-10}$	2 days	8.82	0.3	0.3	0.2	0.2	0.2	0.5
	7 days	6.59	0.4	0.5	0.3	0.3	0.2	0.7
	28 days	5.45	0.5	0.5	0.3	0.3	0.3	0.8
	365 days	3.08	0.8	0.9	0.5	0.6	0.5	1.5
	1 month	4.19	0.6	0.7	0.4	0.5	0.3	1.1
	12 months	3.31	0.8	0.8	0.5	0.6	0.5	1.4

Abbreviations: α, type I error rate; LC; n, number of participants; NK, natural killer cell; PM, particulate matter; SD, standard deviation.
[1]Calculated from the first available visit from WHI-EMPC, WHI-BAA23, and ARIC-AA

C. Specific Aim 2

MDAs represented as a change in DNAm (%) were estimated per 10 μg/m^3 increase in PM in a sample size of 8,983 participants (Table 3-6) with a Bonferroni-corrected type I error rate of 1x10^{-7} that accounts for multiple testing of DNAm at 500,000 CpG sites. With 80% power, MDAs ranged from 0.21% to 0.69% per 10 μg/m^3 increase in PM (Table 4-3), which are similar to those observed in a previous methylome-wide association study.[173]

Table 4-3. Minimum detectable associations (Δ, %) for DNAm per 10 μg/m^3 increase in PM with 80% power (n=8,983)

Exposure			Δ for DNAm (%) DNAm, SD[1]: 0.03 α = 1x10^{-7} [2]
PM	Duration	SD[1]	
PM_{10}	2 days	12.16	0.21
	7 days	9.26	0.25
	28 days	7.17	0.30
	365 days	5.16	0.38
	1 month	5.84	0.35
	12 months	4.11	0.45
$PM_{2.5}$	2 days	7.16	0.30
	7 days	6.1	0.34
	28 days	5.19	0.38
	365 days	3.23	0.65
	1 month	4.4	0.43
	12 months	3.09	0.69
$PM_{2.5-10}$	2 days	8.82	0.26
	7 days	6.59	0.32
	28 days	5.45	0.37
	365 days	3.08	0.69
	1 month	4.19	0.45
	12 months	3.31	0.63

Abbreviations. α, type I error rate; DNAm, DNA methylation; n, number of participants; PM, particulate matter; SD, standard deviation. [1]Calculated from the first available visit from WHI-EMPC, WHI-BAA23, and ARIC-AA. [2]Bonferroni-corrected α for discovery at 500,000 CpG sites

D. Specific Aim 3.1

MDAs **(**Δ, %**)** for log-transformed measures of HRV and interval-scale QT (Δ, ms) were estimated per 10 μg/m^3 increase in PM in a sample of 81,984 participants with a type I error rate of 0.05. With 80% power, MDAs ranged from 0.2% to 0.6% for RR, 0.6% to 2.3% for RMSSD, 0.5% to 2.2% for SDNN, and 0.2 and 0.9 ms for QT (Table 4-4), values similar to those detected in a previous study of the PM-HRV and PM-QT association in WHI[241,339] and a separate meta-analysis of 29 studies.[239] Although well-powered in the cross-sectional setting, analyses will also involve an additional 68653, 62797, and 22437 PM and HRV/ QT measures from participants'

second through fourth visits (Tables 3-4 and 3-5). Therefore, the tabulated MDAs are at the upper bound. Simulation-based analyses leveraging repeated measures data over four visits suggested that power to detect the tabulated MDAs (Table 4-4) ranges from 99% to 100% for RR, 98% to 100% for RMSSD, 99% to 100% for SDNN, and 99% and 100% for QT (data not shown). Power was estimated using 100 simulations and assumed constant covariance of PM and HRV / QT across study visits and loss to follow-up patterns across visits representative of those observed in WHI and ARIC.

Table 4-4. Minimum detectable associations (Δ, %) for HRV and (Δ, ms) for QT per 10 μg/m^3 increase in PM assuming 80% power (n=81,984)

Exposure			Δ % for HRV, Δ ms for QT			
PM	Duration	SD[1]	log(RR), SD[2]: 0.15 α = 0.05	log(RMSSD), SD[2]: 0.70 α = 0.05	log(SDNN), SD[2]: 0.66 α = 0.05	QT, SD[2]: 29.9 α = 0.05
PM_{10}	2 days	12.16	0.1	0.5	0.5	0.2
	7 days	9.26	0.2	0.8	0.7	0.3
	28 days	7.17	0.2	1.0	0.9	0.4
	365 days	5.16	0.3	1.3	1.3	0.6
	1 month	5.84	0.2	1.2	1.1	0.5
	12 months	4.11	0.4	1.7	1.6	0.7
$PM_{2.5}$	2 days	7.16	0.2	1.0	0.9	0.4
	7 days	6.1	0.2	1.1	1.1	0.5
	28 days	5.19	0.3	1.3	1.3	0.6
	365 days	3.23	0.4	2.1	2.0	0.9
	1 month	4.4	0.4	1.6	1.5	0.7
	12 months	3.09	0.5	2.2	2.1	0.9
$PM_{2.5-10}$	2 days	8.82	0.2	0.8	0.7	0.3
	7 days	6.59	0.2	1.1	1.0	0.4
	28 days	5.45	0.3	1.3	1.2	0.5
	365 days	3.08	0.5	2.3	2.1	0.9
	1 month	4.19	0.4	1.7	1.6	0.7
	12 months	3.31	0.5	2.1	2.0	0.9

Abbreviations. α, type I error rate; ms, milliseconds; n, number of participants; QT, QT interval duration; RMSSD, root mean square of successive differences; RR, RR interval; SD, standard deviation; SDNN, SD of NN intervals. [1]Calculated from the first available visit from WHI-EMPC, WHI-BAA23, and ARIC-AA; [2]and WHI CT, WHI OS, and ARIC.

E. Specific Aim 3.2

MDAs (Δ, %) for HRV and (Δ, ms) for QT were estimated per 1% increase in DNAm in a sample of 8,086 participants with Bonferroni-corrected type I error rates of 0.05, 0.01, and 0.005 for associations at one, five, and ten CpG sites. With 80% power, MDAs ranged from 0.2% to 0.2% for RR, 1.1% to 1.6% for RMSSD, 1.0% to 1.4% for SDNN, and 0.3 to 0.4 ms for QT (Table 4-5).

Table 4-5. Minimum detectable associations (Δ, %) for HRV and (Δ, ms) for QT per 1% increase in DNAm assuming 80% power (n=8,086)

Exposure	Δ % for HRV, Δ ms for QT				
DNAm, SD[1]	α	log(RR), SD[2]: 0.15	log(RMSSD), SD[2]: 0.70	log(SDNN), SD[2]: 0.66	QT, SD[2]: 29.9
0.03	0.05	0.2	1.1	1.0	0.3
0.03	0.01	0.2	1.4	1.3	0.4
0.03	0.005	0.2	1.6	1.4	0.4

Abbreviations: α, type I error rate; DNAm, DNA methylation; HRV, heart rate variability; ms, milliseconds; n, number of participants; QT, QT interval duration; RMSSD, root mean square of successive differences; RR, RR interval; SD, standard deviation; SDNN, SD of NN intervals. [1]Calculated from the first available visit from WHI-EMPC, WHI-BAA23, and ARIC-AA; [2]and WHI CT, WHI OS, and ARIC.

F. Specific Aim 3.3

To obtain 80% power to detect mediation, approximately 89.4% power is needed[398] for both PM-DNAm and DNAm-HRV / DNAm-QT analyses. Therefore, MDAs were recalculated, under the same assumptions as specified for Specific Aims 2 and 3.2, but now assuming 89.4% power in the mediation sample (n = 8,086). The MDAs (Δ, %) in DNAm ranged from 0.2% to 0.7% per 10 μg/m^3 increase in PM (Table 4-6). The MDAs (Δ, %) in for RR, RMSSD, SDNN, and (Δ, ms) QT ranged from 0.2% to 0.3%, 1.3% to 1.9%, 1.2% to 1.7%, and 0.4 to 0.4 ms per 1% increase in DNAm (Table 4-7).

Table 4-6. Minimum detectable associations (Δ, %) for DNAm per 10 μg/m^3 increase in PM assuming 89.4% power (n=8,086)

PM	Exposure Duration	SD[1]	Δ for DNAm (%) DNAm, SD[1]: 0.03 α = 1x10^{-7}
PM_{10}	2 days	12.16	0.2
	7 days	9.26	0.3
	28 days	7.17	0.3
	365 days	5.16	0.4
	1 month	5.84	0.4
	12 months	4.11	0.6
$PM_{2.5}$	2 days	7.16	0.3
	7 days	6.1	0.4
	28 days	5.19	0.4
	365 days	3.23	0.7
	1 month	4.4	0.5
	12 months	3.09	0.7
$PM_{2.5-10}$	2 days	8.82	0.3
	7 days	6.59	0.3
	28 days	5.45	0.4
	365 days	3.08	0.7
	1 month	4.19	0.6
	12 months	3.31	0.7

Abbreviations. α, type I error rate; DNAm, DNA methylation; Minimum detectable association; PM, particulate matter; SD, standard deviation; n, number of participants. [1]Calculated from the first available visit from WHI-EMPC, WHI-BAA23, and ARIC-AA

Table 4-7. Minimum detectable associations (Δ, %) for HRV and (Δ, ms) for QT per 1% increase in DNAm assuming 89.4% power (n=8,086)

Exposure	Δ % for HRV, Δ ms for QT				
DNAm, SD[1]	α	log(RR), SD[2]: 0.15	log(RMSSD), SD[2]: 0.70	log(SDNN), SD[2]: 0.66	QT, SD[2]: 29.9
0.03	0.05	0.2	1.3	1.2	0.4
0.03	0.01	0.2	1.7	1.5	0.4
0.03	0.005	0.3	1.9	1.7	0.4

Abbreviations: α, type I error rate; DNAm, DNA methylation; HRV, heart rate variability; n, number of participants; QT, QT interval duration; RMSSD, root mean square of successive differences; RR, RR interval; SD, standard deviation; SDNN, SD of NN intervals. [1]Calculated from the first available visit from WHI-EMPC, WHI-BAA23, and ARIC-AA; [2]and WHI CT, WHI OS, and ARIC.

CHAPTER 5. LEYKOCYTE TRAITS AND EXPOSURE TO AMBIENT PARTICULATE MATTER AIR POLLUTION IN THE WOMEN'S HEALTH INITIATIVE AND ATHEROSCLEROSIS RISK IN COMMUNITIES STUDY

A. Overview

Inflammatory effects of ambient particulate matter (PM) air pollution exposures may underlie PM-related increases in cardiovascular disease risk and mortality, although evidence of PM-associated leukocytosis is inconsistent and largely based on small, cross-sectional, and / or unrepresentative study populations. We therefore estimated PM-leukocyte associations among U.S. women and men in the Women's Health Initiative and Atherosclerosis Risk in Communities study (n=165,675). We based the estimation on up to four study visits per participant, at which peripheral blood leukocytes and geocoded address-specific concentrations of PM≤10, ≤2.5, and 2.5-10 μm in diameter (PM_{10}; $PM_{2.5}$; $PM_{2.5-10}$) were available. We multiply imputed missing data using chained equations and estimated PM-leukocyte count associations over daily to yearly PM exposure averaging periods using center-specific, linear, mixed, longitudinal models weighted for attrition and adjusted for sociodemographic, behavioral, meteorological, and geographic covariates. In a subset of participants with available data (n = 8,457), we also estimated PM-leukocyte proportion associations in compositional data analyses. We found a 12 cell/uL (95% confidence interval: -9, 33) higher leukocyte count, a 1.2% (0.6%, 1.8%) higher granulocyte proportion, and a -1.1% (-1.9%, -0.3%) lower CD8+ T cell proportion per 10 $\mu g/m^3$ increase in 1-month mean $PM_{2.5}$. However, shorter-duration PM_{10} exposures were inversely and only modestly associated with leukocyte count. The estimates, albeit imprecise, suggest that among

racially, ethnically, and environmentally diverse U.S. populations, sustained, ambient exposure to fine PM may induce subclinical, but epidemiologically important inflammatory effects.

B. Introduction

Exposures to airborne particulate matter (PM) ≤ 10, ≤ 2.5 and between 2.5 and 10 um in diameter (PM_{10}; $PM_{2.5;}$ $PM_{2.5-10}$) can trigger inflammatory responses that involve the release and hematogenous redistribution of leukocytes [47,399,400]. Such responses may be key to the pathophysiology underpinning established associations between ambient PM, cardiovascular (CVD) disease risk, and mortality [7,12,13,401,402]. However, evidence of PM-associated leukocytosis is inconsistent and mostly based on small studies and panels with limited generalizability [85-96,400,403,404].

In larger, community- and population-based studies, short-duration PM_{10}-leukocyte count associations are similarly inconsistent [98-101], although longer-duration PM_{10} - and $PM_{2.5}$-leukocyte count associations tend to be positive in published cross-sectional and longitudinal studies [102-104]. Moreover, associations between short- and longer-term PM exposures, leukocyte count, and its differential composition have not been thoroughly evaluated controlling for known relationships among leukocyte traits (count and component proportions).

Associations between ambient PM exposures and leukocyte traits could nevertheless lend support to the hypothesized role of inflammation in PM-related pathogenesis. Furthermore, their magnitude would provide insight into PM associations with leukocyte-derived biomarkers like DNA methylation (DNAm), a heritable but dynamic epigenetic modification that can influence gene expression. Indeed, epidemiologic studies often rely on peripheral blood leukocytes as a source of DNA for DNAm assays given the relative ease with which they are collected and archived in large populations [108,115]. Because DNAm and other epigenetic biomarkers [405] differ among leukocyte subtypes, e.g. granulocytes versus monocytes [178,179], leukocyte composition

may plausibly mediate their associations with environmental exposures.

To expand on prior work evaluating PM-leukocyte count associations, and to address the limitations of studies examining PM-leukocyte compositional associations, we estimated associations of leukocyte traits with short- to longer-duration exposures to ambient $PM_{2.5}$, PM_{10}, and $PM_{2.5-10}$ in large, multi-racial/ethnic, and geographically diverse United States populations enrolled in the Women's Health Initiative (WHI) and the Atherosclerosis Risk in Communities study (ARIC).

C. Methods

C1. Study populations

The WHI is a multicenter prospective study of risk factors for CVD, breast / colorectal cancer, and osteoporotic fractures [197,341]. From forty clinical centers throughout the US, postmenopausal women aged 50-79 years were either randomized in the Clinical Trials (CT, n=68,132) or enrolled in the Observational Study (OS, n=93,676) between 1993 and 1998. The WHI CT included three interventions: hormone therapy (i.e. estrogen with or without progestin vs. placebo), calcium and vitamin D supplementation (vs. placebo), and dietary modification (vs. usual diet). The WHI OS [197,341] recruited participants interested in the dietary modification or hormone therapy trials of the WHI CT, but were otherwise ineligible, unwilling, or unresponsive to a direct invitation.

The WHI CT and OS participants completed a baseline screening visit (SV), at which fasting blood and other demographic, socioeconomic, behavioral, and medical information was collected by trained and certified staff. The present study additionally included WHI CT participant data from annual visits (AVs) at three and six years after randomization (AV3 and AV6) and WHI OS participant data three years after enrollment (AV3), at which fasting blood was re-drawn.

The ARIC study is a prospective epidemiologic study of atherosclerosis and CVD in four U.S. communities: Washington County, Maryland; Forsyth County, North Carolina; selected suburbs of Minneapolis, Minnesota; and Jackson, Mississippi [196]. Participants were selected as a community-stratified probability sample of 15,792 mostly African- and European-American men and women aged 45-64 and participated in a baseline visit (V1; 1987-1989) at which fasting blood and other demographic, socioeconomic, behavioral, and medical information was collected by trained and certified staff. The present study also included participant data from up to three subsequent visits (V2-V4; 1990-1998) during which fasting blood was re-drawn.

Leukocyte composition analyses were conducted in five WHI and ARIC subpopulations with available DNAm data. The three WHI subpopulations included: *Epigenetic Mechanisms of PM-Mediated CVD Risk* (WHI-EMPC; n = 2,200) [345], *Broad Agency Announcement 23* (WHI-BAA23; n = 1,988) [346] and *Ancillary Study 311* (WHI-AS311; n = 860) [351]. WHI-EMPC is a study of epigenetic mechanisms underlying associations between PM and CVD within randomly selected WHI CT participants at the SV, AV3, or AV6. WHI-BAA23, also known as *Integrative Genomics and Risk of CHD and Related Phenotypes in the Women's Health Initiative*, is a case-control study of coronary heart disease. By design, WHI-BAA23 oversampled African Americans and Hispanic/Latino Americans and required all participants to have undergone genome-wide genotyping and profiling of seven CVD biomarkers. DNAm was measured in blood collected at the SV, before the incidence of coronary heart disease. WHI-AS311, also known as the *Bladder Cancer and Leukocyte Methylation* study, is a nested case-control study of bladder cancer. Bladder cancer cases were matched to controls based on enrollment year, age at enrollment, follow-up time, and DNAm extraction method. DNAm was measured in blood collected at the SV, before the incidence of bladder cancer. The two ARIC subpopulations

included 2,796 African Americans from Forsyth County or Jackson (ARIC-AA) with DNA and 1,139 European Americans from Forsyth County or Minneapolis (ARIC-EA) with cerebral magnetic resonance imaging data [352], all at Visits 2 (1990-1992) or 3 (1993-1995).

C2. Leukocyte counts and composition

Leukocyte count was measured among WHI CT and OS participants at the SV, among OS participants at AV3, and among ARIC participants at V1-V2 on automated cell counters at local laboratories following standard quality-assurance procedures [361]. Leukocyte count was re-measured among ARIC participants in Washington County at V3-V4 and Forsyth County at V4.

Leukocyte composition, i.e. the proportions of CD8+ T cells, CD4+ T cells, natural killer (NK) cells, B cells, monocytes, and granulocytes were validly estimated [179] among WHI and ARIC participants with DNAm data using methods that leverage differentially methylated regions, i.e. stably methylated CpG sites within, but variably methylated CpG sites among leukocyte cell types [179,362].

C3. Particulate matter exposure estimation

The study focused on $PM_{2.5}$, PM_{10} and (coarse) $PM_{2.5-10}$, the first two of which are regulated under the Clean Air Act by the U.S. Environmental Protection Agency (EPA) [11]. PM exposures were based on either daily and monthly estimation methods. Daily mean concentrations ($\mu g/m^3$) of PM_{10} were spatially estimated at all geocoded participant addresses [353,354] using EPA Air Quality System (AQS) data and national-scale, log-normal ordinary kriging [355,406]. For each participant, daily mean concentrations of PM_{10} were averaged over 2 and 7 days prior to and including the day of the study visit.

Geocoded participant address-specific monthly mean concentrations ($\mu g/m^3$) of PM_{10} and $PM_{2.5}$ were spatiotemporally estimated using generalized additive mixed models and geographic information system-based predictors. Because EPA AQS monitoring data for $PM_{2.5}$ were not

widely available until 1999, spatiotemporal estimation also involved the log-transformed ratio of $PM_{2.5}$ to predicted PM_{10} between 1987 and 1999 [359]. Monthly mean concentrations were averaged over the 12 months prior to and including examination months to obtain annual means. $PM_{2.5-10}$ concentrations for 1- and 12-month means were defined as the monthly differences between PM_{10} and $PM_{2.5}$ concentrations.

C4. Covariates

Demographic, socioeconomic, behavioral, and medical covariates included study center, visit, self-identified race/ethnicity, age (years), individual-level education (high school education or lower, more than high school), neighborhood socioeconomic status [365], smoking status (current, former, never), alcohol use (current, former, never), body mass index (BMI, kg/m^2), physical activity (metabolic equivalent of task [MET-hours/week]), mean temperature (°C), mean dew point (°C), mean barometric pressure (kPa), season (using sine/cosine functions) [366], and to control for longer-term temporal trends, an interval-scale variable for calendar date. Subpopulation-specific covariates included sex (in ARIC); randomly assigned treatment group (in WHI), case-control status (in WHI-AS311 and WHI-BAA23); and other sampling-related variables in WHI-AS311 (enrollment year, age at enrollment, follow-up time, DNAm extraction method).

C5. Exclusions

Of all observations in WHI and ARIC (n = 285,548), small percentages were excluded because they were made on participants in one WHI center outside of the contiguous 48 states (2%), on study visit dates for which PM was not estimable (2%), among participants with a study-specific leukocyte count > 99.5^{th} percentile (leukocytosis, 0.5%), study-specific leukocyte count < 0.5^{th} percentile (leukopenia, 0.5%), or conditions associated with abnormal leukocyte traits, e.g. hematological malignancy (1.7%) or oral/parenteral use of a granulocyte/macrophage

colony stimulating factor (< 0.01%), lithium (0.2%), glucocorticosteroid (1.1%), or antibiotic use (2.6%).

C6. Multiple imputation

To avoid potential for selection bias in complete-data analyses when data are missing at random [370], multivariate imputation by chained equations (MICE) [371,372] was used to impute missing data (% missing range: 0.6% - 5.8%). Binary and categorical data were imputed using logistic regression while continuous variables were imputed using predictive means matching.

C7. Attrition weights

To address potential for bias due to non-random attrition over time in leukocyte count analyses in WHI and ARIC, stabilized inverse probability weights for each participant were calculated at each examination using logistic regression, where the numerator was the marginal probability of the participant not being lost to follow-up at an examination and the denominator was the probability of the participant not being lost to follow-up at an examination conditional on their covariate patterns at prior examination [373].

C8. Statistical analysis: leukocyte count

Study- and center-stratified, PM-leukocyte count associations were estimated using an attrition-weighted and covariate-adjusted, two-level, linear, mixed-effects, longitudinal model including a random intercept for examination at the participant level. The model was given by

$$LC_{ij} = \beta_0 + \beta_1 PM_{ij} + \beta_2 Z_{ij} + b_{0j}^{P} + \varepsilon_{ij}^{E}, \quad (17)$$

where i and j denote the i^{th} examination (level 1) of the j^{th} participant (level 2), LC is the leukocyte count, β_0 is the intercept, PM is 2- or 7-day mean of PM_{10} or 1- or 12-month mean of $PM_{2.5}$, PM_{10}, or $PM_{2.5\text{-}10}$, and Z is a vector of covariates. The term $(b_0^P) \sim N(O, G)$ is a random

intercept for examination at the participant level and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level. Study- and center-specific measures of association (β_1) and their 95% confidence intervals (CIs) were estimated as $\beta_1 \pm 1.96 * standard\ error\ (SE)$ per 10 μg/m³ increase in PM, forest plotted, and pooled in random-effects meta-analyses [369] after testing homogeneity of associations among strata ($P_{Cochran's\ Q} < 0.10$) [374].

C9. Statistical analysis: leukocyte composition

Subpopulation-stratified, cross-sectional, PM-leukocyte proportion associations were analyzed using multivariate methods for compositional data [375,376], i.e. a set of positive, mutually exclusive components (such as proportions, *p*) that represent parts constituting a whole, are multi-collinear, and collectively sum to 1 within a constrained space called a simplex. Proportions were isometrically log-ratio (ilr)-transformed from the simplex to real (Euclidean geometric) space. Transformation—which allowed for the dependent variation [376,377] and relative positioning of components in the simplex [377,378]—resulted in *p*-1 orthogonal (i.e. non-multi-collinear) coordinates. It also allowed for back-transformation of multivariate results into component proportions [379]. Back-transformation was based on compositional data analysis models, as given by

$$ilr(LP) = \beta_0 + \beta_1 PM + \beta_3 Z + \varepsilon, \quad (18)$$

where $ilr(LP)$ denotes the ilr-transformed estimated leukocyte proportions, β_0 is the intercept, PM is 2- or 7-day mean of PM_{10} or 1- or 12-month mean of $PM_{2.5}$, PM_{10}, or $PM_{2.5\text{-}10}$, Z is a vector of covariates, and $\varepsilon \sim (O, \sigma^2)$ is the random error term. The vector of association measures (β_1) denotes the five orthogonal coordinates, the back-transformation of which represents the corresponding change in each of the six leukocyte proportions per 10 μg/m³

increase in PM. Because the standard errors of β_1 cannot be back-transformed, the standard errors of back-transformed leukocyte proportion associations were estimated using 1,000 bootstrap samples. Subpopulation-specific measures of association were reported as absolute percentage changes (%), forest plotted, and pooled in random effects meta-analyses [369] after testing homogeneity of associations among strata ($P_{Cochran's\ Q} < 0.10$) [374].

C10. Statistical analysis: sensitivity

In leukocyte count analyses, Model 1 adjusted for self-identified race/ethnicity, age, sex (in ARIC), randomly assigned treatment group (in WHI), visit, mean temperature, mean dew point, mean barometric pressure, season to control for within-year variation, and a restricted cubic natural spline function of calendar date [407-409] with one knot per year to control for secular trends in PM and leukocyte count methods. Model 2 also adjusted for potential socioeconomic confounders (individual-level education and neighborhood socioeconomic status). Model 3 additionally adjusted for behavioral variables that explain variation in leukocyte traits or account for residual confounding (smoking status, alcohol use, BMI, and physical activity). Sensitivity of Model 3 results to use of two knots per calendar year, one knot for every two calendar years, and no calendar date adjustment was assessed. Although leukocyte composition analyses also adjusted for subpopulation-specific covariates, the models did not adjust for calendar date because leukocyte proportions were estimated using the same methods across subpopulations. Leukocyte composition models also were not center-stratified due to small sample sizes, and instead adjusted for U.S. Census region (Midwest, Northeast, South, West). Lastly, sensitivity of leukocyte count associations to PM estimation method was examined by substituting spatially estimated 28- and 365-day mean concentrations of PM_{10} for spatiotemporally estimated 1- and 12-month mean concentrations of PM_{10}.

D. Results

Of the 150,328 WHI participants and 15,347 ARIC participants with leukocyte count data (total n = 165,675; Figure 5-1), 96% and 94% had baseline data after exclusions. At baseline, participants were aged 62.3 years on average, mostly female (96%), white (84%), more than high school educated (74%), never/former smokers (91%), and current alcohol users (70%). Mean BMI, physical activity, and leukocyte count were 28.0 kg/m^2, 12.3 MET-hours/week, and 5,908 cells/μL (Table 5-1). Participants in the WHI and ARIC subpopulations with leukocyte composition data (n = 8,457; Table 5-S1) were more likely to be younger (mean age: 61.5 years) and male (16%) and less likely to be white (45%), more than high school educated (52%), never/former smokers (85%), and current alcohol users (52%). Among these subpopulations, mean estimated leukocyte cell type percentages were 9% (CD8+ T cells), 18% (CD4+ T cells), 7% (natural killer cells), 7% (B cells), 10% (monocytes), and 49% (granulocytes).

Mean PM_{10} concentrations in the populations with leukocyte count and composition data were below EPA National Ambient Air Quality Standards (NAAQS) in place during the study period (24-hour $PM_{10} \leq 150$ $\mu g/m^3$; annual $PM_{10} \leq 50$ $\mu g/m^3$) [11]. However, 1- and 12-month mean $PM_{2.5}$ concentrations in ARIC approached or exceeded the annual standard in place during the study period (≤ 15 $\mu g/m^3$) (Table 5-2 and Table 5-S2). PM_{10} and $PM_{2.5}$ concentrations were higher, while $PM_{2.5-10}$ concentrations were lower among subpopulations with leukocyte composition data.

In Models 1-3, short-term mean PM_{10} concentrations were inversely associated with leukocyte count when pooled across study- and center-specific strata. For example, in Model 3, there were 7 (95% confidence interval [CI]: -13, -1) and 11 (-20, -2) cell/μL lower leukocyte count per 10 $\mu g/m^3$ increase in 2- and 7-day mean PM_{10} concentration (Table 5-3; Figure 5-2).

In Model 1, longer-term mean PM_{10}, $PM_{2.5}$, and $PM_{2.5-10}$ concentrations were more strongly and positively, but imprecisely associated with leukocyte count. However, the associations also were attenuated by additional adjustment for potential socioeconomic confounders (Model 2) and behavioral variables (Model 3). For example, there were 114 (65, 163), 64 (15, 114), and 28 (-20, 75) cell/μL higher leukocyte count per 10 $\mu g/m^3$ increase in the 12-month mean $PM_{2.5}$ concentration in Models 1-3 (Table 5-3; Figure 5-S1). In sensitivity analyses, estimates were generally robust to variation in the method of controlling for calendar date (Figure 5-S2). Leukocyte count associations with 28- and 365-day mean PM_{10} concentrations also were imprecise and no different from the null associations (data not shown), like those between leukocyte count and 1- and 12-month mean PM_{10}.

Across PM concentrations and averaging durations, PM-leukocyte compositional associations in Model 3 (Table 5-4) differed little from those in Models 1 and 2 (data not shown). Higher 7-day mean PM_{10} concentrations were associated with somewhat higher, while 1- and 12-month mean PM_{10} concentrations were associated with somewhat lower CD8+ T cell proportions (Table 5-4; Figure 5-S3). One- and 12-month mean concentrations of $PM_{2.5}$ were associated with lower CD8+ T, NK and B cell proportions and higher granulocyte proportions. For example, there was a 1.1% (-1.9%, -0.3%) lower CD8+ T cell proportion and 1.2% (0.6%, 1.8%) higher granulocyte proportion per 10 $\mu g/m^3$ increase in 1-month mean $PM_{2.5}$ (Figure 5-3). In contrast, there were 0.6% (-1.3%, 0.1%) and 1.2% (-2.4%, 0.1%) lower granulocyte proportions per 1- and 12-month mean $PM_{2.5-10}$ (Figure 5-S3).

E. Discussion

Results from this study suggest that mid- to longer-duration exposures to $PM_{2.5}$ concentrations below EPA NAAQS may be associated with a higher leukocyte count, higher

granulocyte proportion, and lower CD8+ T cell proportion among multi-ethnic and geographically diverse populations of U.S. women and men.

While leukocyte count associations also were observed with 1- and 12-month mean PM_{10} and $PM_{2.5-10}$ concentrations, adjusting for potential socioeconomic confounders attenuated them. Indeed, lower socioeconomic status has been related both to increases in CVD risk [410] and higher concentrations of ambient PM [411]. Further attenuation was observed with additional adjustment for behavioral variables (smoking, alcohol use, BMI, physical activity) suggesting that they may account for residual confounding by socioeconomic or other unmeasured characteristics. Taken together with prior evidence suggesting positive [102] and null [103] associations between longer-duration PM_{10} with leukocyte counts, the present results were unable to clarify the relationship. Nevertheless, positive – yet imprecise – leukocyte count estimates remained for $PM_{2.5}$, supporting evidence first reported in the Heinz Nixdorf Recall study [103]. Moreover, the magnitudes of estimates presently observed are on par with those previously associated with a one-cigarette/day increase in smoking [412-414].

$PM_{2.5}$ concentrations also were associated with leukocyte composition; particularly, with higher granulocyte and lower CD8+ T cell proportions. This observation is consistent with results from the Social Environment and Biomarkers of Aging Study (SEBAS) in Taiwan that found positive associations between long-duration $PM_{2.5}$ exposure and the proportion of neutrophils, the most abundant type of granulocyte. SEBAS also detected similar associations with long-duration PM_{10} concentrations, but they were not observed in the present study. Results are also consistent with small-scale occupational studies that found higher neutrophil [415] and lower lymphocyte / CD8+ T cell proportions [415,416] albeit with short-duration exposure to $PM_{2.5}$, which was further demonstrated in rats [417-419]. Indeed, observed lower CD8+ T cell proportions

may be related to PM-responsive migration of CD8+ lymphocytes from the blood to bronchial tissues [85], contraction of the CD8+ regulatory (suppressor) T cell pool, and / or latter phase homeostatic contraction of the CD8+ cytotoxic T cell pool [420].

Persistent systemic inflammation due to longer-duration PM exposure is a biologically plausible mechanism linking PM with adverse health. Indeed, systemic inflammation has been implicated in endothelial injury, atherosclerotic disease progression, and subsequent increases in CVD risk [45]. In the epidemiologic context, systemic inflammation, as measured by leukocyte count, has been consistently and independently associated with CVD and mortality in WHI [61,62], ARIC [60], and other populations [57,63,69].

The results presented herein support the hypothesis that chronic exposure to PM contributes to systemic inflammation and may partly explain the established connection between PM and CVD risk [12,401]. They support prior studies that mechanistically linked atherosclerosis and the inflammatory responses to PM [421-425]. Such studies observed higher pro-inflammatory cytokines following inhalation and deposition of PM in the lungs [47,399,400,426,427] and the activation of coagulation and adhesion molecules [400,428-432], which could ultimately lead to increased leukocyte content within and vulnerability to rupture of atherosclerotic plaques [45,46,422].

Although the inverse relationship between short-duration (i.e. 2- and 7-day mean) ambient PM_{10} exposures and leukocyte counts may be at odds with this suggestion, PM exposure may initiate pulmonary alveolar microvascular sequestration of monocytes and granulocytes [433-435], thereby reducing their concentrations in peripheral blood over the short-term [93,434]. Animal studies of monocytes and acute PM_{10} exposure also suggest that atherosclerotic plaques may recruit leukocytes from the circulation [434]. The inverse PM_{10}-leukocyte count associations with short-duration exposure in the present study are in contrast to null [98,100] and positive [99,101]

epidemiologic associations observed in other contexts. However, they are consistent with observed inverse associations with short-duration exposure to $PM_{2.5}$ in the Normative Aging Study [436].

The characterization of PM-leukocyte associations in the compositional context is particularly relevant given the increasing availability of epigenomic biomarkers that are based on DNA extracted from peripheral blood with leukocyte proportions that can vary widely among participants. However, leukocyte cell types possess distinguishing patterns of DNAm, so measurements of methylation are driven in part by leukocyte composition [178]. Common practice is therefore to restrict measurement of DNAm to a single cell type [171], to statistically adjust associations with DNAm for leukocyte proportions determined via cytometry as part of a complete blood count / differential, or in its absence, to adjust for DNAm-based estimates of CD8+ T cell, CD4+ T cell, NK cell, B cell, monocyte, and granulocyte proportions [173,179]. Mindful of the PM-leukocyte compositional associations detected herein, causal diagrams [437] may benefit from thoughtful consideration of their potential effects on causal association and mediation analyses [180,438] involving DNAm and other leukocyte-derived biomarkers. Indeed, leukocyte composition may itself be a mediator of PM-DNAm associations. As such, DNAm associations with $PM_{2.5}$ – without control for leukocyte composition – may reflect mechanisms that involve inflammation, epigenetics, or both.

The present results are nevertheless limited by the variances of the observed association estimates. The analyses weighted for attrition to avoid potential selection bias due to non-random loss to follow-up, however the loss of bias came at the cost of precision [439]. Furthermore, precision was influenced by technical, temporal, and biological variation of leukocyte count measurements. Participant blood samples were collected, processed, and analyzed by local

laboratories across the U.S. using different automated hematology cell counters. Indeed, secular trends in methods of determining leukocyte count [63] may have affected the precision or accuracy of association estimates. And while lack of adjustment for other cell (erythrocyte; platelet) counts capable of explaining some variation in leukocyte counts may have contributed to the precision of estimates observed herein, there also is evidence to suggest high within-laboratory reliability of leukocyte counts [440] and robustness of study- and center-stratified, longitudinal model results to multiple methods of calendar date adjustment. Moreover, erythrocyte and platelet counts—plausible intermediates of PM-leukocyte count associations—were neither uniformly available nor necessarily appropriate candidates for statistical adjustment [441].

Additional limitations include error in estimated leukocyte proportions and PM concentrations. While cytometrically determined leukocyte proportions for the cell types of interest were not available herein at participant visits with corresponding PM data, estimation of the CD8+ T cell, CD4+ T cell, NK cell, B cell, monocyte, and granulocyte proportions at hand was associated with a low root-mean-square-error (median rMSE: 8.2%, range: 5.4%-11.6%) [179,362]. Furthermore, the validity of spatially estimated daily PM_{10} estimates was demonstrated with an average prediction error and standardized prediction error near zero, a root mean square standardized near one, and a root mean square prediction error near the standard error [355,406]. Similarly, models for spatiotemporally estimated monthly mean PM_{10} and $PM_{2.5}$ estimation performed well, with high squared Pearson correlations between excluded monthly observations and model predictions (R^2 = 0.68-0.77) in a five- to ten-fold, out-of-sample cross-validation [359]. Therefore, outcome and exposure measurement error were less likely to bias observed associations.

Limitations aside, this longitudinal study observed that 1- and 12-month mean ambient $PM_{2.5}$ concentrations were associated with higher leukocyte count. It is the first to do so in large, multi-ethnic and geographically diverse populations of women and men from two well-characterized cardiovascular disease cohorts. Furthermore, this study is the first to use compositional data analysis methods to estimate associations between ambient $PM_{2.5}$ concentrations and leukocyte composition. Its analyses accounted for known relationships among proportions, thereby avoiding methodological biases inherent in conventional analyses that erroneously assume compositional data are independent. Results from them are therefore relatively well-positioned to inform future causal analyses using leukocyte-derived biomarkers.

In conclusion, findings suggest that mid- to longer-duration ambient exposure to fine particulate matter air pollution may induce subclinical, but epidemiologically important inflammatory responses among racially, ethnically, and environmentally diverse U.S. populations in EPA regions 1-10.

F. Tables and Figures

Table 5-1. Characteristics of n=165,675 participants with leukocyte count data, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1986-1998)

Characteristic	WHI SV & ARIC V1 n = 159,162	WHI		ARIC			
		SV n = 144,744	AV3[a] n = 77,096	V1 n = 14,418	V2 n = 13,000	V3[b] n = 3,100	V4[c] n = 5,433
Male, n (%)	6,563 (4.1)	0 (0.0)	0 (0.0)	6,563 (45.5)	5,892 (45.3)	1,470 (47.4)	2,497 (46.0)
Age (years), mean (SD)	62.3 (7.6)	63.2 (7.2)	66.5 (7.3)	54.2 (5.8)	57.0 (5.7)	60.6 (5.6)	63.3 (5.7)
Race / ethnicity, n (%)							
American Indian or Alaskan Native	658 (0.4)	647 (0.4)	315 (0.4)	11 (0.1)	10 (0.1)	2 (0.1)	5 (0.1)
Asian or Pacific islander	1,633 (1.0)	1,601 (1.1)	1,018 (1.3)	32 (0.2)	29 (0.2)	9 (0.3)	16 (0.3)
Black or African American	15,809 (10.0)	11,990 (8.3)	5,675 (7.4)	3,819 (26.5)	3,221 (24.8)	25 (0.8)	244 (4.5)
Hispanic/Latino	5,967 (3.8)	5,967 (4.1)	2,681 (3.5)	--[d]	--[d]	--[d]	--[d]
Other	1,353 (0.9)	1,353 (0.9)	740 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
White (not of Hispanic origin) or European American	133,400 (84.0)	122,844 (85.1)	66,457 (86.4)	10,556 (73.2)	9,740 (74.9)	3,064 (98.8)	5,168 (95.1)
Education, n (%)							
High school education or lower	40,473 (25.6)	32,358 (22.5)	15,677 (20.5)	8,115 (56.4)	7,136 (55.0)	2,115 (68.3)	3,148 (58.0)
More than high school	117,654 (74.4)	111,377 (77.5)	60,818 (79.5)	6,277 (43.6)	5,842 (45.0)	982 (31.7)	2,279 (42.0)
Smoking status, n (%)							
Never	78,794 (50.1)	72,760 (50.9)	37749 (51.1)	6,034 (41.9)	5,173 (39.9)	1,378 (44.5)	2,270 (41.9)
Former	64,941 (41.2)	60,314 (42.2)	32,708 (44.2)	4,627 (32.1)	4,897 (37.7)	1,259 (40.6)	2,331 (43.0)
Current	13,564 (8.6)	9,821 (6.9)	3,465 (4.7)	3,743 (26.0)	2,909 (22.4)	463 (14.9)	822 (15.2)
Alcohol use, n (%)							
Never	18,683 (11.8)	15,101 (10.5)	6,807 (9.1)	3,582 (24.9)	2,917 (22.5)	783 (25.3)	1,273 (23.5)
Former	28,972 (18.3)	26,274 (18.3)	15,040 (20.1)	2,698 (18.8)	2,678 (20.6)	761 (24.6)	1,680 (31.0)
Current	110,366 (69.8)	102,289 (71.2)	5,2866 (70.8)	8,077 (56.3)	7,384 (56.9)	1,554 (50.2)	2,474 (45.6)

Characteristic	WHI SV & ARIC V1 n = 159,162	WHI		ARIC			
		SV n = 144,744	AV3[a] n = 77,096	V1 n = 14,418	V2 n = 13,000	V3[b] n = 3,100	V4[c] n = 5,433
Body mass index (kg/m^2), mean (SD)	28.0 (5.8)	28.9 (5.9)	27.4 (5.7)	27.7 (5.3)	28.0 (5.4)	28.8 (5.5)	28.3 (5.4)
Physical activity (MET-hours/week), mean (SD)	12.3 (13.7)	12.5 (13.7)	13.7 (14.6)	10.2 (12.7)	10.7 (11.5)	10.7 (12.9)	11.7 (12.9)
Leukocyte count (cell/uL), mean (SD)	5,908 (1,553)	5,882 (1,529)	5,794 (1,500)	6,076 (1,761)	5,952 (1,677)	6,435 (1,680)	6,394 (1,671)

[a]WHI Observational Study participants only
[b]Participants from Washington County only
[c]Participants from Forsyth County (46%) or Washington County (54%)
[d]ARIC recruitment and data collection occurred before the National Instutite of Health required collection of information about Hispanic/Latino ethnicity

Table 5-2. Mean (SD) particulate matter concentrations among n=165,675 participants with leukocyte count data, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1986-1998)

PM ($\mu g/m^3$)	WHI SV & ARIC	WHI		ARIC			
	V1	SV	AV3[a]	V1	V2	V3[b]	V4[c]
	n = 159,162	n = 144,744	n = 77,096	n = 14,418	n = 13,000	n = 3,100	n = 5,433
PM_{10}							
2-day	29.5 (11.9)	28.4 (11.1)	28.4 (11.2)	39.8 (14.1)	35.4 (12.3)	31.9 (11.9)	28.2 (10.1)
7-day	28.7 (9.3)	27.6 (8.3)	27.6 (8.6)	39.2 (10.3)	34.4 (8.5)	30.9 (8.1)	27.4 (7.4)
1-month	20.9 (6.7)	20.6 (6.6)	20.6 (6.6)	25.2 (7.1)	22.0 (5.7)	24.3 (6.5)	21.2 (5.4)
12-month	20.9 (5.1)	20.8 (5.1)	20.7 (5.0)	24.4 (4.4)	22.6 (3.8)	23.4 (2.6)	21.1 (2.1)
$PM_{2.5}$							
1-month	12.2 (4.3)	12.0 (4.1)	12.0 (4.2)	15.2 (5.2)	13.6 (4.2)	15.2 (4.2)	15.2 (4.0)
12-month	12.1 (3.0)	12.0 (3.0)	12.0 (2.9)	14.7 (3.6)	13.8 (3.2)	14.9 (1.4)	14.8 (1.3)
$PM_{2.5-10}$							
1-month	8.7 (4.7)	8.6 (4.8)	8.6 (4.8)	10.0 (3.4)	8.4 (2.7)	9.0 (3.0)	6.0 (2.5)
12-month	8.7 (3.9)	8.7 (4.0)	8.7 (4.0)	9.7 (2.1)	8.8 (1.7)	8.5 (1.5)	6.2 (1.7)

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CI, confidence intervals; PM, particulate matter; PM_{10}, PM < 10 μm in diameter; $PM_{2.5-10}$, PM > 2.5 and < 10 μm in diameter; WHI, Women's Health Initiative

[a]WHI Observational Study participants only

[b]Participants from Washington County only

[c]Participants from Forsyth County (46%) or Washington County (54%)

Table 5-3. Pooled change in leukocyte count (Δ, cell/μL) per 10 μg/m^3 increase in PM concentrations among n=165,675 participants, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1986-1998)

	Model 1[a]			Model 2[b]			Model 3[c]		
	Δ cell/μL	95% CI	$P_{Cochran's\ Q}$	Δ cell/μL	95% CI	$P_{Cochran's\ Q}$	Δ cell/μL	95% CI	$P_{Cochran's\ Q}$
PM_{10} (μg/m^3)									
2-day mean	-6	-12, 0	0.89	-7	-12, -1	0.90	-7	-13, -1	0.91
7-day mean	-10	-19, -1	0.49	-10	-20, -1	0.53	-11	-20, -2	0.42
1-month mean	22	3, 41	2.5E-03	8	-8, 25	0.08	-2	-18, 14	0.08
12-month mean	65	26, 103	6.5E-04	32	4, 59	0.37	8	-17, 33	0.56
$PM_{2.5}$ (μg/m^3)									
1-month mean	33	9, 56	0.21	21	0, 43	0.51	12	-9, 33	0.45
12-month mean	114	65, 163	0.59	64	15, 114	0.99	28	-20, 75	0.99
$PM_{2.5-10}$ (μg/m^3)									
1-month mean	18	-8, 44	0.01	-1	-24, 21	0.13	-13	-36, 9	0.12
12-month mean	67	8, 127	6.5E-06	18	-30, 66	0.04	-5	-47, 36	0.15

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CI, confidence intervals; PM, particulate matter; PM_{10}, PM < 10 μm in diameter; PM2.5, PM < 2.5 μm in diameter; $PM_{2.5-10}$, PM > 2.5 and < 10 μm in diameter; WHI, Women's Health Initiative

[a]Model 1 adjusted for race/ethnicity, age, gender (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, and a restricted cubic natural spline function of calendar time with one knot per calendar year

[b]Model 2 adjusted for all covariates in Model 1 and additionally for individual-level education and neighborhood socioeconomic status

[c]Model 3 adjusted for all covariates in Model 2 and additionally for smoking status, alcohol use, body mass index, and physical activity

Table 5-4. Pooled change in estimated leukocyte proportion (Δ, %) per 10 μg/m3 increase in PM concentrations among n=8,457 participants, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1990-1995)

	CD8+ T cells			CD4+ T cells			Natural Killer cells			B cells			Monocytes			Granulocytes		
	Δ %[a]	95% CI	$P_{Cochran's\ Q}$	Δ %[a]	95% CI	$P_{Cochran's\ Q}$	Δ %[a]	95% CI	$P_{Cochran's\ Q}$	Δ %[a]	95% CI	$P_{Cochran's\ Q}$	Δ %[a]	95% CI	$P_{Cochran's\ Q}$	Δ %[a]	95% CI	$P_{Cochran's\ Q}$
PM_{10} (μg/m^3)																		
2-day mean	0.1	-0.4, 0.6	0.15	-0.1	-0.4, 0.2	0.12	0.0	-0.2, 0.3	0.93	-0.2	-0.4, 0.0	0.46	0.0	-0.1, 0.2	0.49	0.1	-0.1, 0.3	0.69
7-day mean	0.3	-0.3, 0.8	0.28	-0.2	-0.5, 0.1	0.63	-0.1	-0.6, 0.4	0.18	-0.4	-0.7, -0.1	0.93	-0.1	-0.3, 0.2	0.49	-0.2	-0.5, 0.2	0.25
1-month mean	-0.4	-1.2, 0.3	0.30	0.0	-0.5, 0.5	0.26	-0.3	-1.0, 0.4	0.16	-0.2	-0.7, 0.2	0.64	0.2	-0.4, 0.8	0.06	0.4	-0.1, 0.9	0.28
12-month mean	-0.5	-1.4, 0.4	0.63	0.1	-0.5, 0.6	0.38	-0.3	-1.5, 0.8	0.14	-0.5	-1.1, 0.2	0.58	-0.3	-0.7, 0.1	0.41	0.0	-0.9, 1.0	0.13
$PM_{2.5}$ (μg/m^3)																		
1-month mean	-1.1	-1.9, -0.3	0.58	-0.2	-1.0, 0.6	0.18	-0.6	-2.2, 1.0	0.00	-0.5	-1.1, 0.1	0.72	-0.1	-0.5, 0.3	0.44	1.2	0.6, 1.8	0.75
12-month mean	-1.3	-2.4, -0.1	0.84	0.2	-0.7, 1.2	0.34	-1.4	-3.7, 0.8	0.03	-0.9	-1.9, 0.2	0.42	-0.4	-0.9, 0.2	0.65	1.1	-0.2, 2.4	0.25
$PM_{2.5-10}$ (μg/m^3)																		
1-month mean	0.5	-0.9, 1.8	0.23	0.0	-0.6, 0.6	0.80	-0.1	-0.8, 0.7	0.63	-0.2	-0.9, 0.5	0.80	0.1	-0.7, 0.8	0.14	-0.6	-1.3, 0.1	0.33
12-month mean	0.0	-2.4, 2.3	0.13	-0.2	-1.0, 0.6	0.51	0.3	-0.9, 1.4	0.80	-0.3	-1.3, 0.7	0.71	-0.2	-0.9, 0.4	0.60	-1.2	-2.4, 0.1	0.17

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CI, confidence intervals; PM, particulate matter; PM_{10}, PM < 10 μm in diameter; PM2.5, PM < 2.5 μm in diameter; $PM_{2.5-10}$, PM > 2.5 and < 10 μm in diameter; WHI, Women's Health Initiative

[a]Model adjusted for race/ethnicity, age, gender (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, individual-level education, neighborhood socioeconomic status, smoking status, alcohol use, body mass index and physical activity

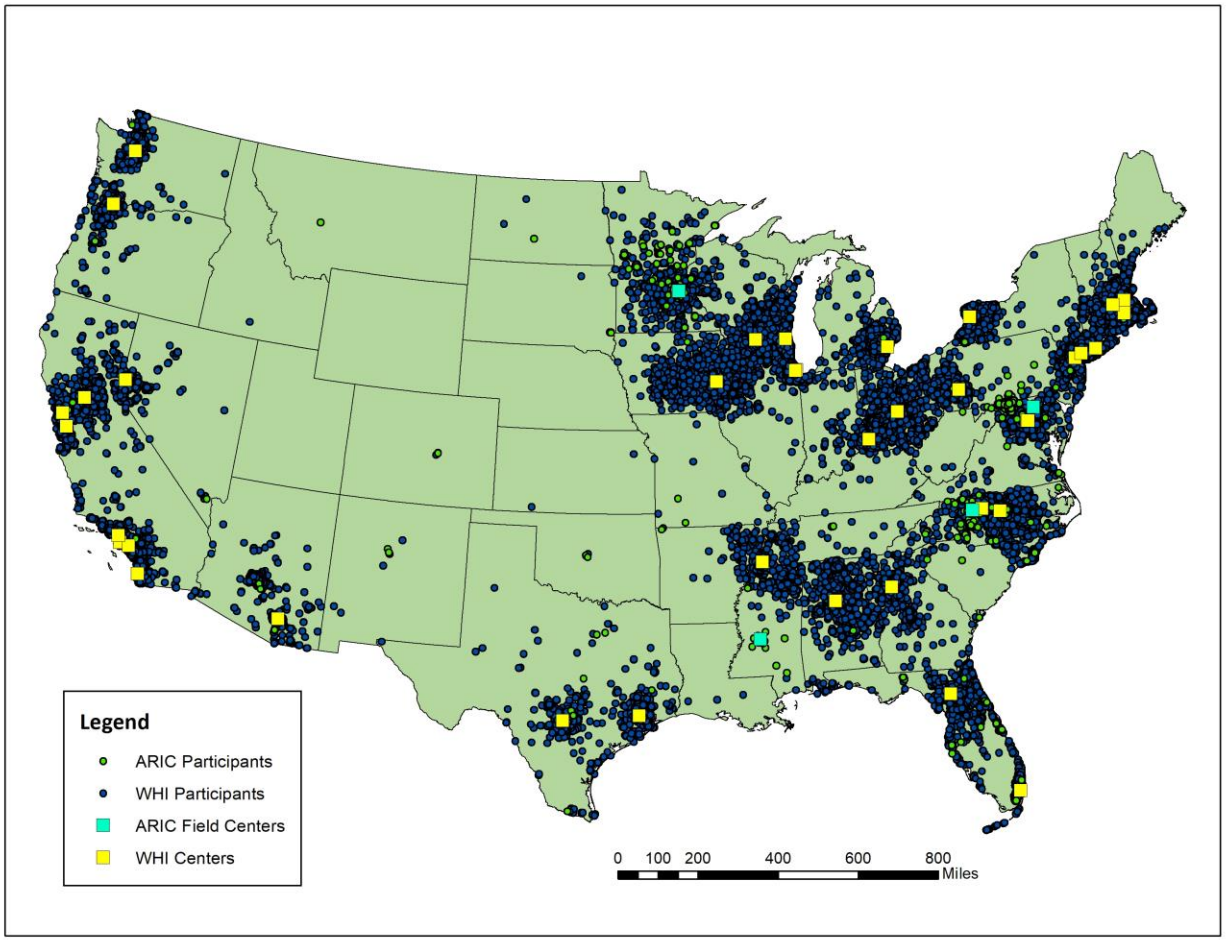

Figure 5-1. Map of geocoded Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1986-1998) participants and centers at baseline. WHI centers (n=39) followed 1,238-3,690 participants. ARIC centers followed 3,588-3,943 participants. WHI and ARIC centers were co-located in Minneapolis, MN and Winston-Salem, NC.

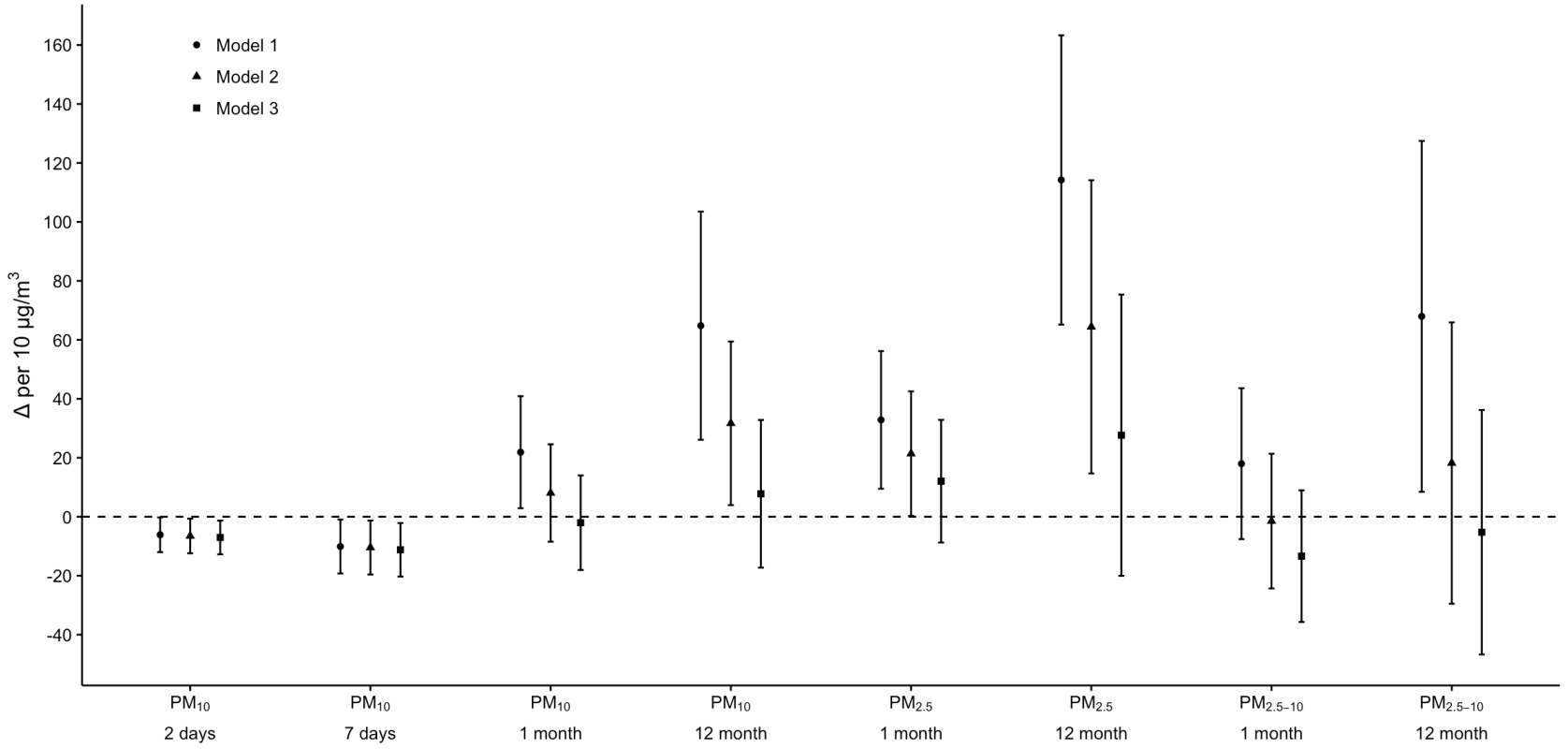

Figure 5-2. Pooled change in leukocyte count (Δ, cell/µL) per 10 µg/m^3 increase in PM concentrations among n=165,675 participants, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1986-1998). Model 1 adjusted for race/ethnicity, age, sex (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, and a restricted cubic natural spline function of calendar date with one knot per year. Model 2 adjusted for all covariates in Model 1 plus individual-level education and neighborhood socioeconomic status. Model 3 adjusted for all covariates in Model 2 plus smoking status, alcohol use, body mass index, and physical activity.

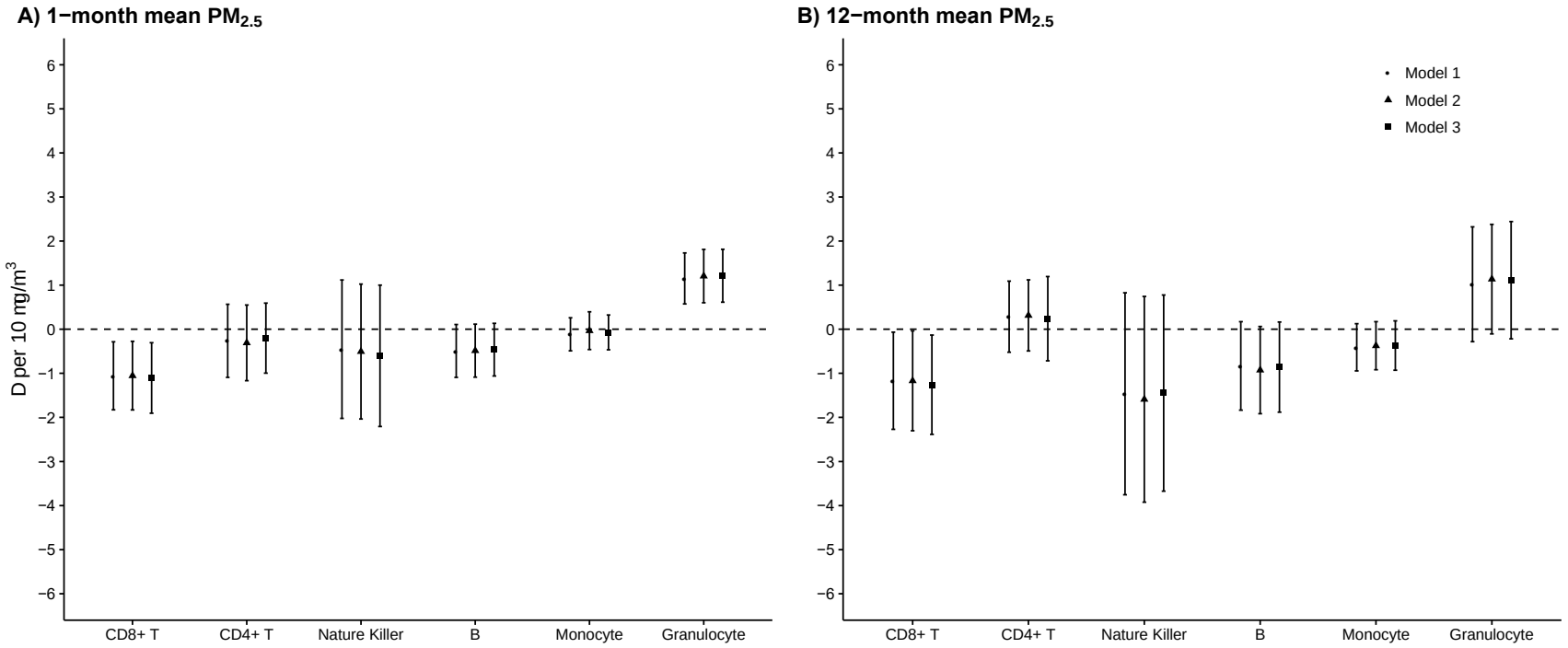

Figure 5-3. Pooled change in leukocyte composition (Δ, %) per 10 μg/m^3 increase in A) 1- and B) 12-month mean $PM_{2.5}$ concentrations among n=8,457 participants, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1990-1995). Model 1 adjusted for race/ethnicity, age, sex (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, and subpopulation-specific covariates. Model 2 also adjusted for individual-level education and neighborhood socioeconomic status. Model 3 additionally adjusted for smoking status, alcohol use, body mass index, and physical activity.

G. Supplement

Table 5-S1. Characteristics of n=8,457 participants with estimated leukocyte composition data, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1990-1995)

Characteristic	WHI & ARIC n = 8,457	WHI-EMPC n = 2,160	WHI-AS311 n = 822	WHI-BAA23 n = 1,910	ARIC-AA[a] n = 2,534	ARIC-EA[b] n = 1,031
Male, n (%)	1,380 (16.3)	0 (0.0)	0 (0.0)	0 (0.0)	943 (37.2)	437 (42.4)
Age (years), mean (SD)	61.5 (7.4)	63.7 (7.0)	65.4 (7.1)	64.8 (7.1)	56.6 (5.9)	59.9 (5.5)
Race / ethnicity, n (%)						
American Indian or Alaskan Native	51 (0.6)	50 (2.3)	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Asian or Pacific islander	147 (1.7)	132 (6.1)	15 (1.8)	0 (0.0)	0.0 (0.0)	0 (0.0)
Black or African American	3,737 (44.2)	544 (25.2)	58 (7.1)	601 (31.5)	2534 (100.0)	0 (0.0)
Hispanic/Latino	717 (8.5)	314 (14.5)	24 (2.9)	379 (19.8)	--[c]	--[c]
Other	42 (0.5)	33 (1.5)	9 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)
White (not of Hispanic origin) or European American	3763 (44.5)	1,087 (50.3)	715 (86.9)	930 (48.7)	0 (0.0)	1,031 (100.0)
Education, n (%)						
High school education or lower	4,030 (47.9)	591 (28.6)	177 (21.7)	609 (32.2)	1,562 (61.8)	479 (46.5)
More than high school	4,378 (52.1)	1,551 (71.4)	640 (78.3)	1,283 (67.8)	964 (38.2)	552 (53.5)
Smoking status, n (%)						
Never	4,044 (48.5)	1,126 (53.3)	367 (45.5)	1,007 (53.6)	1,113 (44.9)	431 (41.8)
Former	3,042 (36.5)	828 (39.2)	371 (46.0)	685 (36.4)	756 (30.1)	402 (39.0)
Current	1,255 (15.0)	158 (7.5)	69 (8.6)	188 (10.0)	642 (25.6)	198 (19.2)
Alcohol use, n (%)						
Never	1,883 (22.4)	289 (13.6)	90 (11.0)	307 (16.1)	876 (34.9)	321 (31.1)
Former	2,156 (25.7)	610 (28.7)	155 (18.9)	450 (23.6)	795 (31.6)	146 (14.2)
Current	4,351 (51.9)	1,225 (57.7)	575 (70.1)	1,146 (60.2)	841 (33.5)	564 (54.7)
Body mass index (kg/m^2), mean (SD)	29.2 (6.1)	29.5 (5.9)	28.0 (6.2)	29.9 (6.1)	30.1 (6.3)	26.2 (4.4)
Physical activity (MET-hours/week), mean (SD)	12.3 (12.9)	10.3 (12.5)	11.6 (12.6)	10.0 (12.6)	12.8 (11.3)	20.6 (14.2)

Characteristic	WHI & ARIC n = 8,457	WHI-EMPC n = 2,160	WHI-AS311 n = 822	WHI-BAA23 n = 1,910	ARIC-AA[a] n = 2,534	ARIC-EA[b] n = 1,031
Leukocyte count (cell/uL), mean (SD)	5,846 (1,607)	5,864 (1,507)	5,924 (1,481)	6,074 (1,610)	5,609 (1,675)	5,972 (1,611)
CD8+ T cells (%)	9 (6)	10 (7)	9 (4)	5 (5)	12 (5)	10 (4)
CD4 +T cells (%)	18 (7)	20 (7)	17 (7)	21 (7)	16 (7)	16 (6)
Nature killer cells (%)	7 (5)	2 (2)	9 (5)	9 (5)	7 (5)	7 (4)
B cells (%)	7 (4)	5 (4)	6 (3)	9 (4)	8 (3)	6 (3)
Monocytes (%)	10 (3)	12 (3)	11 (3)	8 (3)	9 (3)	8 (3)
Granulocytes (%)	49 (12)	50 (12)	49 (12)	48 (12)	48 (13)	54 (12)

Abbreviations: AA, African Americans; ARIC, Atherosclerosis Risk in Communities; AS311, Ancillary Study 311; BAA23, Broad Agency Award 23; EA, European Americans; EMPC, Epigenetic Mechanisms of Particulate Matter-Mediated CVD Risk; SD, standard deviation; WHI, Women's Health Initiative

[a]Participants were from Jackson (90%) or Forsyth County (10%)

[b]Participants were from Forsyth County (90%), Minneapolis (8%) or Washington County (2%)

[c]ARIC recruitment and data collection occurred before the National Institute of Health required collection of information about Hispanic/Latino ethnicity

Table 5-S2. Mean (SD) particulate matter concentrations among n=8,457 with estimated leukocyte composition data, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1990-1995)

PM ($\mu g/m^3$)	WHI & ARIC n = 8,457	WHI-EMPC n = 2,160	WHI-AS311 n = 822	WHI-BAA23 n = 1,910	ARIC-AA[a] n = 2,534	ARIC-EA[b] n = 1,031
PM_{10}						
2-day	31.7 (12.1)	28.5 (11.1)	28.2 (10.8)	28.4 (10.9)	36.1 (12.4)	35.9 (11.5)
7-day	30.8 (9.3)	27.6 (7.9)	27.2 (8.2)	27.8 (8.3)	35.2 (9.3)	34.9 (8.2)
1-month	21.90 (5.9)	20.8 (6.6)	20.1 (6.1)	20.9 (6.5)	20.5 (4.6)	23.1 (5.2)
12-month	21.0 (4.2)	21.1 (5.3)	20.4 (4.8)	21.1 (4.8)	19.9 (1.7)	23.7 (2.4)
$PM_{2.5}$						
1-month	13.3 (4.5)	13.8 (5.7)	12.1 (3.9)	12.2 (4.1)	13.1 (3.1)	15.4 (4.3)
12-month	13.2 (3.2)	13.8 (4.4)	12.1 (2.8)	12.2 (2.9)	12.7 (1.3)	15.9 (2.1)
$PM_{2.5-10}$						
1-month	7.7 (4.0)	7.0 (5.2)	8.0 (4.3)	8.8 (4.8)	7.3 (2.1)	7.7 (2.5)
12-month	7.8 (3.2)	7.3 (4.2)	8.3 (3.8)	8.9 (4.0)	7.2 (0.8)	7.8 (1.4)

Abbreviations: AA, African Americans; ARIC, Atherosclerosis Risk in Communities; AS311, Ancillary Study 311; BAA23, Broad Agency Award 23; CI, confidence intervals; EA, European Americans; EMPC, Epigenetic Mechanisms of Particulate Matter-Mediated Cardiovascular Disease; PM, particulate matter; PM_{10}, PM < 10 μm in diameter; $PM_{2.5-10}$, PM > 2.5 and < 10 μm in diameter; SD, stadard deviation; WHI, Women's Health Initiative

[a]Participants were from Jackson (90%) or Forsyth County (10%)

[b]Participants were from Forsyth County (90%), Minneapolis (8%) or Washington County (2%)

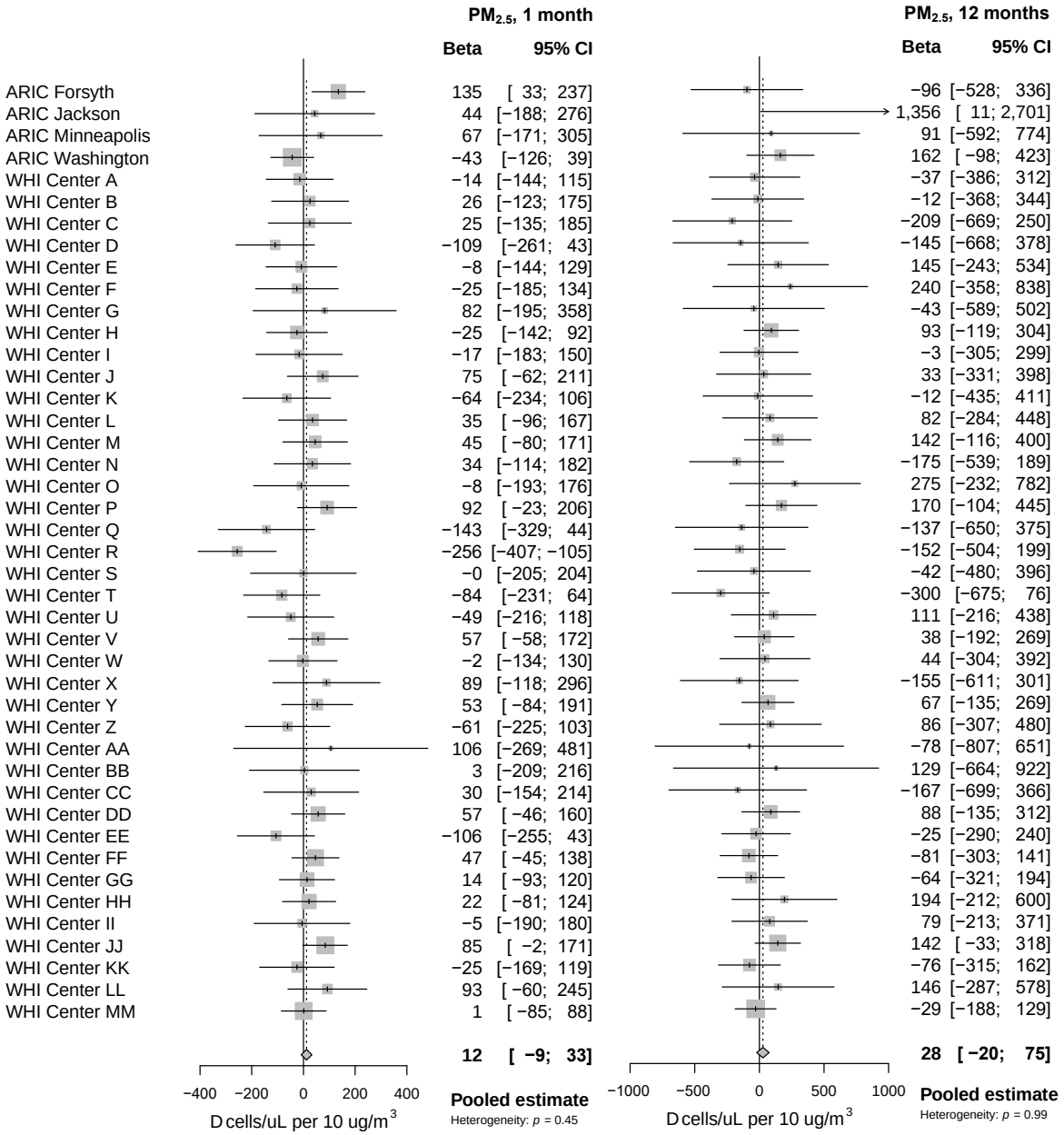

Figure 5-S1. Center-specific and pooled change in leukocyte count (Δ, cell/μL) per 10 μg/m^3 increase in 1- and 12-month mean concentrations of $PM_{2.5}$ among n=165,675 participants, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1986-1998). The models adjusted for race/ethnicity, age, sex (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, and a restricted cubic natural spline function of calendar date with one knot per year, individual-level education, neighborhood socioeconomic status, smoking status, alcohol use, body mass index, and physical activity.

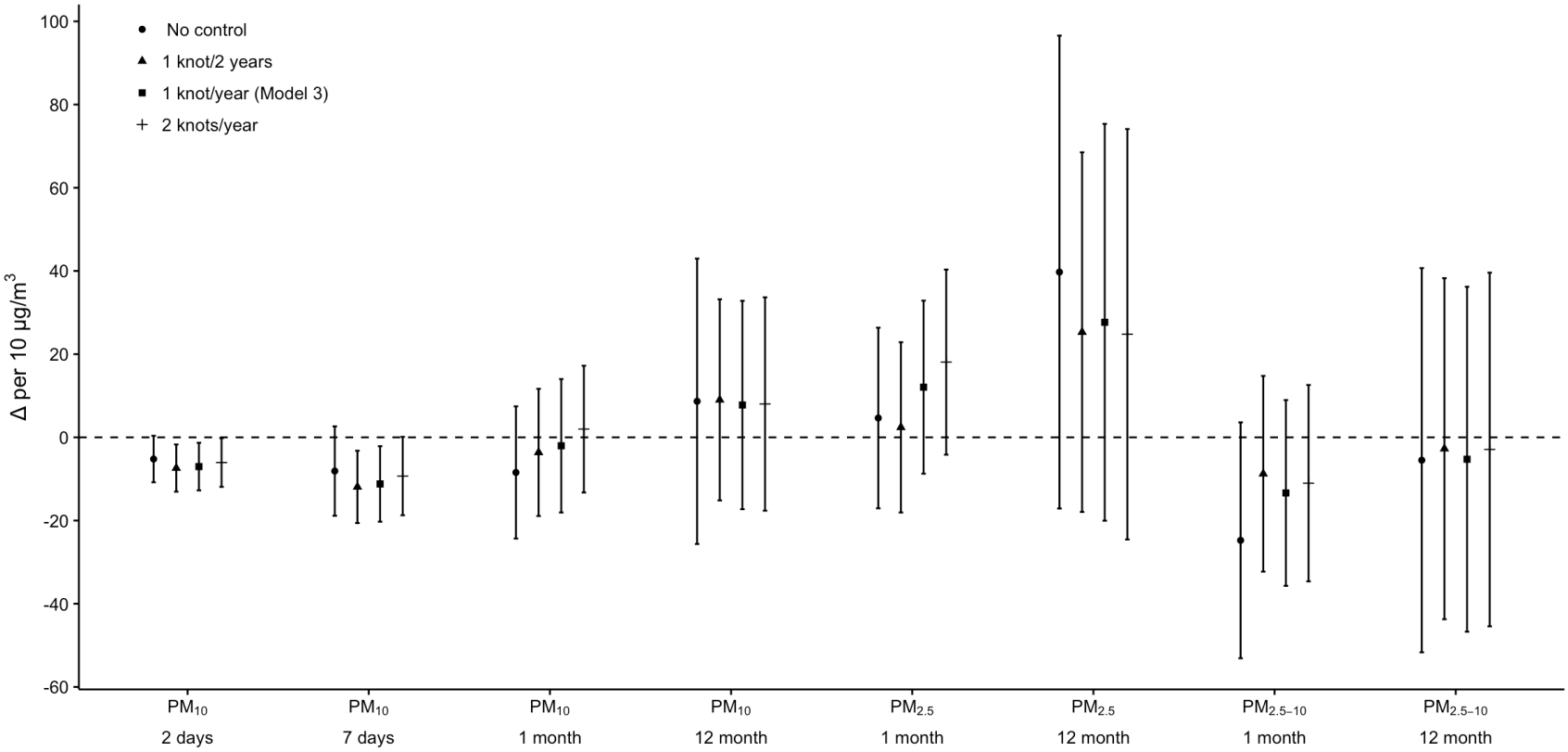

Figure 5-S2. Pooled change in leukocyte count (Δ, cell/μL) per 10 μg/m^3 increase in PM among n=165,675 participants, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1986-1998). The models adjusted for race/ethnicity, age, sex (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, individual-level education, neighborhood socioeconomic status, smoking status, alcohol use, body mass index, and physical activity (●), with additional adjustment for a restricted cubic natural spline function of calendar date with 1 knot for every 2 years (▼), 1 knot per year (■), and 2 knots per year (+).

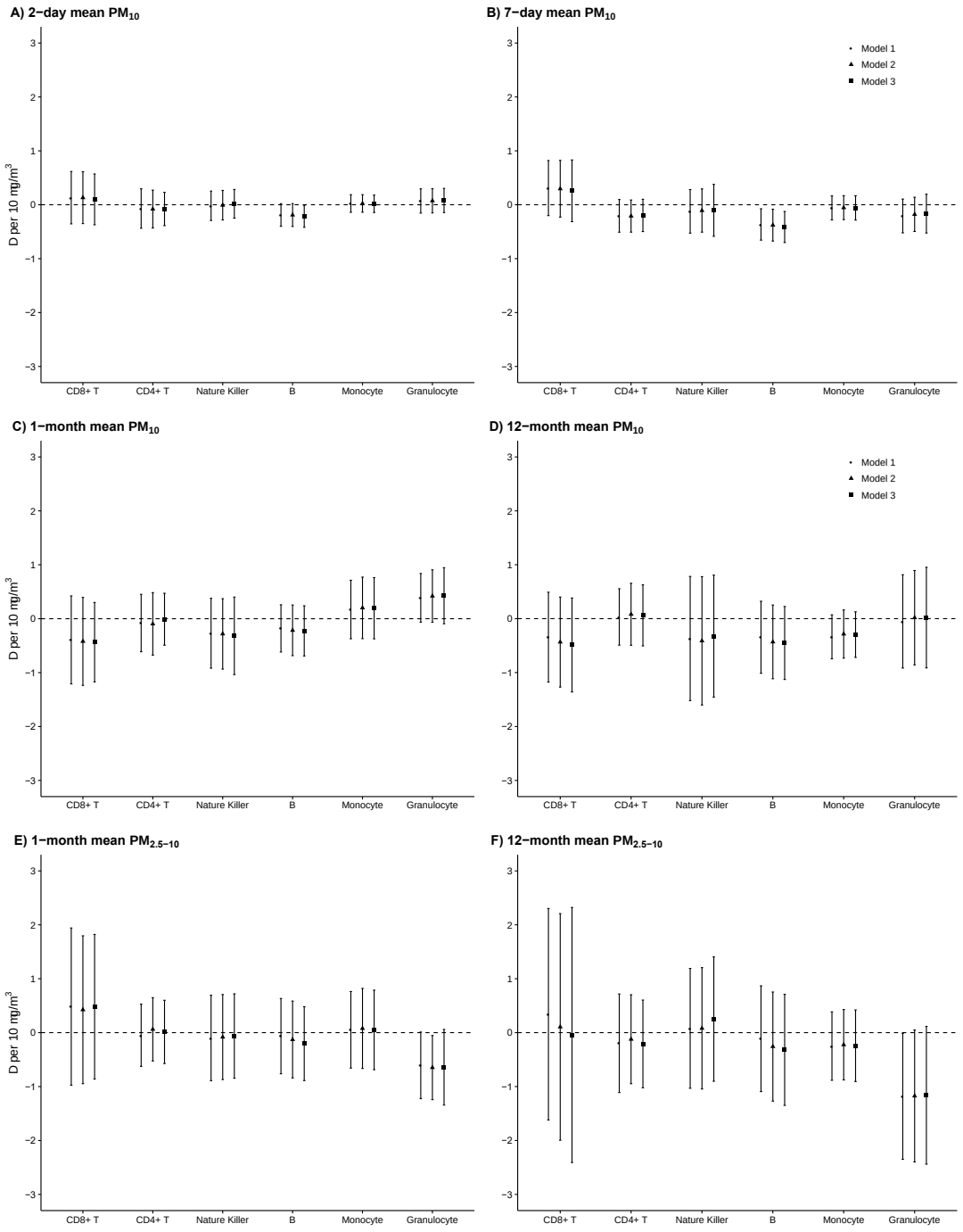

Figure 5-S3. Pooled change in leukocyte composition (Δ, % point) per 10 μg/m3 increase in A) 2- and B) 7-day mean PM10; C) 1- and B) 12-month mean PM10; and E) 1- and F) 12-month mean PM2.5-10 concentrations among n=8,457 participants, Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1990-1995). Model 1 adjusted for race/ethnicity, age, sex (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, and subpopulation-specific covariates. Model 2 also adjusted for individual-level education and neighborhood socioeconomic status. Model 3 additionally adjusted for smoking status, alcohol use, body mass index, and physical activity.

CHAPTER 6. METHYLOME-WIDE ASSOCIATION STUDY PROVIDES EVIDENCE OF PARTICULATE MATTER AIR POLLUTION-ASSOCIATED DNA METHYLATION

A. Overview

DNA methylation (DNAm) may contribute to processes that underlie associations between air pollution and poor health. Therefore, our objective was to evaluate associations between DNAm and ambient concentrations of particulate matter (PM) ≤2.5, ≤10, and 2.5-10 μm in diameter ($PM_{2.5}$; PM_{10}; $PM_{2.5-10}$). We conducted a methylome-wide association study among twelve cohort- and race/ethnicity-stratified subpopulations from the Women's Health Initiative and the Atherosclerosis Risk in Communities study (n = 8,397; mean age: 61.5 years; 83% female; 45% African American; 9% Hispanic/Latino American). We averaged geocoded address-specific estimates of daily and monthly mean PM concentrations over 2, 7, 28, and 365 days and 1 and 12 months before exams at which we measured leukocyte DNAm in whole blood. We estimated subpopulation-specific, DNAm-PM associations at approximately 485,000 Cytosine-phosphate-Guanine (CpG) sites in multi-level, linear, mixed-effects models. We combined subpopulation- and site-specific estimates in fixed-effects, inverse variance-weighted meta-analyses, then for associations that exceeded methylome-wide significance and were not heterogeneous across subpopulations ($P < 1.0x10^{-7}$; $P_{Cochran's\ Q} > 0.10$), we characterized associations using publicly accessible genomic databases and attempted replication in the Cooperative Health Research in the Region Augsburg (KORA) study. Analyses identified significant DNAm-PM associations at three CpG sites. Twenty-eight-day mean PM_{10} was positively associated with DNAm at cg19004594 (chromosome 20; *MATN4;* $P = 3.33x10^{-8}$). One-month mean PM_{10} and $PM_{2.5-10}$ were positively associated with DNAm at cg24102420 (chromosome 10; *ARPP21;* $P = 5.84x10^{-8}$) and inversely associated with DNAm at cg12124767 (chromosome 7; *CFTR*; $P =$

$9.86x10^{-8}$). The PM-sensitive CpG sites mapped to neurological, pulmonary, endocrine, and cardiovascular disease-related genes, but DNAm at those sites was not associated with gene expression in blood cells and did not replicate in KORA. Ambient PM concentrations were associated with DNAm at genomic regions potentially related to poor health among racially, ethnically and environmentally diverse populations of U.S. women and men. Further investigation is warranted to uncover mechanisms through which PM-induced epigenomic changes may cause disease.

B. Introduction

Ambient particulate matter (PM) air pollution is a modifiable exposure that has been consistently associated with morbidity and mortality [5,12,13] attributed to cardiovascular disease [6,7], respiratory disease [21,442,443], and lung cancer [444,445]. Despite the ubiquity of air pollution exposure and the continued population burden of PM [5], the causal mechanisms underlying PM associations with poor health have not been adequately investigated.

One such mechanism could involve methylation of deoxyribonucleic acids (DNAm), conventionally measured at Cytosine-phosphate-Guanine (CpG) sites. DNAm is a heritable, but dynamic epigenetic modification that can influence gene expression without altering the DNA sequence [106,107] and may be central to mediation of PM-associated disease risk [105,108,109]. Indeed, PM exposure has been implicated in whole blood DNAm near candidate genes involved in inflammation, oxidative stress, coagulation and vasoconstriction [154-156,162,163], abnormalities of which have established associations with cardiovascular and respiratory disease. A few studies have agnostically evaluated DNAm associations with PM on a methylome-wide scale [173,174,446], but none have done so in large, sociodemographically and environmentally diverse, well-characterized populations of adult women and men.

The present study therefore examined methylome-wide associations between DNAm and ambient concentrations of PM ≤ 2.5, ≤ 10, and 2.5-10 μm in diameter ($PM_{2.5}$, PM_{10}, and $PM_{2.5-10}$) within the Women's Health Initiative (WHI) and the Atherosclerosis Risk in Communities study (ARIC) cohorts, and their replication in subpopulations of the Cooperative Health Research in the Region Augsburg (KORA) study.

C. Methods

C1. Study design and populations

The study included 8,397 consenting participants from subpopulations within the WHI and ARIC cohorts who had available peripheral blood leukocyte DNA.

The WHI is a multicenter prospective study of risk factors for cardiovascular disease (CVD), cancer, osteoporotic fractures, and other causes of morbidity and mortality among postmenopausal women [197,341]. Between 1993 and 1998, women aged 50-79 years from forty WHI clinical centers throughout the United States (US) were enrolled in the Clinical Trials (CT) (n = 68,132) or Observational Study (OS) (n = 93,676). All WHI participants completed a screening visit (SV). CT participants also completed an annual visit (AV) at one, three, six, and nine years after randomization (AV1, AV3, AV6, AV9), and OS participants three years after enrollment (AV3). An additional visit of CT and OS participant subsets occurred between 2011 and 2012 (ranging from 14 to 19 years after enrollment) as part of the WHI Long Life Study (LLS) [343].

For the current study, WHI participants were drawn from three ancillary studies: *Epigenetic Mechanisms of PM-Mediated CVD Risk* (WHI-EMPC) [345], *Broad Agency Announcement 23* (WHI-BAA23) [346] and *Ancillary Study 311* (WHI-AS311) [447]. WHI-EMPC is a study of epigenetic mechanisms underlying associations between ambient PM air pollution and CVD within the WHI CT. From this population, DNAm was measured in 2,200 randomly

selected participants (stage 1: SV, AV3, or AV6), remeasured in 200 participants at a second visit (stage 2: AV3 or AV6), and remeasured again in 43 participants at a third visit among those who participated in the WHI Long Life Study (stage 3: LLS), yielding 2,443 total observations. WHI-BAA23, also known as *Integrative Genomics and Risk of CHD and Related Phenotypes in the Women's Health Initiative*, is a case-control study of coronary heart disease within the WHI CT (n = 1,546) and OS (n = 442). By design, WHI-BAA23 oversampled African Americans and Hispanic/Latino Americans and required all participants to have undergone genome-wide genotyping and profiling of seven cardiovascular disease biomarkers. DNAm was measured in blood collected at the SV, before the incidence of coronary heart disease. WHI-AS311 is a matched case-control study of bladder cancer among women within the WHI CT (n = 405) and OS (n = 455). Bladder cancer cases were matched to controls based on enrollment year, age at enrollment, follow-up time, and DNAm extraction method. DNAm was measured in blood collected at the SV, before the incidence of bladder cancer.

ARIC is a community-based prospective study of atherosclerosis and its clinical outcomes in four US communities: Washington County, Maryland; Forsyth County, North Carolina; selected suburbs of Minneapolis, Minnesota; and Jackson, Mississippi [196]. Enrollment in 1987-1989 (Visit 1) was followed by five subsequent visits (Visits 2-6) between 1990-2017. The present study included all 2,796 African Americans from Forsyth County or Jackson (ARIC-AA) with DNA and 1,139 European Americans from Forsyth County or Minneapolis (ARIC-EA) with cerebral magnetic resonance imaging data [352], all at Visits 2 (1990-1992) or 3 (1993-1995).

Replication involved up to 2,176 participants from two studies of the population-based KORA cohort: F3 (n = 464) and F4 (n = 1,712). KORA F3 (2004-2005) and F4 (2006-2008) are

follow-up studies of the KORA S3 and S4 cohort participants, including German nationals aged 25-74 years from Augsburg, Germany [390,391].

C2. Particulate matter exposure estimation

The study focuses on three ambient particulate matter (PM) air pollutants, including two ($PM_{2.5}$ and PM_{10}) that are regulated under the Clean Air Act by the US Environmental Protection Agency (EPA) according to its National Ambient Air Quality Standards (NAAQS) [11].

PM exposures were estimated at all geocoded WHI and ARIC participant addresses [353,354] in the contiguous US since the baseline examinations using two exposure modeling approaches, both based on US EPA Air Quality System (AQS) monitoring data for PM_{10} (since 1987) and $PM_{2.5}$ (since 1999). In the WHI, the median distance from geocoded participant addresses to PM_{10} and $PM_{2.5}$ EPA monitors was 7.8 and 7.6 kilometers. In ARIC, it was 4.8 and 7.2 kilometers. Geocoded address-specific daily mean PM_{10} concentrations ($\mu g/m^3$) were spatially estimated using national-scale, log-normal ordinary kriging. Exposure measurement error using kriging methods may yield misclassification and increase variance or bias associations [448,449], therefore validity of the estimation was assessed, using standard cross-validation statistics: average prediction error (PE), standardized prediction error (SPE), root mean square standardized (RMSS), and standard error (SE). Observed values of PE and SPE near zero, RMSS near one, and RMS near SE have provided evidence of model validity [355,406].

Also, geocoded address-specific monthly mean concentrations ($\mu g/m^3$) were spatiotemporally estimated using generalized additive mixed models and geographic information system-based predictors. Because EPA AQS monitoring data for $PM_{2.5}$ were not widely available until 1999, spatiotemporal estimation also involved the log-transformed ratio of $PM_{2.5}$ to predicted PM_{10} between 1987 and 1999. A five- or ten-fold, out-of-sample cross-validation of the estimates in which the squared Pearson correlation between excluded monthly observations

and model predictions (R^2 = 0.68-0.77) indicated that estimation models performed well [359].

Daily mean concentrations of PM_{10} were averaged over the 2-, 7-, 28-, and 365-day periods ending on (including) the examination day. Monthly mean concentrations of $PM_{2.5}$ and PM_{10} were averaged over the 12-month period ending on (including) the calendar month of examination. Finally, coarse PM ($PM_{2.5-10}$) concentrations for each averaging duration were calculated as differences between PM_{10} and $PM_{2.5}$ concentrations.

C3. DNA methylation

Peripheral blood leukocytes were isolated from visit-specific, fasting blood drawn from study participants. DNA was extracted from the peripheral blood leukocytes and then DNAm was measured on a methylome-wide scale at 485,577 CpG sites using the Illumina 450K Infinium Methylation BeadChip (Illumina Inc.; San Diego, CA, USA). Methylation was quantitatively represented by beta, the proportion of methylated cytosines over the sum of methylated and unmethylated cytosines across the same loci. The data from all studies were quality controlled (Table S1), Beta Mixture Quantile (BMIQ)-normalized to adjust for probe bias [360], and in WHI-EMPC, ComBat-adjusted for stage and plate using empirical Bayes methods [450]. Otherwise, technical covariates (assay plate, chip, and row) were available to control for batch effects; and leukocyte proportions (CD8+ T cell, CD4+ T cell, B cell, natural killer cell, monocyte, and granulocyte) to account for leukocyte composition [179]. Among ARIC-AA participants, missing lymphocyte, monocyte, neutrophil, eosinophil, and basophil proportions were imputed based on measured proportions. Analyses excluded CpG sites at which DNAm distributions were multi-modal [451] in at least one study.

C4. Multiple imputation

To avoid potential for selection bias in complete-data analysis when data are missing at random [370], multivariate imputation by chained equations (MICE) [371,372] as implemented in SAS

9.3 (Cary, NC) was used to impute infrequently missing $PM_{2.5}$, PM_{10}, and $PM_{2.5-10}$ concentrations (missing range: 3.3%, 3.5%) and other covariates (missing range: 0%, 10.4%), excluding methylome-wide DNAm. Binary and categorical data were imputed using the logistic and discriminant functions whereas interval-scale data were imputed using predictive means matching with a k-nearest neighbor (k=5) approach.

C5. Statistical analysis

All analyses were stratified by cohort and race/ethnicity (African-, European-, and Hispanic/Latino-American) and adjusted for age (years) at blood draw, education (high school education or lower, more than high school), smoking status (current, former, never), alcohol use (current, former, never), physical activity (metabolic equivalent of task [MET-hours/week]), body mass index (BMI, kg/m^2), neighborhood socioeconomic status [365], mean temperature (°C), mean dew point (°C), mean barometric pressure (kPa), season, and methylation-related variables, which included ten principal components (PCs) for genetic ancestry (when available), leukocyte proportions, and technical covariates. Analyses additionally controlled for cohort-specific covariates, including binary sex (male, female) in ARIC; randomly assigned treatment group (CT subpopulations of WHI-AS311, WHI-BAA23, WHI-EMPC); case-control status (WHI-AS311, WHI-BAA23); and control matching criteria (WHI-AS311).

In each subpopulation, covariate-adjusted, multi-level, linear, mixed-effects models (LMMs) were used to estimate DNAm-PM associations. In WHI-EMPC, three-level, longitudinal models had a random intercept for examination at the participant level, a random intercept and slope for PM at the WHI center level, and a random intercept for chip, as given by

$$DNAm_{ijk} = \beta_0 + \beta_1 PM_{ijk} + \beta_2 Z_{ijk} + b_{0k}^{C} + b_{1k}^{C} PM_{ijk} + b_{0jk}^{P} + b_{0ijk}^{E} + \varepsilon_{ijk}^{E}. \quad (19)$$

In WHI-BAA23 CT & OS, and WHI-AS311 CT & OS, two-level cross-sectional models had a random intercept and slope for PM at the WHI center level and a random intercept for plate and chip, as given by

$$(20) \qquad DNAm_{ik} = \beta_0 + \beta_1 PM_{ik} + \beta_2 Z_{ik} + b_{0k}^{C} + b_{1k}^{C} PM_{ik} + b_{0ik}^{E} + \varepsilon_{ik}^{E}.$$

In ARIC-AA and ARIC-EA, one-level cross-sectional models had a random intercept for plate and chip, as given by

$$(21) \qquad DNAm_{i} = \beta_0 + \beta_1 PM_{i} + \beta_2 Z_{i} + b_{0i}^{E} + \varepsilon_{i}^{E}.$$

Above, i, j and k denote the i^{th} examination of the j^{th} participant in the k^{th} center; $DNAm$ is the CpG site-specific beta value; β_0 is the intercept; PM is the 2-, 7-, 28-, 365-day, or 1- or 12-month mean of $PM_{2.5}$, PM_{10}, or $PM_{2.5\text{-}10}$; and Z is a vector of covariates. The terms $(b_0^C, b_1^C) \sim N(O, G)$ are a random intercept and a random slope for PM at the center level, $(b_0^P) \sim N(O, G)$ is a random intercept for examination at the participant level, $(b_0^E) \sim N(O, G)$ are random intercepts for technical covariates, and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level. Measures of association (β_1) and their 95% confidence intervals ($\beta_1 \pm 1.96\ x\ standard\ error$) were reported as an absolute percentage change in DNAm per 10 μg/m³ increase in PM.

Given the focus on fixed effects, LMMs were fit with maximum likelihood using the MixedModels package [452] in Julia v0.6 [453]. Stratum-specific results were combined using fixed-effects, inverse-variance weighted meta-analysis. Homogeneity of associations was assessed using Cochran's Q test statistic [374]. A $P_{Cochran's\ Q} < 0.10$ and Bonferroni-corrected threshold of $P < 1 \times 10^{-7}$ (i.e. assuming 500,000 independent CpG tests) were used to identify significant CpG associations. The threshold of suggestive significance was $P < 1 \times 10^{-5}$.

Examination of stratified and meta-analyzed results included reviewing quantile-quantile (QQ) plots of the observed -$\log_{10}$-transformed *P* values for each CpG site against the expected values from a theoretical χ^2 distribution and estimating the associated genomic inflation factor (λ), where λ is defined as the ratio of the observed to expected median -$\log_{10}P$ values [380].

C6. Technical validation

In a random subset of 200 WHI-EMPC participants, bisulfite pyrosequencing was used to validate the Illumina 450K measures of DNAm at ten PM_{10}- or $PM_{2.5}$-sensitive CpG sites ($P < 1 \times 10^{-5}$). CpG sites with poor next generation sequencing data or situated in CpG-rich, repetitive element, or low sequence complexity regions of the genome were not candidates for pyrosequencing. Site-specific comparisons of DNAm measures were based on mean Illumina 450K minus bisulfite pyrosequencing differences (**Δ**), Pearson correlation coefficients (r), and Deming regression estimates of their intercepts (α) and slopes (β) [382]. When the two measures are nearly identical, **Δ**, r, α, and β approach values of 0, 1, 0, and 1, respectively.

C7. Functional annotation

Published genotype-phenotype associations for variants annotated to or within 100 kilobases of genes containing statistically significant PM-sensitive CpG sites were identified in the National Human Genome Research Institute (NHGRI) Genome-Wide Association Study (GWAS) Catalog [383]. Tissue-specific gene expression was assessed using the Genotype-Tissue Expression (GTEx) database [384] and associations between DNAm and gene expression in human blood cells were obtained from a study of approximately 400,000 CpG sites and > 13,000 transcripts in the *Multi-Ethnic Study of Atherosclerosis* (MESA) and *Grady Trauma Project* (GTP) cohorts [385]. PM-sensitive CpG sites ($P < 1 \times 10^{-5}$) were functionally characterized using experimentally derived Functional element Overlap analysis of ReGions from EWAS (eFORGE) v2.0 [386] with data from the Encyclopedia of DNA elements (ENCODE) [387], Roadmap

Epigenomics Project [388], and BLUEPRINT [389]. Overlap of CpG site-specific PM sensitivity, histone modification, and DNase I hypersensitivity were evaluated in eFORGE with a false discovery rate (FDR) threshold of 0.05.

C8. Replication

Significant CpG sites that were not heterogeneous across subpopulations ($P < 1.0x10^{-7}$; $P_{Cochran's\ Q} > 0.10$) underwent replication and meta-analyses in KORA F3 and F4. Pollutant- and averaging duration-specific replication thresholds were Bonferroni-corrected by dividing the conventional alpha level (0.05) by the number of CpG sites carried into replication.

D. Results

The study consisted of twelve ARIC and WHI subpopulations, collectively representing 8,397 participants, of whom 45.8% were African American, 8.4% were Hispanic/Latino American, and 83.0% were female (Table 1). Participants were on average 61.3 years of age and contributed methylation data at ≥ 461,014 CpG sites. One-month mean concentrations of PM_{10}, $PM_{2.5}$, and $PM_{2.5-10}$ were 20.9, 13.2, and 7.7 $\mu g/m^3$; varied by subpopulation and race/ethnicity (Tables 1 and S2); and did not exceed NAAQS in place at the time of data collection. Between-pollutant Pearson correlation coefficients depended on size fraction and averaging duration (Table 2). Overall, the median (range) was 0.35 (-0.14, 0.79) and among 2-, 7-, 28, and 365-day mean PM_{10} concentrations, it was 0.64 (0.43, 0.79). Correlations between PM_{10} and $PM_{2.5}$ concentrations were 0.73 and 0.64 when they were averaged over 1 and 12 months.

QQ plots (Fig. 1) based on the trans-ethnic, fixed-effects, inverse variance-weighted meta-analyses provided little evidence of inflation across pollutants and averaging durations: median (range) $\lambda = 1.01$, (0.89-1.07). Manhattan plots (Fig. 2) show three significant ($P < 1 \times 10^{-7}$) and 55 suggestively significant ($1 \times 10^{-5} < P < 1 \times 10^{-7}$) PM-sensitive CpG sites (Tables 3 and S3). The three significant CpG sites (cg19004594; cg24102420; cg12124767) were neither

within ten base pairs of single nucleotide polymorphisms (minor allele frequency > 1%) nor previously identified as cross-reactive probes [454].

On chromosome 20 within an exonic CpG island of *MATN4*, a 10 $\mu g/m^3$ increase in 28-day mean PM_{10} was associated with a 0.3% (95% confidence interval [CI]: 0.2, 0.4) higher DNAm at cg19004594 ($P = 3.33 \times 10^{-8}$; Fig. 3A). On chromosome 3 intronic to *ARPP21*, a 10 $\mu g/m^3$ increase in 1-month mean PM_{10} was associated with a 0.5% (95% CI: 0.3, 0.7) lower DNAm at cg24102420 ($P = 5.84 \times 10^{-8}$; Fig. 3B). Cg24102420 is approximately 200 base pairs upstream from the transcriptional start site for microRNA 128-2 (*miR128-2*). On chromosome 7 intronic to *CFTR*, a 10 $\mu g/m^3$ increase in 1-month mean $PM_{2.5-10}$ was associated with a 0.5% (95% CI: 0.3, 0.7) lower DNAm at cg12124767 ($P = 9.86 \times 10^{-8}$; Fig. 3C). Furthermore, PM associations with cg19004594, cg24102420, and cg12124767 were similar across race/ethnic strata (Fig. S1). Complete annotations for all PM-sensitive CpG sites ($P < 1 \times 10^{-7}$) are available in Excel Table S1.

D1. Technical validation

Overall, bisulfite pyrosequencing and Illumina 450K-based DNAm measures were similar (Table S4). The medians (interdecile ranges) of $\mathbf{\Delta}$, r, α and β were: 0.01 (-0.06, 0.07), 0.73 (0.20, 0.83), 0.04 (-0.27, 0.24), and 0.98 (0.09, 1.62). Corresponding estimates (95% CIs) for cg24102420 were -0.04 (-0.04, -0.03), 0.79 (0.73, 0.83), -0.16 (-0.38, 0.07) and 1.13 (0.88, 1.39). Cg19004594 and cg12124767 were not pyrosequenced.

D2. Functional annotation

MATN4 is highly expressed in the pancreas, reproductive tract, and skin (Fig. S2), but variants of this gene have not been significantly associated ($P < 5 \times 10^{-8}$) with any phenotypes in prior GWAS. *ARPP21* is primarily expressed in the brain (Fig. S3), is significantly associated with neuroticism and severe H1N1 influenza, and suggestively associated ($5 \times 10^{-8} < P < 5 \times 10^{-}$

[6]) with entorhinal cortical thickness and childhood-onset asthma in prior GWAS. *CFTR* is expressed in various tissues, including the pancreas, colon, minor salivary gland, digestive tract, and lung (Fig. S4). *CFTR* polymorphisms are associated with cystic fibrosis (CF), Barrett's esophagus / esophageal carcinoma, and coronary artery disease.

Differential methylation at cg19004594, cg24102420, or cg12124767 was not associated with gene expression in blood cells at any of the > 13,000 transcripts evaluated ($P > 10^{-5}$) in the MESA/GTP cohorts. Although genomic regions around PM-sensitive CpG sites were associated with tri-methylation of histone 3 at lysine 9 (H3K9me3) in natural killer cells, derived mesenchymal stem cells, the fetal adrenal gland, fetal lung fibroblasts, and foreskin fibroblasts (FDR < 0.05; Fig. 4), they were not associated with mono- or tri-methylation of histone 3 at lysine 4, 27, or 36 (H3K4me1, H3K4me3, H3K27me3, or H3K36me3) or DNase I hypersensitivity in any tissues catalogued by eFORGE.

D3. Replication

The three statistically significant, non-heterogeneous PM-sensitive CpG sites (cg19004594; cg24102420; cg12124767) did not replicate in KORA F3 / F4 (Table S5).

E. Discussion

This methylome-wide association study (MWAS) discovered three CpG sites at which higher levels of monthly mean ambient particulate matter air pollution concentrations were associated with DNAm. The DNAm-PM associations at all three CpG sites were homogeneous across the twelve subpopulations and each site was annotated to a neurological, pulmonary, endocrine, or cardiovascular disease-related gene (*MATN4*, *ARPP21* or *CFTR*). Although a recent MWAS also implicated cigarette smoking in DNA methylation at *ARPP21* and *CFTR* [140]—two genes that may underlie epigenetically mediated responses to inhalable environmental exposures—the CpG sites discovered herein are in

different regions of *ARPP21* and *CFTR*, suggesting varied responses to particulate exposures, and none of them were associated with gene expression of blood cells in MESA/GTP.

Methylation of cg19004594 (exon of *MATN4*) was positively associated with 28-day mean PM_{10} concentrations. Although *MATN4*-encoded Matrilin 4, a von Willebrand factor A domain-containing protein, contributes to cardiac remodeling [455] and inhibits the proliferation of hematopoietic stem cells at rest, environmental stressors trigger expression of the *CXCL12*-encoded chemokine (SDF1) [456], activation of its G protein-coupled receptor (CXCR4), inhibition of Matrilin 4, and subsequent expansion of hematopoietic stem cell pools [457]. SDF1-activated CXCR4 also inhibits beta-adrenergically activated calcium influx through myocardial L-type calcium ion channels [458], a process that may affect PM_{10}-associated ventricular action potential and electrocardiographic QT interval duration [339]. Methylation of *MATN4* may therefore underlie commonly observed hematological and electrocardiographic of effects of PM_{10}.

Methylation at cg24102420 (intron of *ARPP21*) was positively associated with 1-month mean PM_{10} concentrations. *ARPP21* encodes a neuronal cAMP-regulated phosphoprotein, a regulator of calmodulin signaling (RCS) that is highly enriched in medium spiny neurons within the basal ganglia, cerebral cortex, and other regions of the brain [459], with dual evidence of expression in cardiac tissues [460-462]. Variants of *ARPP21* have been associated with entorhinal cortical thickness [463]. Calmodulin signaling [464], entorhinal cortical thickness [465], and PM air pollution [466] are all associated with Alzheimer's disease progression, suggesting a potential epigenetic mechanism of PM_{10}-related neuropathology.

Indeed, *ARPP21* and *miR128-2*, a microRNA within *ARPP21*, are both regulators of dendritic growth [467]. In a study of rats, exposure to ammonium sulfate, a major component of $PM_{2.5}$, was associated with diminished dendritic complexity in hippocampal neurons [468]. Additionally, *miR128* expression in peripheral blood of steel plant workers increased with

increases in PM exposure, as was confirmed by an *in vitro* study of PM-treated pulmonary tissue [469]. Additional roles of *miR128* include the inhibition of *ABCA1* and *ABCG1*, adenosine triphosphate-binding cassette (ABC) transporter genes also involved in homeostasis of cholesterol [470], an established risk factor for stroke, myocardial infarction, and other common forms of cardiovascular disease.

Methylation at cg12124767 (intron of *CFTR*) was inversely associated with 1-month mean $PM_{2.5-10}$ concentrations. *CFTR* encodes a transmembrane conductance regulator; specifically, an ABC transporter of chloride and thiocyanate ions. The *CFTR*-encoded ABC transporter controls fluid secretion and absorption in epithelial tissues [471]. Its most common mutation impairs folding and trafficking of the encoded protein in pulmonary and pancreatic epithelia, causing CF and CF-related diabetes [472]. However, cigarette smoke and chronic inflammation also reduce *CFTR* chloride channel function [473], a hypothesized molecular pathway underlying the development of chronic obstructive pulmonary disease [474]. Furthermore, CFTR chloride channel currents in the myocardium shorten action potential and QT interval duration [475]. Their activation by cAMP protein kinase A (PKA), protein kinase C (PKC), or extracellular adenosine triphosphate (ATP) through purinergic receptors [475,476] can be arrhythmogenic [477-481]. Hypomethylation of *CFTR* at this site therefore highlights another epigenetic mechanism that may underlie PM_{10}-related pulmonary and electrocardiographic manifestations of disease.

While the putative mechanisms described above are biologically plausible, analyses on which they are based are limited by their reliance on DNAm derived from leukocytes. Although other (e.g. heart, lung, nervous) tissues may be more appropriate for studying the role of DNAm on human disease, their collection is highly invasive [108,115]; as such, leukocytes extracted from peripheral blood are widely used surrogate tissues [108] with demonstrated consistency of DNAm

patterns across relevant tissues types [116-118]. Still, DNAm at cg19004594, cg24102420, cg12124767 was not associated with gene expression of blood cells in GTP/MESA [385]. Unlike DNAm patterns though, gene expression is highly variable by tissue type [482], and *MATN4*, *ARPP21* and *CFTR* are primarily expressed in other tissues.

The inability to replicate associations in KORA F3 and F4 participants is noteworthy. Although independent from the discovery populations, KORA represents a population of white, European men and women living in Augsburg, Germany, one distinct from that of the environmentally diverse, multi-racial/ethnic U.S. populations in the discovery. In addition, PM composition in ARIC and WHI (1990-2012) may differ from that in Augsburg during KORA F3 and F4 (2004-2006). Furthermore, PM concentrations in KORA were measured at community monitors, while those in WHI and ARIC were spatially or spatiotemporally estimated at participant geocoded addresses from monitoring networks in the 48 contiguous US states.

DNAm associations with $PM_{2.5}$ – often cited as the driver for PM-associated disease [7] – were not detected in this study. Inability to do so may be due to lower power to detect $PM_{2.5}$ versus PM_{10} associations with DNAm given lower-variance $PM_{2.5}$ exposure estimates, lack of short-duration $PM_{2.5}$ data before 1999 when EPA AQS started monitoring it, and / or induction of $PM_{2.5}$ health effects that are not epigenetically mediated.

The analyses also were limited by predominantly cross-sectional data, high multiple testing burden, small effect sizes, and residual need for functional characterization. However, repeated measures of PM and DNAm over time were leveraged in WHI-EMPC to increase statistical power. Among-pollutant correlations also were moderate in this context, so the multiple comparisons made were not strictly independent. Similarly, the Bonferroni-corrected threshold used herein ($P < 1 \times 10^{-7}$) is conservative because of methylome-wide correlations

among CpG sites [483,484], decreasing the likelihood of false positivity. Moreover, observed effect sizes were consistent with those seen in other epigenetic studies of particulate matter exposure [173,174,446] and smoking [140]. Further investigation is nonetheless needed to determine the clinical impact of CpG-specific changes in methylation although functional validation of epigenetic associations was outside the scope of presently funded work. Still, this is a well-powered study of geographically diverse, multi-racial/ethnic populations of women and men with methylome-wide DNAm and geocoded address-specific PM data, that leveraged multivariate imputation to minimize selection-related biases otherwise known to affect epidemiologic associations in complete data analyses.

F. Conclusions

Findings from this large, racially/ethnically and environmentally diverse methylome-wide association study of women and men in EPA regions 1-10 suggest that ambient particulate matter air pollution affects DNAm at regions of the genome potentially related to neurological, pulmonary, endocrine, and cardiovascular disease. Although the discovered associations are biologically plausible, functional characterization in relevant tissues or animal models remain necessary to validate associations and elucidate putative epigenetic mechanisms of PM-associated disease.

G. Tables and Figures

Table 6-1. Characteristics of the study participants, by subpopulation

Subpopulation			Race / ethnicity	n	% female	Age, yrs $\bar{x}$ (SD)	Maximum CpGs	PM (μg/m^3), 1 mo $\bar{x}$ (SD)		
								PM_{10}	$PM_{2.5}$	$PM_{2.5-10}$
ARIC			AA	2,664	63%	56.6 (5.9)	463,431	20.5 (4.6)	13.2 (3.1)	7.3 (2.1)
			EA	1,100	58%	59.9 (5.4)	462,543	23.2 (5.3)	15.4 (4.3)	7.8 (3.5)
WHI	AS311	CT	EA	351	100%	64.7 (7.1)	461,136	19.8 (6.6)	11.9 (3.82)	7.9 (4.6)
		OS	EA	395	100%	66.2 (6.9)	461,136	19.9 (5.7)	12.0 (3.9)	7.9 (4.1)
	BAA23	CT	AA	371	100%	61.8 (6.3)	461,014	22.6 (6.2)	14.3 (4.2)	8.3 (3.8)
			EA	926	100%	67.8 (6.2)	461,014	19.7 (5.7)	11.7 (3.7)	8.0 (4.4)
			HLA	220	100%	60.7 (6.4)	461,014	21.4 (8.1)	10.3 (4.1)	11.1 (5.7)
		OS	AA	259	100%	62.8 (6.8)	461,014	22.3 (5.9)	14.0 (4.0)	8.3 (4.2)
			HLA	174	100%	62.8 (7.3)	461,014	23.0 (8.1)	11.0 (4.2)	11.9 (6.4)
	EMPC[a]		AA	553	100%	62.7 (6.9)	463,916	22.2 (6.2)	15.2 (5.1)	7.0 (4.7)
			EA	1,072	100%	64.6 (7.1)	463,916	19.4 (6.0)	13.0 (5.0)	6.4 (5.2)
			HLA	312	100%	61.5 (6.1)	463,916	21.9 (7.1)	12.8 (6.3)	9.1 (5.3)
All			AA (45.8%) HLA (8.4%) EA (45.8%)	8,397	83%	61.3 (7.4)	463,916	20.9 (5.8)	13.2 (4.3)	7.7 (4.0)

Abbreviations: AA, African American; ARIC, Atherosclerosis Risk in Communities; AS311, Ancillary Study 311; BAA23, Broad Agency Award 23; CpG, Cytosine-phosphate-Guanine; CT, Clinical Trial; EA, European American; EMPC, Epigenetic Mechanisms of PM-Mediated CVD Risk; HLA, Hispanic/Latino American; mo, month; OS, Observational Study; PM_{10}, PM < 10 μm in diameter; $PM_{2.5}$, PM < 2.5 μm in diameter; $PM_{2.5-10}$, PM > 2.5 and < 10 μm in diameter; SD, standard deviation; WHI, Women's Health Initiative; $\bar{x}$, mean

[a]At the 1st visit. Methylation data also were available among 185 & 43 WHI-EMPC participants @ the 2nd & 3rd visits

Table 6-2. Particulate matter concentration (μg/m³) means and Pearson correlations in the total population (n = 8,397)

		PM_{10} 2 d	PM_{10} 7 d	PM_{10} 28 d	PM_{10} 365 d	PM_{10} 1 mo	PM_{10} 12 mo	$PM_{2.5}$ 1 mo	$PM_{2.5}$ 12 mo	$PM_{2.5-10}$ 1 mo	$PM_{2.5-10}$ 12 mo
	$\bar{x}$ (SD)	31.9 (12.1)	31.1 (9.2)	30.9 (7.1)	31.2 (5.1)	20.9 (5.8)	20.9 (4.0)	13.2 (4.3)	13.2 (3.0)	7.7 (4.0)	7.8 (3.1)
PM_{10}	2 d	1.00									
PM_{10}	7 d	0.74	1.00								
PM_{10}	28 d	0.58	0.79	1.00							
PM_{10}	365 d	0.43	0.56	0.70	1.00						
PM_{10}	1 mo	0.39	0.48	0.54	0.27	1.00					
PM_{10}	12 mo	0.15	0.18	0.24	0.35	0.62	1.00				
$PM_{2.5}$	1 mo	0.29	0.36	0.41	0.17	0.73	0.39	1.00			
$PM_{2.5}$	12 mo	0.11	0.12	0.15	0.23	0.40	0.64	0.66	1.00		
$PM_{2.5-10}$	1 mo	0.25	0.31	0.35	0.21	0.67	0.48	-0.02	-0.13	1.00	
$PM_{2.5-10}$	12 mo	0.08	0.12	0.17	0.23	0.41	0.67	-0.14	-0.14	0.74	1.00

Abbreviations: d, day; mo, month; PM, particulate matter; PM_{10}, PM < 10 μm in diameter; $PM_{2.5}$, PM < 2.5 μm in diameter; $PM_{2.5-10}$, PM > 2.5 and < 10 μm in diameter; SD, standard deviation; $\bar{x}$, mean

Table 6-3. Findings from trans-ethnic, fixed-effects meta-analyses ($P < 1 \times 10^{-7}$, $P_{Cochran's\ Q} > 0.10$)

Chr	Position[a]	CpG	Exposure	%Δ (95% CI)[b]	*P*	n_{obs}	Gene
20	43926884	cg19004594	PM_{10}, 28 d	0.3 (0.2, 0.4)	3.33×10^{-8}	8,622	*MATN4*
3	35785890	cg24102420	PM_{10}, 1 mo	-0.5 (-0.7, -0.3)	5.84×10^{-8}	8,575	*ARPP21 / MIR128-2*
7	117299297	cg12124767	$PM_{2.5-10}$, 1 mo	-0.5 (-0.7, -0.3)	9.96×10^{-8}	8,577	*CFTR*

Abbreviations: **Δ**, change; Chr, chromosome; CI, confidence interval; CpG, Cytosine-phosphate-Guanine; d, days; mo, month; PM_{10}, PM < 10 μm in diameter; $PM_{2.5}$, PM < 2.5 μm in diameter; $PM_{2.5-10}$, PM > 2.5 and < 10 μm in diameter

[a]Build 37

[b]Absolute percentage point per 10 $\mu g/m^3$ increase in PM_{10}

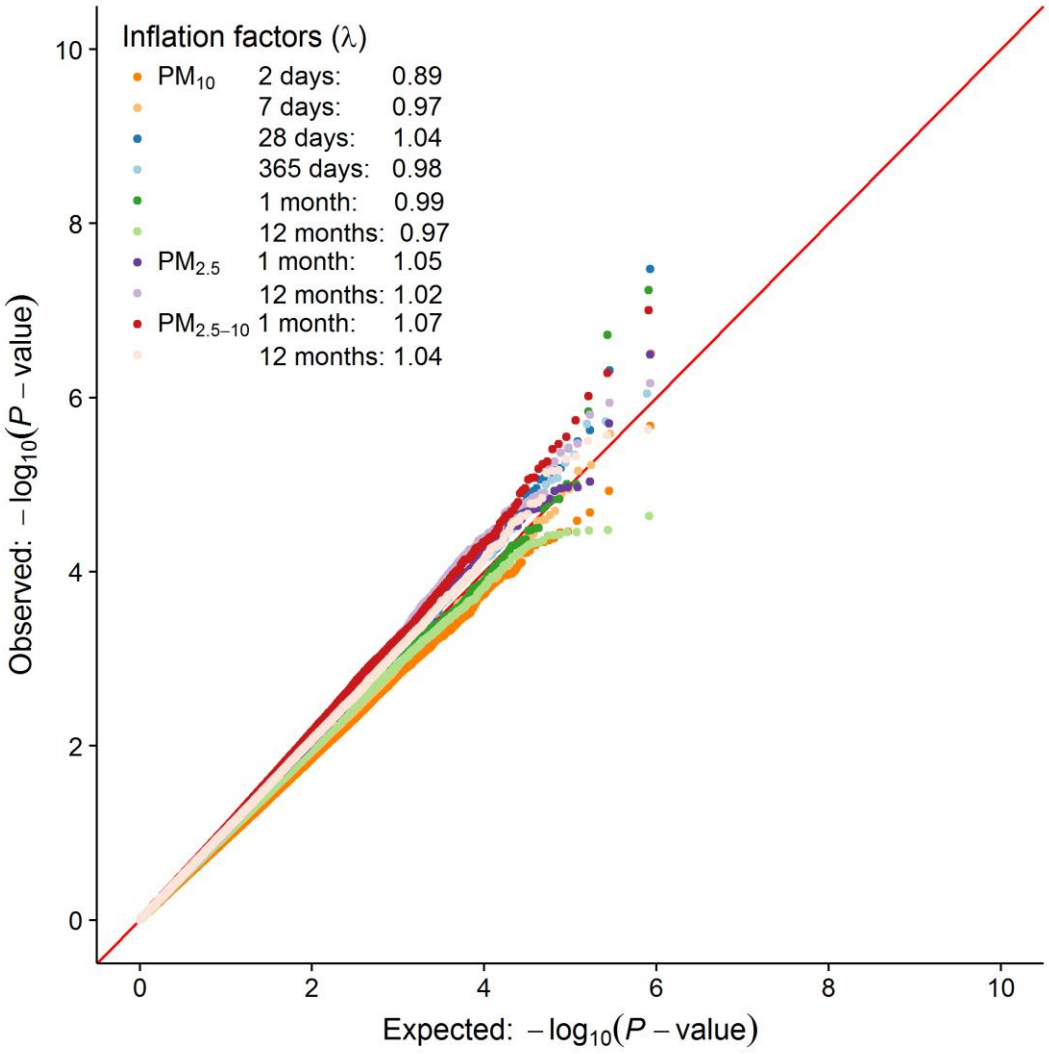

Figure 6-1. Quantile-quantile (QQ) plot of observed vs. expected -$\log_{10}$ *p*-value of each CpG site from trans-ethnic, fixed-effects meta-analyses of 2-, 7-, 28-, and 365-day PM_{10} and 1- and 12-month PM_{10} and $PM_{2.5}$. The red diagonal line references the methylome-wide significance threshold ($P < 1.0\times10^{-7}$). Lambda (λ) is the inflation factor.

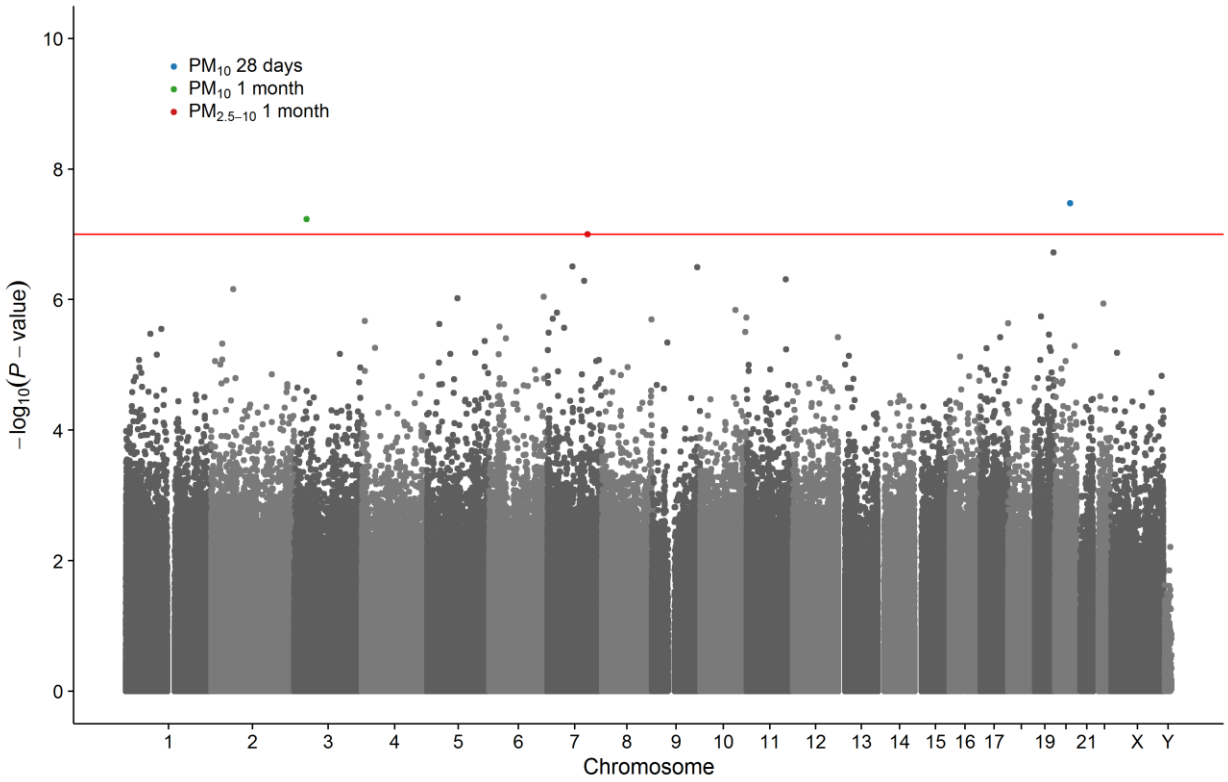

Figure 6-2. Manhattan plot of −log$_{10}$ *p*-value vs. chromosomal position of each CpG site from trans-ethnic, fixed-effects meta-analyses of 2-, 7-, 28-, and 365-day PM_{10} and 1- and 12-month PM_{10} and $PM_{2.5}$. The red line references the methylome-wide significance threshold ($P < 1.0 \times 10^{-7}$)

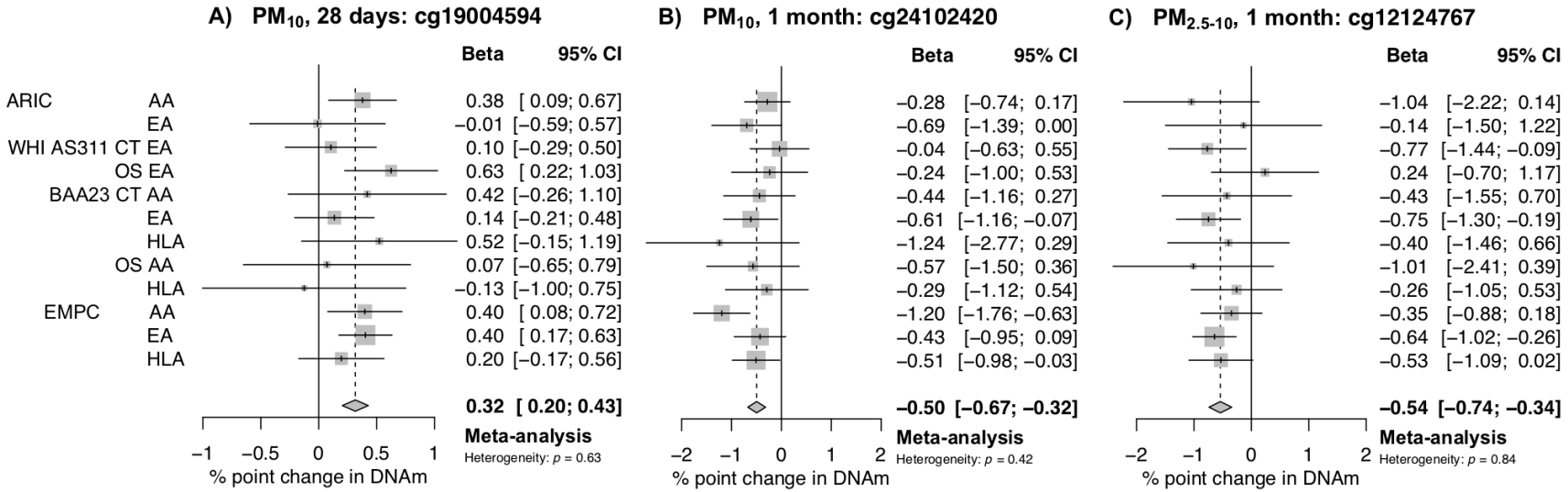

Figure 6-3. Forest plots of PM-CpG associations (95% confidence intervals) for A) cg19004594, B) cg2410240, and C) cg12124767 with a 10 µg/m³ increase in PM by subpopulation and overall after fixed-effects meta-analysis

Figure 6-4. Enrichment of PM-sensitive CpG sites in regions overlapping H3K9me3 using Roadmap data

H. Supplement

The Cooperative Health Research in the Region of Augsburg (KORA) study is a population-based cohort from the region of Augsburg, Southern Germany. Replication analyses involved data from the F3 (n = 3,006; 2004-2005) and F4 (n = 3,080; 2006-2008) follow-up studies of the KORA S3 and S4 participants [485,486].

DNA methylation was analyzed from whole blood samples in 500 (F3) and 1799 (F4) participants using the Infinium HumanMethylation450 BeadChip Array (Illumina). Probes with signals from less than three functional beads, a detection *P* value > 0.05 in > 1% of samples, or covered single nucleotide polymorphisms (minor allele frequency in Europeans > 5%) were excluded. Sample exclusions included participants with a detection *P* value > 0.05 for > 1% of probes and those with a gender mismatch. DNAm measures were Beta Mixture Quantile (BMIQ)-normalized to adjust for probe bias [360]. DNAm at three CpG sites was analyzed: cg19004594, cg24102420, and cg12124767. Analyses controlled for technical variation by adjusting for CD4 T-cells, plasmablasts, natural killer cells, CD8 naive T-cells, monocytes, granulocytes, and a linear combination of CD8, CD45RA, and CD28 T-cells [487]. Analyses also controlled for plate and batch effects using 20 principal components calculated from the control probes. Moreover, analyses controlled for demographic and clinical variables collected via standardized questionnaires at each visit, as well as meteorological variables: age, sex, years of education, smoking status (current regular, current irregular, former, never), alcohol consumption (alcohol usage, no alcohol usage), physical activity (active, inactive), body mass index [485], mean temperature, mean barometric pressure, and mean relative humidity.

Table 6-S1. Methylome-wide DNAm data exclusions in WHI and ARIC

	Sample Exclusions		Probe Exclusions				
Study	N after exclusions[a]	Detection p-value	n CpGs after exclusions[b]	Detection p-value	Y Chr	Bead Count	Non-CpG CH_3
WHI-EMPC[c]	1,937	> 0.01 in > 1%[d]	463,916	> 0.01 in > 10%[e]	Yes	No	No
WHI-BAA23	1,950	No	461,014	> 0.01 in > 10%[e]	Yes	No	Yes
WHI-AS311	746	No	461,136	> 0.01 in > 1%[e]	Yes	< 3 in > 10%[e]	Yes
ARIC-AA	2,664	> 0.01 in > 1%[d]	463,431	> 0.01 in > 1%[e]	No	< 3 in > 5%[e]	No
ARIC-EA	1,100	> 0.01 in > 1%[d]	462,543	> 0.01 in > 5%[e]	No	< 3 in >5%[e]	No

[a]Additional study-specific sample exclusions: gender mismatch or SNP discordance with previous genotyping, and / or outliers in principal component analysis

[b]Additional probe exclusion: CpG sites with multi-modal DNAm distributions in ≥ 1 study

[c]185 participants had a second and 43 had a third DNAm measure at a subsequent visit (n observations = 2,165)

[d]Of probes

[e]Of samples

Table 6-S2. Mean concentrations (μg/m^3) of particulate matter (PM) by study

Study	Race / Ethnicity	PM_{10}						$PM_{2.5}$		$PM_{2.5-10}$	
		2 d $\bar{x}$ (SD)	7 d $\bar{x}$ (SD)	28 days $\bar{x}$ (SD)	365 d $\bar{x}$ (SD)	1 mo $\bar{x}$ (SD)	12 mo $\bar{x}$ (SD)	1 mo $\bar{x}$ (SD)	12 mo $\bar{x}$ (SD)	1 mo $\bar{x}$ (SD)	12 mo $\bar{x}$ (SD)
ARIC	AA	36.0 (12.3)	35.1 (9.1)	34.8 (6.3)	35.5 (3.3)	20.5 (4.6)	19.9 (1.69)	13.2 (3.1)	12.7 (1.3)	7.3 (2.1)	7.2 (0.8)
ARIC	EA	36.1 (11.5)	34.9 (8.2)	34.4 (5.8)	34.8 (3.0)	23.2 (5.3)	23.7 (2.4)	15.4 (4.3)	15.9 (2.1)	7.8 (3.5)	7.8 (1.4)
WHI-AS311[a]	EA	28.0 (11.0)	27.1 (7.9)	27.4 (6.5)	27.5 (4.1)	19.8 (6.6)	20.0 (4.8)	11.9 (3.82)	11.9 (2.7)	7.9 (4.6)	8.1 (3.8)
WHI-AS311[b]	EA	28.7 (11.1)	27.7 (8.9)	27.6 (6.6)	27.6 (4.2)	19.9 (5.7)	20.2 (4.5)	12.0 (3.9)	12.0 (2.6)	7.9 (4.1)	8.2 (3.5)
WHI-BAA23[a]	AA	28.2 (12.2)	27.0 (7.5)	27.8 (5.6)	28.3 (2.8)	22.6 (6.2)	22.3 (3.7)	14.3 (4.2)	14.1 (2.2)	8.3 (3.8)	8.2 (2.6)
WHI-BAA23[a]	EA	28.1 (10.7)	27.2 (8.4)	27.2 (6.4)	27.5 (4.0)	19.7 (5.7)	20.0 (4.5)	11.7 (3.7)	11.8 (2.5)	8.0 (4.4)	8.2 (3.7)
WHI-BAA23[a]	HLA	28.9 (10.4)	29.3 (8.3)	29.3 (6.8)	29.2 (4.1)	21.4 (8.1)	21.5 (5.9)	10.3 (4.1)	10.3 (3.0)	11.1 (5.7)	11.2 (4.5)
WHI-BAA23[b]	AA	28.8 (11.1)	28.8 (8.5)	28.1 (6.1)	28.1 (2.3)	22.3 (5.9)	22.6 (3.7)	14.0 (4.0)	14.1 (2.2)	8.3 (4.2)	8.5 (3.1)
WHI-BAA23[b]	HLA	30.2 (10.7)	29.3 (8.6)	29.9 (7.2)	30.0 (4.7)	23.0 (8.1)	23.1 (6.1)	11.0 (4.2)	10.9 (3.2)	11.9 (6.4)	12.2 (5.2)
WHI-EMPC[a,c]	AA	29.2 (11.2)	27.9 (7.3)	27.7 (5.5)	28.1 (3.0)	22.2 (6.2)	22.4 (4.3)	15.2 (5.1)	15.1 (3.8)	7.0 (4.7)	7.3 (3.4)
WHI-EMPC[a,c]	EA	28.3 (11.5)	27.3 (8.1)	27.2 (6.4)	27.5 (3.8)	19.4 (6.0)	19.8 (5.8)	13.0 (5.0)	12.9 (3.6)	6.4 (5.2)	6.8 (4.1)
WHI-EMPC[a,c]	HLA	28.5 (9.8)	28.4 (8.3)	28.3 (6.2)	28.3 (4.2)	21.9 (7.1)	22.3 (6.1)	12.8 (6.3)	12.9 (5.4)	9.1 (5.3)	9.4 (4.9)
All		31.9 (12.1)	31.1 (9.2)	30.9 (7.1)	31.2 (5.1)	20.9 (5.8)	20.9 (4.0)	13.2 (4.3)	13.2 (3.0)	7.7 (4.0)	7.8 (3.1)

Abbreviations: AA, African American; ARIC, Atherosclerosis Risk in Communities; AS311, Ancillary Study 311; BAA23OS, Broad Agency Award 23; CpG, Cytosine-phosphate-Guanine site; d, day; EA, European American; EMPC, Epigenetic Mechanisms of PM-Mediated CVD Risk; HLA, Hispanic/Latino American; mo, month; PM, particulate matter; SD, standard deviation; WHI, Women's Health Initiative

[a]WHI clinical trials participants

[b]WHI observational study participants

[c]Data from the first visit are presented for WHI-EMPC; 185 participants had a second and 43 had a third DNAm measure from a subsequent visit

Table 6-S3. Findings from trans-ethnic, fixed-effects inverse variance-weighted meta-analyses ($P < 1 \times 10^{-5}$, $P_{Cochran's\ Q} > 0.10$) with Illumina 450K Infinium Methylation

Chr	Position (B37)	CpG	Pollutant	Duration	Beta	SE	*P* value
20	43926884	cg19004594	PM_{10}	28 days	3.16E-04	5.72E-05	3.33E-08
3	35785890	cg24102420	PM_{10}	1 month	-4.96E-04	9.14E-05	5.84E-08
7	117299297	cg12124767	$PM_{2.5-10}$	1 month	-5.40E-04	1.01E-04	9.96E-08
19	55013954	cg19547155	PM_{10}	1 month	-1.21E-03	2.31E-04	1.90E-07
7	73183394	cg12169661	PM_{10}	7 days	7.15E-04	1.40E-04	3.11E-07
9	132383003	cg09731694	$PM_{2.5}$	1 month	-5.23E-04	1.02E-04	3.18E-07
11	114493710	cg20057398	PM_{10}	28 days	2.66E-04	5.29E-05	4.88E-07
7	107385716	cg14590325	$PM_{2.5-10}$	1 month	-8.26E-04	1.65E-04	5.20E-07
2	64682236	cg01948201	$PM_{2.5}$	12 months	-6.41E-04	1.29E-04	6.89E-07
6	159466542	cg16180082	PM_{10}	365 days	-9.85E-04	2.01E-04	9.02E-07
5	88307760	cg02412399	$PM_{2.5-10}$	1 month	-4.69E-04	9.57E-05	9.60E-07
22	30639730	cg07316313	$PM_{2.5}$	12 months	-6.73E-04	1.38E-04	1.15E-06
10	102473022	cg13583895	PM_{10}	1 month	-4.10E-04	8.50E-05	1.44E-06
7	27225396	cg24988255	$PM_{2.5}$	12 months	-7.74E-04	1.61E-04	1.58E-06
19	18549689	cg01065977	$PM_{2.5-10}$	1 month	1.03E-03	2.16E-04	1.82E-06
10	135342413	cg25330361	PM_{10}	365 days	9.91E-04	2.08E-04	1.88E-06
7	15726411	cg18580296	$PM_{2.5}$	1 month	-2.96E-04	6.23E-05	1.97E-06
8	144790656	cg09754549	PM_{10}	365 days	1.74E-03	3.66E-04	2.01E-06
4	8230847	cg01945624	PM_{10}	2 days	-2.28E-04	4.81E-05	2.12E-06
18	11855	cg25023094	$PM_{2.5-10}$	12 months	1.03E-03	2.17E-04	2.29E-06
5	35617730	cg11438448	PM_{10}	28 days	-3.88E-04	8.23E-05	2.38E-06
6	30421218	cg26690915	PM_{10}	7 days	-3.37E-04	7.17E-05	2.60E-06
7	48963408	cg05563813	$PM_{2.5-10}$	12 months	-5.37E-04	1.14E-04	2.70E-06
1	104068488	cg07878955	$PM_{2.5-10}$	1 month	-3.09E-04	6.60E-05	2.81E-06
10	131592483	cg02656060	$PM_{2.5-10}$	12 months	3.44E-04	7.37E-05	3.13E-06
7	2968595	cg22989995	PM_{10}	28 days	2.13E-04	4.58E-05	3.21E-06
1	71512973	cg15201877	$PM_{2.5}$	12 months	-6.54E-04	1.41E-04	3.35E-06
19	40736427	cg15153957	$PM_{2.5-10}$	1 month	-6.57E-04	1.42E-04	3.44E-06
17	57233042	cg02573089	$PM_{2.5}$	12 months	1.22E-04	2.64E-05	3.77E-06
12	131865279	cg00014484	PM_{10}	28 days	3.41E-04	7.37E-05	3.80E-06
6	49755105	cg21080533	$PM_{2.5-10}$	1 month	-5.13E-04	1.11E-04	3.92E-06
5	167719548	cg15232798	$PM_{2.5}$	12 months	-4.70E-04	1.02E-04	4.33E-06
9	44745070	cg14641231	PM_{10}	365 days	-6.35E-04	1.39E-04	4.55E-06
2	32853039	cg19757253	$PM_{2.5-10}$	12 months	-1.39E-04	3.04E-05	4.74E-06
20	57583188	cg12787553	$PM_{2.5-10}$	12 months	3.86E-04	8.47E-05	5.11E-06
19	43969886	cg24950222	$PM_{2.5-10}$	1 month	7.46E-04	1.64E-04	5.44E-06

Chr	Position (B37)	CpG	Pollutant	Duration	Beta	SE	*P* value
4	38667430	cg22453435	$PM_{2.5}$	12 months	6.65E-04	1.46E-04	5.46E-06
17	17929033	cg21187669	PM_{10}	365 days	-7.29E-04	1.60E-04	5.60E-06
11	115088907	cg16061656	$PM_{2.5-10}$	1 month	-5.33E-04	1.18E-04	5.79E-06
7	958244	cg27572072	PM_{10}	7 days	-2.24E-04	4.94E-05	5.98E-06
19	46456210	cg21632975	$PM_{2.5}$	12 months	-7.03E-04	1.56E-04	6.11E-06
5	140700583	cg15351446	PM_{10}	28 days	-6.11E-04	1.35E-04	6.52E-06
X	19141251	cg16641638	$PM_{2.5-10}$	1 month	9.81E-04	2.18E-04	6.53E-06
5	67583609	cg24797508	PM_{10}	28 days	-5.73E-04	1.27E-04	6.80E-06
3	133393119	cg24405999	$PM_{2.5-10}$	12 months	-1.15E-03	2.55E-04	6.84E-06
6	28698008	cg09294156	PM_{10}	7 days	4.05E-04	9.00E-05	6.93E-06
1	90231240	cg12857520	$PM_{2.5-10}$	12 months	7.55E-04	1.68E-04	6.96E-06
13	30088615	cg09750646	$PM_{2.5-10}$	12 months	2.46E-04	5.48E-05	7.30E-06
16	31235824	cg10085057	PM_{10}	28 days	2.66E-04	5.94E-05	7.52E-06
2	32852828	cg26189067	$PM_{2.5-10}$	1 month	2.12E-04	4.77E-05	8.29E-06
1	38260988	cg02851558	$PM_{2.5-10}$	1 month	8.69E-04	1.95E-04	8.41E-06
7	149809179	cg21015808	PM_{10}	365 days	1.21E-03	2.72E-04	8.42E-06
19	15488783	cg03654623	PM_{10}	28 days	-5.16E-04	1.16E-04	8.44E-06
7	142045032	cg12155684	PM_{10}	28 days	-5.36E-04	1.20E-04	8.74E-06
20	30619137	cg27531587	PM_{10}	365 days	-1.10E-03	2.47E-04	8.82E-06
5	33936752	cg02341815	$PM_{2.5}$	1 month	-3.83E-04	8.64E-05	9.21E-06
13	19919758	cg03941975	PM_{10}	1 month	-3.26E-04	7.37E-05	9.85E-06
2	27166550	cg18990157	PM_{10}	1 month	-5.09E-04	1.15E-04	9.85E-06

Chr	FDR	n_{obs}	Direction	$P_{Cochran's\ Q}$	Infinium Design Type	Strand	Probe SNPs	Probe SNPs within 10 bases	UCSC RefGene Name
20	0.008	8,622	+-++++-+++++	0.63	I	R			MATN4;MATN4;MATN4
3	0.027	8,575	----------	0.42	II	F			MIR128-2;ARPP-21
7	0.046	8,577	---------+--	0.84	II	R			CFTR
19	0.044	7,476	-?----------	1.00	II	R			LAIR2;LAIR2
7	0.145	8,619	+-+++--+--++	0.11	II	R			CLDN3;CLDN3
9	0.049	8,580	-+++-+------	0.20	II	F			C9orf50;C9orf50
11	0.057	8,617	++++++++++++	0.51	II	F			
7	0.121	8,574	--+---------	0.75	II	F			CBLL1;CBLL1
2	0.245	8,575	+-----+-+---	0.27	II	F			HSPC159
6	0.210	8,617	---+-+----+-	0.12	II	F			TAGAP;TAGAP;TAGAP
5	0.149	8,575	-++---------	0.21	II	F			
22	0.245	8,568	------+-----	0.73	II	R			LIF
10	0.224	8,582	------+--+--	0.94	II	F			
7	0.245	8,581	+----------	0.45	II	F		rs61741589	HOXA11AS;HOXA11
19	0.169	8,578	+++-++-+++++	0.56	I	F			ISYNA1;ISYNA1;ISYNA1
10	0.233	8,617	-++++++++++	0.69	I	R			CYP2E1
7	0.229	8,578	-----+------	0.40	II	R	rs917442		MEOX2
8	0.233	8,618	+--++-++++++	0.40	II	F			LOC100130274
4	0.985	8,619	-----++-+---	0.51	II	R			SH3TC1
18	0.364	8,578	+++-++++-+++	0.59	I	F	rs6505962		
5	0.222	8,625	----------	0.97	II	F			SPEF2;SPEF2
6	0.606	8,621	-----++-----	0.37	II	R			
7	0.364	8,554	----------	0.50	II	R			CDC14C
1	0.218	8,581	+-----+-----	0.58	II	R			RNPC3
10	0.364	8,575	+-++++++++	0.72	II	F			
7	0.249	8,620	++++++-+++++	0.67	II	F			CARD11
1	0.250	8,577	-+-+----+---	0.58	II	F			PTGER3;PTGER3;PTGER3;PTGER3;PTGER3;PTGER3;PTGER3;PTGER3;PTGER3;PTGER3
19	0.228	8,576	----------	0.31	II	F	rs79228552		AKT2
17	0.250	8,578	++-++++++-+	0.53	I	F	rs80342392		PRR11;SKA2;SKA2
12	0.253	8,601	+++++-++++++	0.56	I	F			
6	0.228	8,575	----+-------	0.46	II	R			PGK2

Chr	FDR	n_{obs}	Direction	$P_{Cochran's\ Q}$	Infinium Design Type	Strand	Probe SNPs	Probe SNPs within 10 bases	UCSC RefGene Name
5	0.252	8,580	+--+-++-----	0.18	II	F			WWC1;WWC1;WWC1
9	0.379	8,613	----------	0.62	II	F	rs10908153		
2	0.377	8,570	-----+------	0.89	I	R			TTC27
20	0.377	8,568	++-+++++++++	0.35	II	F		rs79270271	CTSZ
19	0.269	8,577	++++++++++++	0.80	II	F			LYPD3
4	0.282	8,578	++++++++-+++	0.28	II	F			FLJ13197;KLF3
17	0.379	8,618	----------	0.66	II	R			ATPAF2
11	0.269	8,569	---------+--	0.73	II	R			CADM1;CADM1
7	0.645	8,618	---+-+------	0.53	II	F	rs1534410		ADAP1
19	0.284	8,577	---+--+-----	0.96	II	R			NOVA2
5	0.339	8,619	------+-----	0.88	II	R			TAF7
X	0.276	8,565	+++-++++++++	0.71	I	R			GPR64;GPR64;GPR64;GPR64
5	0.339	8,620	----------	0.93	II	R			PIK3R1;PIK3R1
3	0.377	8,579	---+--------	0.80	II	R		rs79353799	
6	0.645	8,619	++--++++-+++	0.10	I	R			
1	0.377	8,568	+--+-++-++++	0.52	II	R			
13	0.377	8,577	-+++++++-+-+	0.11	II	F			SLC7A1
16	0.339	8,614	++++++++++++	0.95	II	R			TRIM72
2	0.289	8,582	+-+-+-++++++	0.11	II	R			TTC27
1	0.289	8,577	-++-+++++-++	0.10	II	F			MANEAL;MANEAL;MANEAL
7	0.379	8,604	+-+++-++++++	0.33	II	R			
19	0.339	8,620	--++----+---	0.49	II	F	rs34428373		AKAP8
7	0.339	8,620	-++------+--	0.37	II	F	rs2855868		
20	0.379	8,587	+--+-+---+--	0.22	II	F			C20orf160
5	0.466	8,562	-+----------	0.94	II	R			RXFP3;RXFP3
13	0.916	8,576	---------+--	0.71	II	F			LOC100101938
2	0.916	8,575	----+----+--	0.34	II	R		rs34064589	DPYSL5

Chr	UCSC RefGene Accession	UCSC RefGene Group	UCSC CpG Islands Name	Relation to UCSC CpG Island	Phantom	Enhancer	HMM Island	Regulatory Feature Name	Regulatory Feature Group	DHS
20	NM_030592;NM_030590;NM_003833	Body;Body;Body	chr20:43926594-43927171	Island			20:43360020-43360585			
3	NR_029824;NM_016300	TSS200;Body								
7	NM_000492	Body				TRUE				
19	NM_002288;NM_021270	TSS200;TSS200								
7	NM_001306;NM_001306	3'UTR;1stExon	chr7:73183379-73185115	Island		TRUE	7:72820924-72823026			
9	NM_199350;NM_199350	1stExon;5'UTR	chr9:132382432-132383004	Island		TRUE	9:131421972-131422825	9:132381722-132383326	Unclassified	
11							11:11399890-11399055			
7	NM_024814;NR_024199	Body;Body	chr7:107383657-107385021	S_Shore						
2	NM_014181	Body	chr2:64681011-64682237	Island			2:64534584-64535741			
6	NM_152133;NM_138810;NM_054114	TSS1500;TSS1500;TSS1500						6:159465693-159466558	Promoter_Associated	

Chr	UCSC RefGene Accession	UCSC RefGene Group	UCSC CpG Islands Name	Relation to UCSC CpG Island	Phantom	Enhancer	HMM Island	Regulatory Feature Name	Regulatory Feature Group	DHS
5						TRUE				
22	NM_002309	Body	chr22:30639729-30639994	Island		TRUE				
10			chr10:102473206-102474026	N_Shore						
7	NR_002795;NM_005523	Body;TSS1500	chr7:27225050-27225629	Island			7:27190555-27192145			
19	NM_016368;NM_001170938;NM_001170939	TSS1500;TSS1500;TSS1500	chr19:18543828-18549161	S_Shore		TRUE				
10	NM_000773	Body	chr10:135341255-135342561	Island			10:13519225 7-13519 2927	10:135342406-135343123	Unclassified_Cell_type_specific	TRUE
7	NM_005924	TSS200					7:15692873-15693735			
8	NM_001162914	TSS1500	chr8:144788491-144791059	Island			8:144860480-144863032			
4	NM_018986	Body				TRUE		4:8229970-8231542	Unclassified	TRUE
18			chr18:11708-12372	Island			18:1668-2363	18:11623-11949	Unclassified_Cell_type_	TRUE

Chr	UCSC RefGene Accession	UCSC RefGene Group	UCSC CpG Islands Name	Relation to UCSC CpG Island	Phantom	Enhancer	HMM Island	Regulatory Feature Name	Regulatory Feature Group	DHS
									specific	
5	NM_024867;NM_144722	TSS1500;TSS1500	chr5:35617855-35618339	N_Shore				5:35617465-35618744	Promoter_Associated	
6			chr6:30418844-30419630	S_Shore			6:30528709-30529646	6:30420915-30421864	Unclassified	
7	NR_003595	TSS1500	chr7:48963967-48964348	N_Shore						
1	NM_017619	TSS200	chr1:104068487-104068913	Island	high-CpG:10384040904-103841449		1:10384040702-103841560	1:104067619-104069204	Promoter_Associated_Cell_type_specific	
10			chr10:131592393-131592616	Island			10:13148238 4-13148 2606			
7	NM_032415	Body	chr7:2968234-2968596	Island						
1	NM_198718;NR_028294;NM_198714;NR_028293;NM_198716;NM_198717;NR_028292;NM_001126044;NM_198719;NM_198715	1stExon;Body;1stExon;Body;1stExon;1stExon;Body;1stExon;1stExon;1stExon	chr1:71512224-71513804	Island		TRUE	1:71284815-71286418	1:71512684-71513685	Unclassified_Cell_type_specific	TRUE
19	NM_001626	3'UTR	chr19:40732075-40732665	S_Shelf						

Chr	UCSC RefGene Accession	UCSC RefGene Group	UCSC CpG Islands Name	Relation to UCSC CpG Island	Phantom	Enhancer	HMM Island	Regulatory Feature Name	Regulatory Feature Group	DHS
17	NM_018304;NM_001100595;NM_182620	TSS200;TSS1500;TSS1500	chr17:57231855-57232655	S_Shore			17:54586469-54588061	17:57232097-57233543	Promoter_Associated	TRUE
12						TRUE	12:13043123 0-13043 1297			
6	NM_138733	TSS200								
5	NM_001161662;NM_015238;NM_001161661	Body;Body;Body	chr5:167718523-167719688	Island			5:167651074-167652234			
9			chr9:44743987-44744258	S_Shore						
2	NM_017735	TSS200	chr2:32853007-32853270	Island			2:32706435-32707015	2:32852291-32853612	Promoter_Associated	
20	NM_001336	TSS1500	chr20:57581902-57582595	S_Shore						
19	NM_014400	TSS200	chr19:43967247-43968625	S_Shore						
4	NR_026804;NM_016531	TSS1500;5'UTR	chr4:38664649-38666531	S_Shore						
17	NM_145691	Body				TRUE				TRUE
11	NM_001098517;NM_014333	Body;Body								
7	NM_006869	Body	chr7:959724-	N_Shore						

Chr	UCSC RefGene Accession	UCSC RefGene Group	UCSC CpG Islands Name	Relation to UCSC CpG Island	Phantom	Enhancer	HMM Island	Regulatory Feature Name	Regulatory Feature Group	DHS
			959959							
19	NM_002516	Body	chr19:46456209-46456503	Island		TRUE	19:51147814-51148279			TRUE
5	NM_005642	TSS1500	chr5:140699898-140700495	S_Shore			5:140680760-140680833	5:140699328-140700860	Promoter_Associated	TRUE
X	NM_005756;NM_001079859;NM_001079860;NM_001079858	TSS1500;TSS1500;TSS1500;TSS1500	chrX:19139937-19140629	S_Shore			X:19049688-19051204			
5	NM_181523;NM_181524	Body;TSS1500	chr5:67584213-67584451	N_Shore				5:67583598-67584704	Promoter_Associated	
3			chr3:133393118-133393657	Island			3:134875552-134876347			
6							6:28805742-28805992			
1			chr1:90228744-90229173	S_Shelf				1:90231189-90231313	Unclassified_Cell_type_specific	

Chr	UCSC RefGene Accession	UCSC RefGene Group	UCSC CpG Islands Name	Relation to UCSC CpG Island	Phantom	Enhancer	HMM Island	Regulatory Feature Name	Regulatory Feature Group	DHS
13	NM_003045	3'UTR	chr13:30088558-30088772	Island			13:28986598-28986848			
16	NM_001008274	Body	chr16:31235525-31236104	Island			16:31143042-31143605	16:31235797-31235920	Unclassified_Cell_type_specific	
2	NM_017735	TSS1500	chr2:32853007-32853270	N_Shore			2:32706200-32706369	2:32852291-32853612	Promoter_Associated	
1	NM_001113482;NM_001031740;NM_152496	Body;Body;TSS200	chr1:38259095-38260427	S_Shore						
7							7:149439733-149440113			
19	NM_005858	Body	chr19:15489856-15491258	N_Shore				19:15488773-15489081	Unclassified_Cell_type_specific	
7										
20	NM_080625	3'UTR	chr20:30618793-30619138	Island		TRUE	20:30082455-30082986	20:30618596-30620114	Unclassified	
5	NM_016568;NM_016568	1stExon;5'UTR	chr5:33936168-33938309	Island		TRUE	5:33971926-33974929			TRUE

Chr	UCSC RefGene Accession	UCSC RefGene Group	UCSC CpG Islands Name	Relation to UCSC CpG Island	Phantom	Enhancer	HMM Island	Regulatory Feature Name	Regulatory Feature Group	DHS
13	NR_027248	TSS1500	chr13:19918585-19919221	S_Shore						
2	NM_020134	Body				TRUE				

Abbreviations: B37, build 37; Chr, chromosome; CpG, cytosine-phosphate-guanine site; DHS, DNase 1 hypersensitivity site; F, forward; FDR, false discovery rate;HMM, Hiden Markov Model; PM, particulate matter; R, reverse; SE, standard error; SNP, single nucleotide polymorphism; UCSC, University of California Santa Cruz

Table 6-S4. Comparison of DNA methylation measures from the Illumina 450K Infinium Methylation BeadChip versus bisulfite pyrosequencing

Chr	Position (B37)	CpG	Δ (95% CI)	r (95% CI)	α (95% CI)	β (95% CI)
4	8230847	cg01945624	0.07 (0.06, 0.07)	0.83 (0.78, 0.87)	0.17 (0.14, 0.20)	0.76 (0.68, 0.83)
2	64682236	cg01948201	0.02 (0.02, 0.02)	0.71 (0.63, 0.77)	0.04 (0.01, 0.08)	0.91 (0.78, 1.04)
22	30639730	cg07316313	-0.15 (-0.15, -0.14)	0.75 (0.68, 0.81)	-0.58 (-0.74, -0.42)	1.52 (1.33, 1.72)
9	132383003	cg09731694	0.08 (0.08, 0.08)	0.78 (0.72, 0.83)	0.03 (0.01, 0.06)	1.36 (1.21, 1.51)
8	144790656	cg09754549	-0.03 (-0.04, -0.02)	0.86 (0.82, 0.90)	-0.06 (-0.21, 0.09)	1.04 (0.85, 1.23)
6	159466542	cg16180082	0.04 (0.04, 0.05)	0.61 (0.52, 0.69)	-0.23 (-0.35, -0.12)	2.49 (1.90, 3.07)
7	15726411	cg18580296	0 (-0.01, 0.00)	0.22 (0.08, 0.34)	0.08 (0.08, 0.09)	0.09 (0.04, 0.15)
7	2968595	cg22989995	-0.05 (-0.06, -0.05)	0.04 (-0.10, 0.18)	0.82 (0.60, 1.04)	0.07 (-0.17, 0.30)
3	35785890	cg24102420	-0.04 (-0.04, -0.03)	0.79 (0.73, 0.83)	-0.16 (-0.38, 0.07)	1.13 (0.88, 1.39)
7	27225396	cg24988255	0.07 (0.07, 0.08)	0.4 (0.27, 0.51)	0.12 (0.09, 0.15)	0.55 (0.27, 0.82)

Abbreviations: B37, build 37; **Δ**, mean Illumina 450K minus bisulfite pyrosequencing difference in DNAm; Chr, chromosome; CI, confidence interval; CpG, cytosine-phosphate-guanine site; ICC, intra-class correlation; r, Pearson correlation coefficient

Table 6-S5. Findings from Cooperative Health Research in the Region Augsburg study (KORA)

Chr	Position[a]	CpG	Exposure	%Δ (95% CI)[b]	*P*	n_{obs}	Gene
20	43926884	cg19004594	PM_{10}, 28 d	-0.1 (-0.3, 0.1)	0.42	2,168	*MATN4*
3	35785890	cg24102420	PM_{10}, 30 d	-0.2 (-0.6, 0.1)	0.13	2,176	*ARPP21 / MIR128-2*
7	117299297	cg12124767	$PM_{2.5-10}$, 30 d	0.4 (-0.2, 1.0)	0.21	2,036	*CFTR*

Abbreviations: **Δ**, change; Chr, chromosome; CI, confidence interval; CpG, Cytosine-phosphate-Guanine; d, days; PM_{10}, PM < 10 μm in diameter; $PM_{2.5}$, PM < 2.5 μm in diameter; $PM_{2.5-10}$, PM > 2.5 and < 10 μm in diameter

[a]Build 37

[b]Absolute percentage point per 10 $\mu g/m^3$ increase in PM_{10}

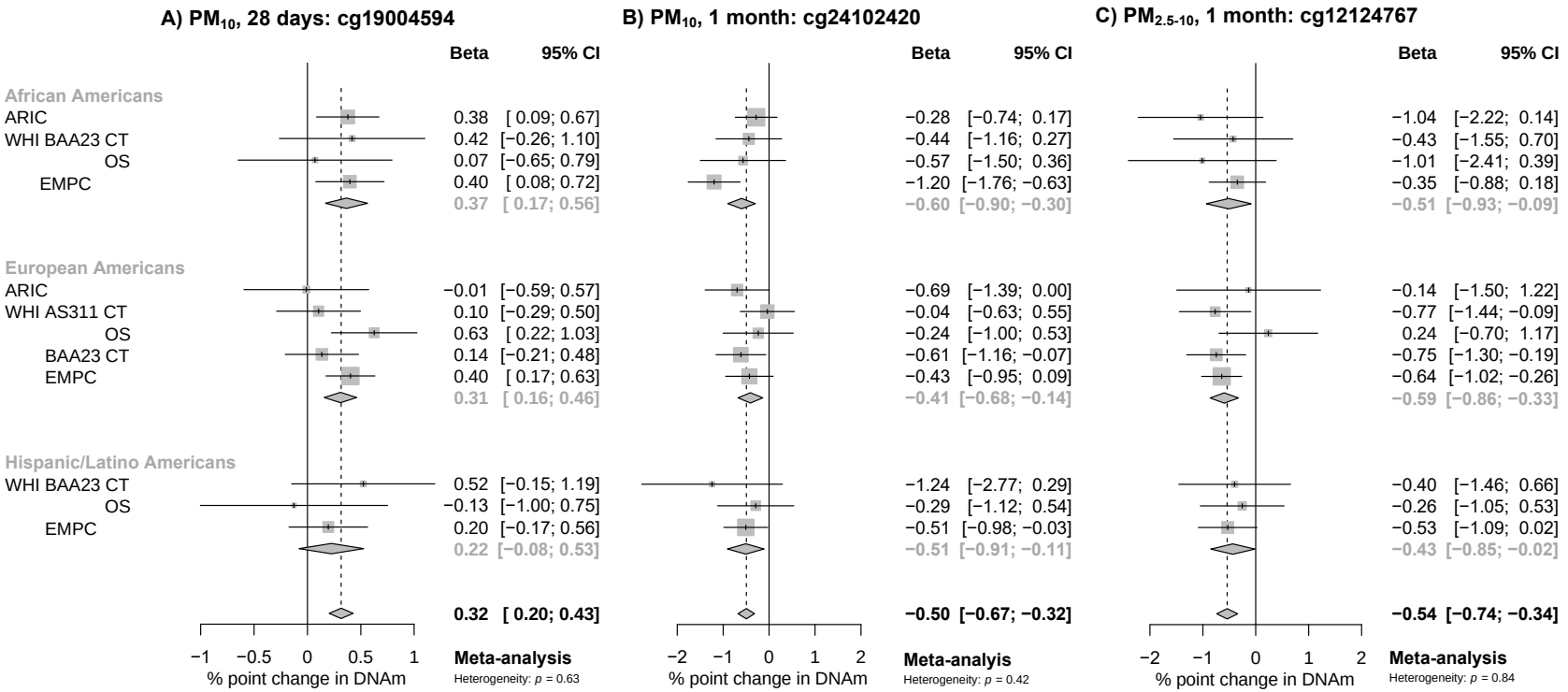

Figure 6-S1. Forest plots of PM-CpG associations (95% confidence intervals) for A) cg19004594, B) cg2410240, and C) cg12124767 with a 10 μg/m³ increase in PM by subpopulation and by race/ethnicity and overall after fixed-effects meta-analysis.

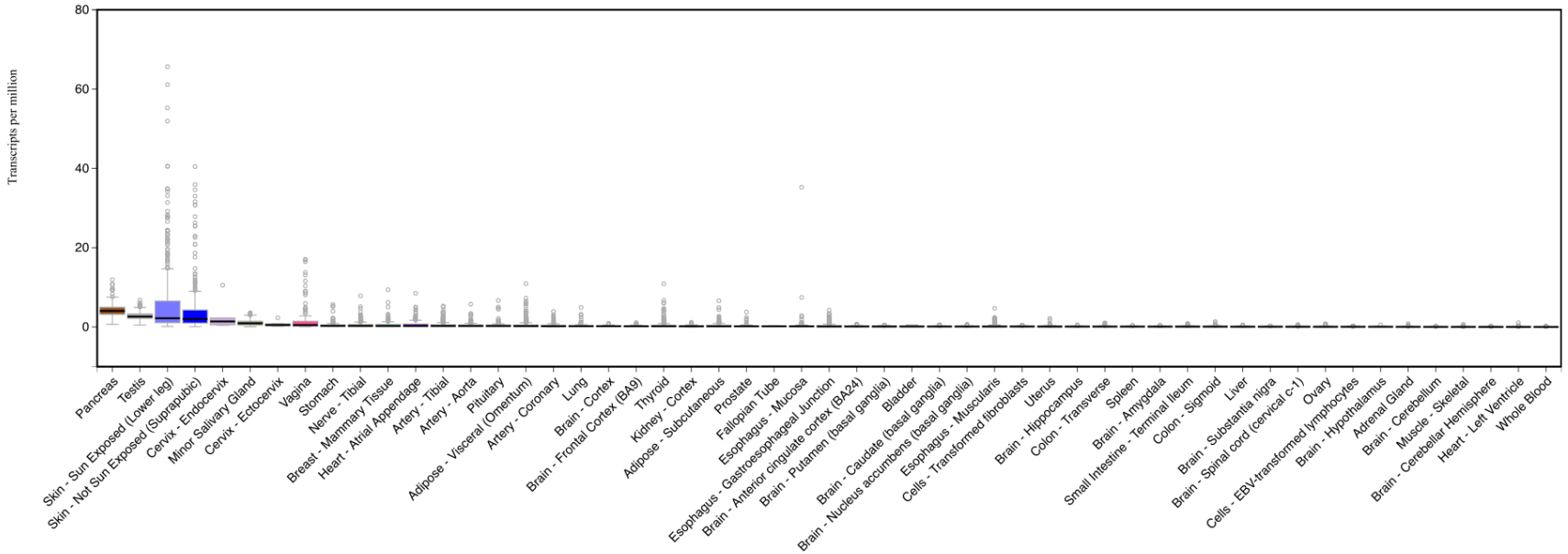

Figure 6-S2. Gene expression for *MATN4*

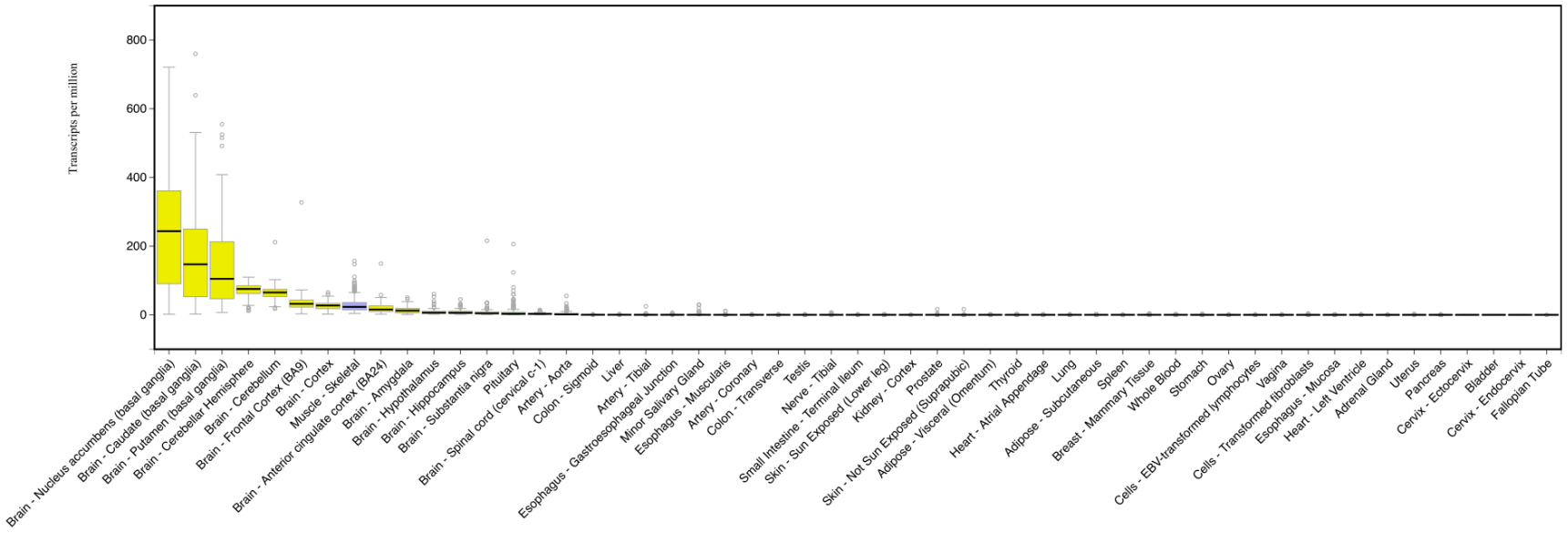

Figure 6-S3. Gene expression for *ARPP21*

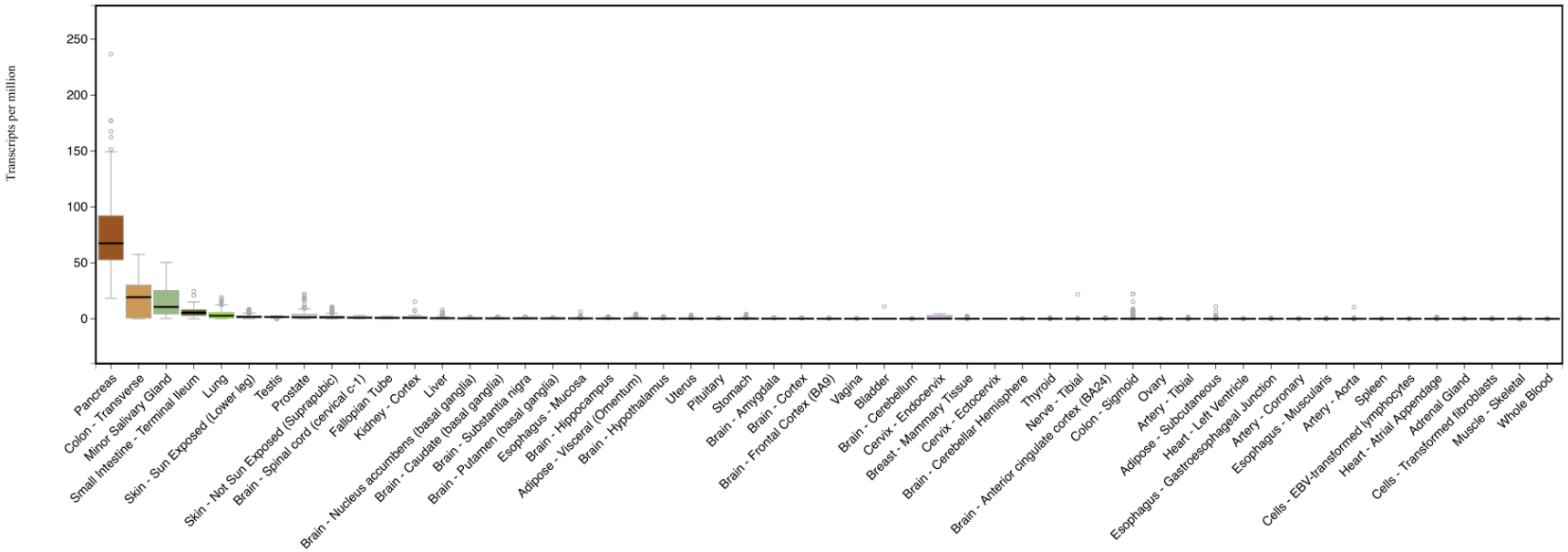

Figure 6-S4. Gene expression for *CFTR*

CHAPTER 7. EPIGENETICALLY MEDIATED ELECTROCARDIOGRAPHIC MANIFESTATIONS OF SUB-CHRONIC EXPOSURES TO AMBIENT PARTICULATE MATTER AIR POLLUTION

A. Overview

Short-duration exposure to ambient particulate matter (PM) air pollution is associated with cardiac autonomic dysfunction and prolonged ventricular repolarization. However, associations with sub-chronic exposures to coarser particulates are relatively poorly characterized as are molecular mechanisms underlying their potential relationships with cardiovascular disease. We therefore estimated associations between monthly mean concentrations of PM < 10μm and 2.5-10μm in diameter ($PM_{10;}$ $PM_{2.5\text{-}10}$) with time-domain measures of heart rate variability (HRV) and QT interval duration (QT) among U.S. women and men in the Women's Health Initiative and Atherosclerosis Risk in Communities Study (n_{HRV} = 82,107; n_{QT} = 76,711). Then we examined mediation of the PM-HRV and PM-QT associations by DNA methylation (DNAm) at three Cytosine-phosphate-Guanine (CpG) sites (cg19004594, cg24102420, cg12124767) with known sensitivity to monthly mean PM concentrations in a subset of the participants (n_{HRV} = 7,169; n_{QT} = 6,895). After multiply imputing missing data, we estimated associations using attrition-weighted, linear, mixed, longitudinal models adjusting for sociodemographic, behavioral, meteorological, and clinical characteristics. We assessed mediation by estimating the proportions of PM-HRV and PM-QT associations mediated by DNAm. Overall, we found little evidence of PM-HRV association, PM-QT association, or mediation by DNAm. The findings suggest that among racially / ethnically and environmentally diverse U.S. populations, sub-chronic exposures to coarser particulates may not exert

appreciable, epigenetically mediated effects on cardiac autonomic function or ventricular repolarization, although further investigation of shorter duration exposures to finer particulates and non-electrocardiographic outcomes among relatively susceptible populations is warranted.

B. Introduction

Exposure to ambient particulate matter (PM) air pollution has been consistently associated with increases in cardiovascular disease (CVD) risk.[6,7,12] For example, short-duration exposures to PM have been associated with decreased heart rate variability (HRV)[239,241,242] and increased QT interval duration (QT)[319,321,325], both of which are established cardiovascular disease risk factors.[202,226,227,291,293,295,296,488] However, most epidemiologic studies of PM, HRV and QT rely on short-duration (≤ 2-day) exposure averaging and electrocardiographic recordings. Moreover, studies of longer (monthly) exposures to coarser particulates, i.e. PM ≤ 10 and 2.5-10 μm in diameter (PM_{10}; $PM_{2.5\text{-}10}$) remain uncommon.

Although molecular mechanisms underlying PM-associated effects also remain inadequately characterized to date, methylation of deoxyribonucleic acids (DNAm) at Cytosine-phosphate-Guanine (CpG) sites is an environmentally modifiable process by which epigenetic modifications may affect gene expression, cardiac electrophysiology, and their electrocardiographic manifestations.[105,108,109,173,174] Indeed, we recently discovered that DNAm was associated with higher monthly mean PM_{10} and $PM_{2.5\text{-}10}$ concentrations at three PM-sensitive CpG sites annotated to neurological, pulmonary, endocrine, and / or cardiovascular disease-related genes (*MATN4*; *ARPP21*; *CFTR*) that can affect cardiac electrophysiology.[489] However, the actual role of DNAm at these sites in PM-associated, quantitative electrocardiographic traits is unclear.

In the present study, we therefore estimated the associations between monthly mean ambient PM_{10} and $PM_{2.5\text{-}10}$ concentrations, HRV, and QT in two large, racially, ethnically, and

geographically diverse U.S. populations enrolled in the Women's Health Initiative (WHI) and the Atherosclerosis Risk in Communities study (ARIC), then examined mediation of the monthly mean PM-HRV and PM-QT associations by DNAm.

C. Methods

C1. Study populations

The WHI is a multicenter, prospective study of risk factors for cardiovascular disease, breast / colorectal cancer, and osteoporotic fractures.[197,341] From forty clinical centers throughout the U.S., postmenopausal women aged 50-79 years were either randomized in the Clinical Trials (CT, n = 68,132) or enrolled in the Observational Study (OS, n = 93,676) between 1993 and 1998. The WHI CT included three interventions: hormone therapy (i.e. estrogen with or without progestin), calcium and vitamin D supplementation, and dietary modification. The WHI OS[197,341] recruited participants interested in the dietary modification or hormone therapy trials of the WHI CT, but were otherwise ineligible, unwilling, or unresponsive to a direct invitation.

All WHI participants completed a baseline screening visit (SV; 1993-1998) at which demographic, socioeconomic, behavioral, and medical information was collected by trained and certified staff. WHI CT participants also completed annual visits three, six, and nine years after randomization (AV3, AV6, AV9; 1996-2005) and WHI OS participants three years after enrollment (AV3). A resting, supine, ten-second, standard twelve-lead electrocardiogram (ECG) was collected at each visit in the WHI CT and an ambulatory, 24-hour, three-lead ECG was collected at the baseline exam of the *Myocardial Ischemia and Migraine Study*[344] (MIMS, n = 3,369), an ancillary study of WHI OS participants enrolled by ten clinical centers (SV or AV3; 1997-2000).

The ARIC study is a prospective, epidemiologic study of atherosclerosis and CVD in four U.S. communities: Washington County, Maryland; Forsyth County, North Carolina; selected

suburbs of Minneapolis, Minnesota; and Jackson, Mississippi.[196] Participants were selected as a community-stratified probability sample of 15,792 African- and European-American men and women aged 45-64 years. Participants completed a baseline visit (V1; 1987-1989) and follow-up visits (V2-V4; 1990-1998) at which resting, supine, ten-second, standard twelve-lead ECGs and demographic, socioeconomic, behavioral, and medical information were collected by trained and certified staff.

Three WHI CT subpopulations contributed DNAm data to the present study: *Epigenetic Mechanisms of PM-Mediated CVD Risk* (WHI-EMPC; n = 2,200)[345], *Broad Agency Announcement 23* (WHI-BAA23; n = 1,546)[346], and *Ancillary Study 311* (WHI-AS311; n = 405)[351]. WHI-EMPC is study of epigenetic mechanisms underlying associations between PM and CVD within randomly selected participants at the SV, AV3, or AV6. WHI-BAA23, also known as *Integrative Genomics and Risk of CHD and Related Phenotypes in the Women's Health Initiative*, is a case-control study of coronary heart disease. By design, WHI-BAA23 oversampled African Americans and Hispanic/Latino Americans and required all participants to have undergone genome-wide genotyping and profiling of seven CVD biomarkers. DNAm was measured in blood collected at the SV, before the incidence of coronary heart disease. WHI-AS311, also known as the *Bladder Cancer and Leukocyte Methylation* study, is a nested case-control study of bladder cancer. Bladder cancer cases were matched to controls based on enrollment year, age at enrollment, follow-up time, and DNAm extraction method. DNAm was measured in blood collected at the SV, before the incidence of bladder cancer. Two ARIC subpopulations also contributed DNAm data to the present study, one involving African Americans (ARIC-AA; n = 2,796) from Forsyth County or Jackson with DNA and another involving European Americans (ARIC-EA; n = 1,139) from Forsyth County or Minneapolis with

DNA and cerebral magnetic resonance imaging data[352], all at Visits 2 (1990-1992) or 3 (1993-1995).

C2. Heart rate variability and QT interval duration measurement

In the WHI CT and ARIC, ten-second, resting, supine, standard twelve-lead ECGs[363,364] were recorded by MAC PCs (MAC PC, GE Marquette Electronics Inc., Milwaukee, WI), then transmitted to a central laboratory (Epidemiological Cardiology Research Center, Wake Forest School of Medicine, Winston-Salem, NC) for visual inspection, identification of technical errors / inadequate quality, and analysis using the 2001 version of the GE Marquette 12-SL program (GE Marquette, Milwaukee, WI). HRV and QT were reliably measured from ECGs in the WHI CT and ARIC[195,287]. The measures included the mean RR interval duration (RR, ms), i.e. unit-corrected inverse of mean heart rate; standard deviation of normally conducted RR intervals (SDNN, ms); square root of mean squared differences in successive, normally conducted RR intervals (RMSSD, ms); and median QT (ms) from orthogonal XYZ leads. In WHI MIMS, ambulatory, 24-hour, three-lead (Holter) ECGs were digitally recorded (Zymed Model 3100–001) then RR and SDNN were measured from them.

C3. Particulate matter exposure estimation

The study focused on ambient PM_{10} and (coarse) $PM_{2.5\text{-}10,}$ the first of which is regulated under the Clean Air Act by the U.S. Environmental Protection Agency (EPA).[11] Daily mean PM_{10} concentrations ($\mu g/m^3$) were spatially estimated at all geocoded participant addresses[353,354] using U.S. EPA Air Quality System (AQS) data and national-scale, log-normal ordinary kriging. Daily mean concentrations of PM_{10} were averaged over 28 days prior to and including the day of the study visit.

Because EPA AQS monitoring data for $PM_{2.5}$ were not widely available until 1999, geocoded participant address-specific monthly mean PM_{10} and $PM_{2.5}$ concentrations ($\mu g/m^3$) also

were spatiotemporally estimated using generalized additive mixed models and geographic information system-based predictors. Spatiotemporal estimation involved the log-transformed ratio of $PM_{2.5}$ to predicted PM_{10} between 1987 and 1999 [359]. Monthly mean concentrations of $PM_{2.5-10}$ concentrations were defined as the differences between PM_{10} and $PM_{2.5}$ concentrations.

C4. DNA methylation

Peripheral blood leukocytes were isolated from visit-specific, fasting blood drawn from study participants in WHI-EMPC, WHI-BAA23, WHI-AS311, ARIC-AA, and ARIC-EA. DNA was extracted from the peripheral blood leukocytes and then DNAm was measured on a methylome-wide scale at 485,577 potentially relevant Cytosine-phosphate-Guanine (CpG) sites using the Illumina 450K Infinium Methylation BeadChip (Illumina Inc.; San Diego, CA, USA). Methylation was quantitatively represented by beta, the proportion of methylated cytosines over the sum of methylated and unmethylated cytosines. The data were quality-controlled, Beta Mixture Quantile (BMIQ)-normalized to adjust for probe bias [360], and in WHI-EMPC, ComBat-adjusted for stage and plate using empirical Bayes methods.[450] Otherwise, WHI-AS311 control matching criteria (enrollment year, age at enrollment, follow-up time, DNAm extraction method) were available to control for variation in study design; technical covariates (assay plate, chip, and row) to control for batch effects; and leukocyte (CD8+ T cell, CD4+ T cell, B cell, natural killer cell, monocyte, and granulocyte) proportions to adjust for leukocyte composition [179]. Analyses focused on DNAm at three CpG sites previously identified as PM-sensitive: cg19004594, cg24102420, and cg12124767.[489]

C5. Covariates

Demographic, socioeconomic, behavioral, and meteorological covariates included clinical center, visit, race/ethnicity, age (years), individual-level education (high school education or lower, more than high school), neighborhood socioeconomic status [365], smoking

status (current, former, never), alcohol use (current, former, never), body mass index (BMI, kg/m^2), physical activity (MET-hours/week), mean temperature (°C), mean dew point (°C), mean barometric pressure (kPa), and season (using sine/cosine functions).[366] Clinical covariates included coronary heart disease (CHD: anti-anginal medication use; history of angina, myocardial infarction, or coronary artery revascularization; or interim CHD presentation, based on physician review of medical records, incident event classification, and adjudication), diabetes (anti-diabetic medication use; history; or in ARIC, fasting glucose ≥ 126 mg/dL), hyperlipidemia (anti-hyperlipidemic medication use; history; or in ARIC, total cholesterol > 240 mg/dL), hypertension (anti-hypertensive medication use, history, systolic blood pressure ≥ 140 mmHg, or diastolic blood pressure ≥ 90 mmHg), chronic lung disease (history of asthma, emphysema, or lung cancer), and heart failure (HF: cardiac glycoside and loop or potassium-sparing diuretic use; history of HF; or interim HF presentation, based on physician review of medical records, incident event classification, and adjudication). Subpopulation-specific covariates included sex (in ARIC), randomly assigned treatment group (in WHI), case-control status (in WHI-AS311 and WHI-BAA23), and other sampling-related variables in WHI-AS311 (enrollment year, age at enrollment, follow-up time, DNAm extraction method).

C6. Exclusions

Of all observations in WHI and ARIC with ECG data (n = 234,344), 2% made on participants at a WHI clinical center outside of the contiguous 48 states and 3% with conditions affecting the availability or accuracy of ECG measures (electronic pacers; poor quality grades; Wolff Parkinson White syndrome; atrial fibrillation; atrial flutter; atrioventricular block; antiarrhythmic medication) were excluded. HRV analyses excluded an additional 1% of observations made on participants with ventricular or supraventricular tachycardia, supraventricular rhythm, pauses, < 5 or 50% normal-to-normal RR intervals, or ventricular

ectopy. QT analyses excluded an additional 7% of observations made on participants with heart failure or QRS interval > 120 ms.

C7. Multiple imputation

To avoid potential for selection bias in complete-data analyses when data are missing at random[370], multivariate imputation by chained equations (MICE)[371,372] was used to impute missing data (range: 0.1% - 6.0%). Binary and categorical data were imputed using the logistic and discriminant functions whereas interval-scale data were imputed using predictive means matching.

C8. Attrition weights

To address potential bias due to non-random attrition over time in longitudinal analyses, stabilized inverse probability of attrition weights for each participant were calculated at each examination using logistic regression, where the numerator was the marginal probability of the participant not being lost to follow-up at an examination and the denominator was the probability of the participant not being lost to follow-up at an examination conditional on their covariate patterns at the prior examination.[373]

C9. Statistical analysis: PM-HRV and PM-QT associations

In each subpopulation, the right-skewed HRV measures were log-transformed, then attrition-weighted, covariate-adjusted, multi-level, linear, mixed-effects models were used to estimate PM-HRV and PM-QT associations. In the WHI CT, three-level, longitudinal models had a random intercept for examination at the participant level and a random intercept and slope for PM at the clinical center level, as given by

$$ECG_{ijk} = \beta_0 + \beta_1 PM_{ijk} + \beta_3 Z_{ijk} + b_{0k}^{C} + b_{1k}^{C} PM_{ijk} + b_{0jk}^{P} + \varepsilon_{ijk}^{E} \quad (22)$$

In ARIC, two-level, longitudinal models adjusted for clinical center as a fixed effect and had a random intercept for examination at the participant level, as given by

$$(23) \qquad ECG_{ij} = \beta_0 + \beta_1 PM_{ij} + \beta_3 Z_{ij} + b_{0j}^{P} + \varepsilon_{ij}^{E}$$

In WHI-MIMS, two-level, cross-sectional models had a random intercept and slope for PM at the clinical center level, as given by

$$(24) \qquad ECG_{ik} = \beta_0 + \beta_1 PM_{ik} + \beta_3 Z_{ik} + b_{0k}^{C} + b_{1k}^{C} PM_{ik} + \varepsilon_{ik}^{E}$$

where i, j, and k denote the i^{th} examination (level 1) of the j^{th} participant (level 2) in the k^{th} clinical center (level 3); ECG is a measure of RR, SDNN, RMSSD, or QT; β_0 is the intercept; PM is 28-day or 1-month mean PM_{10} or 1-month mean $PM_{2.5\text{-}10}$; and Z is a vector of covariates. The terms $(b_0^C, b_1^C) \sim N(O, G)$ are a random intercept and a random slope for PM at the clinical center level, $(b_0^P) \sim N(O, G)$ is a random intercept for examination at the participant level, and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level.

Measures of association (β_1) and 95% confidence intervals (CI) were reported as millisecond changes (Δ, ms) in QT analyses and percent changes $(\Delta, \%)$ in log-transformed HRV analyses, per 10 μg/m^3 increase in PM, where

$$\Delta, \% = 100\left(10^{10\beta_1} - 1\right), 95\%\ CI: 100\left(10^{10(\beta_1 \pm 1.96SE)} - 1\right).$$

Subpopulation-specific measures of Δ and their 95% CIs were combined in fixed-effects inverse variance-weighted meta-analyses[369] after testing homogeneity of associations ($P_{Cochran's\ Q} < 0.10$).[374]

C10. Statistical analysis: mediation

Mediation analyses were implemented in subpopulations with available DNAm and ECG data: WHI-EMPC, WHI-BAA23 CT, ARIC-AA, and ARIC-EA. All mediation analysis models were subpopulation-stratified and covariate-adjusted. Standard errors were estimated in 500 bootstrapped samples. Subpopulation-specific results were then combined using fixed-effects, inverse variance-weighted meta-analysis after testing homogeneity of associations ($P_{Cochran's\ Q} < 0.10$).[374]

A detailed description of the mediation analysis is reported in the Supplement. Briefly, associations of 28-day mean PM_{10}, 1-month mean PM_{10}, and 1-month mean $PM_{2.5\text{-}10}$ with DNAm at cg19004594, cg24102420, and cg12124767 were estimated. Estimated PM-DNAm (exposure-mediator) associations and their 95% CIs were reported as absolute percentage changes ($\Delta, \%$) in DNAm per 10 μg/m³ increase in PM. Then associations between DNAm and ECG measures were estimated. Estimated DNAm-ECG measure (mediator-outcome) associations and their 95% CIs were reported as millisecond changes (Δ, ms) in QT analyses and percent changes ($\Delta, \%$) in HRV analyses, per 10% increase in DNAm. Lastly, for CpG sites at which methylation was associated with at least one ECG trait and one PM exposure after Bonferroni correction ($P < 0.016$; $P_{Cochran's\ Q} < 0.10$), mediation methods[180,392,393] were used to decompose the total effect (TE) of PM on the ECG measure into its natural direct effect (NDE), i.e. effect of PM on the ECG measure independent of DNAm; and natural indirect effect (NIE), i.e. mediated effect of PM on the ECG measure through DNAm; where the sum of NDE and NIE is the TE. If the NDE and NIE were both positive or both negative (i.e. identically signed), the proportion mediated (%) was estimated as the NIE divided by the TE.[394,395]

C11. Statistical analysis: sensitivity

All PM-HRV and PM-QT models adjusted for race/ethnicity, age, sex (in ARIC), randomly assigned treatment group (in WHI), study visit, mean temperature (°C), mean dew point (°C), mean barometric pressure (kPa), season, and RR (in QT analyses). Model 2 additionally adjusted for other potential confounders (individual-level education; neighborhood socioeconomic status); Model 3, for variables that explain variation in ECG traits or may account for residual confounding (smoking status; alcohol use; BMI; physical activity); and Model 4, for health conditions (coronary heart disease; diabetes; hyperlipidemia; hypertension; chronic lung disease; heart failure, in HRV analyses). Model 5 also assessed sensitivity of $PM_{2.5\text{-}10}$ results from Model 4 to additional adjustment for 1-month mean $PM_{2.5}$ concentrations. Mediation models relied on Model 4 adjustments plus methylation-related variables (ten principal components for genetic ancestry, when available; leukocyte proportions; technical covariates) and subpopulation-specific covariates including case-control status (WHI-AS311; WHI-BAA23) and case selection criteria (AS311; enrollment year; age at enrollment; follow-up time; DNAm extraction method).

D. Results

Of the 82,107 and 76,711 participants included in analyses of HRV and QT, 91% (72,820 and 69,857) had baseline data after exclusions. On average at baseline, participants were aged 61 years, mostly female (91%), white (82%), more than high school educated (70%), never smokers (49%) and current alcohol users (68-69%). Mean physical activity and BMI were 10.6 MET-hours/week and 28.6 kg/m^2 (Table 7-1). Participants with DNAm data (n_{HRV} = 7,169; n_{QT} = 6,895; Table 7-S1) were less likely to be female (81%), white (46%), more than high school educated (55%) and current alcohol users (50%). RR was relatively low and SDNN, high in the WHI MIMS subpopulation with longer duration ECGs. QT was relatively high in the ARIC

subpopulations. In all subpopulations, monthly mean PM_{10} concentrations were below EPA National Ambient Air Quality Standards (NAAQS) for 24-hour and annual mean PM_{10} in place during the study period, i.e. ≤ 150 and ≤ 50 $\mu g/m^3$. [11]

After meta-analysis, PM-HRV associations were mostly homogenous among subpopulations ($P_{Cochran's}$ $Q < 0.10$) and generally null among Models 1-4, varying only slightly among exposures and HRV measures (Figure 7-1A-C). For example in Model 4, SDNN was 1.0 ms (-0.1, 2.0) higher per 10 $\mu g/m^3$ increase in $PM_{2.5-10}$ concentration (Table 7-2), but the estimate fell to 0.7 ms (-0.4, 1.8) after adjusting for 1-month mean $PM_{2.5}$ concentration in Model 5. Although RR also was -0.8% (-1.6%, 0.0%) and -1.2% (-2.1%, -0.2%) lower per 10 $\mu g/m^3$ increase in 1-month PM_{10} and $PM_{2.5-10}$ concentrations in WHI-MIMS participants with 24-hour ECGs, meta-analyses combining information on ARIC and WHI-CT participants with ten-second ECGs also attenuated these estimates. Moreover, QT was -0.2 ms (-0.3, 0.0) and -0.4 ms (-0.6, -0.1) lower per 10 $\mu g/m^3$ increase in 28-day and 1-month mean PM_{10} (Figure 7-1D; Table 7-2). Results for SDNN and RMSSD with were robust to additional adjustment for RR (data not shown).

In participants with available DNAm and HRV data, DNAm was 0.2% (0.1%, 0.3%) higher at cg19004594, -0.4% (-0.6%, -0.2%) lower at cg24102420, and -0.3% (-0.5%, 0.0%) lower at cg12124767 per 10 $\mu g/m^3$ increase in 28-day mean PM_{10}, 1-month mean PM_{10}, and 1-month mean $PM_{2.5-10}$, respectively, (Table 7-3). Estimates were similar in participants with available DNAm and QT data. DNAm associations with RR, SDNN, RMSSD, and QT did not meet statistical significance at $\alpha = 0.016$; however, SDNN was 3.9% (-0.2%, 8.2%; $P = 0.6$) higher and QT was -0.9 ms (-2.0, 0.2; $P = 0.09$) lower per 10% increase in DNAm at cg24102420 (Table 7-4). Estimates of natural indirect (i.e. DNAm-mediated) effects of PM on

the ECG measures and proportions mediated by DNAm were imprecise and non-significant (Table 7-5).

E. Discussion

This multi-center, longitudinal study represents the culmination of an innovative attempt to examine epigenetically mediated electrocardiographic effects of PM in a racially, ethnically and environmentally diverse population of U.S. women and men. Sound motivation for that attempt was provided by the recent identification of PM-sensitive epigenomic loci capable of affecting cardiac electrophysiology in the same populations.[489] Despite that motivation, we found little evidence of PM-HRV association, PM-QT association, or mediation by DNAm in the present study. Indeed, the findings suggest that sub-chronic exposures to coarser particulates may not exert appreciable or epigenetically mediated effects on cardiac autonomic function or ventricular repolarization.

The lack of an observed PM-HRV association in this context is at odds with evidence of negative associations with shorter duration exposures to ambient $PM_{2.5}$, $PM_{10,}$ and $PM_{2.5-10}$ in a variety of other settings. For example, a large meta-analysis of PM-HRV associations found that RMSSD and SDNN was 0.1% to 2.0% lower per 10 $\mu g/m^3$ increase in 2-hour to 3-day mean $PM_{2.5}$ or PM_{10} concentrations.[239] Two, small-scale controlled exposure panel studies of shorter duration $PM_{2.5-10}$-HRV associations also found similarly inverse associations.[243,246] Although studies of longer duration exposures to PM are limited, results from the Multi-Ethnic Study of Atherosclerosis (MESA) and Normative Aging Study (NAS) of monthly and yearly exposures to ambient $PM_{2.5}$ and $PM_{2.5-10}$ also identified only slightly negative to slightly positive associations with HRV[200,249,490]. In jointly suggesting that cardiac autonomic function as measured by brief ECG recordings may well be more sensitive to acute than sub-chronic PM exposure,[249] these studies offer a plausible explanation for the absence of PM-HRV association herein.

Lack of population-wide susceptibility to PM effects in WHI and ARIC provides an equally plausible explanation for the absence of an observed PM-HRV association. Indeed, a Swiss study of middle-aged adults found that 10-year exposures to PM_{10} were associated with lower HRV *only* among participants taking angiotensin-converting enzyme inhibitors, suggesting that underlying health conditions or their treatments may confer susceptibility.[250] Susceptibility to shorter duration $PM_{2.5}$- and PM_{10}-associated decreases in HRV also have been observed in e.g. elderly adults with cardiovascular conditions[251] as well as middle-aged adults with hypertension[242], diabetes[241], or metabolic syndrome.[200] Other susceptible groups have been identified in small-scale studies of $PM_{2.5-10}$, including elderly adults[245,491] and populations with asthma[244] or coronary heart disease.[245]

Scant evidence of PM-QT association in this study also may be related to its explicit focus on exposures to PM_{10} and $PM_{2.5-10}$ in a racially, ethnically and environmentally diverse population. In prior studies, for example, an array of shorter[321,323] to longer duration[319,325] $PM_{2.5}$ exposures have been consistently associated with higher QT. Notable in this regard is the 7.0 ms per 3.4 $\mu g/m^3$ increase in 28-day mean $PM_{2.5}$ in the NAS,[319] a geographically and demographically homogenous population, by comparison. Although shorter duration PM_{10} exposures also have been associated with QT-related risk of ventricular arrhythmias[24,30], generalizable results from epidemiologic studies of PM_{10}, $PM_{2.5-10}$, and QT remain relatively uncommon. Their rarity suggests that the study of epigenetically mediated, QT-prolonging effects of coarser particulates in diverse populations may be especially challenging.

Despite the challenge, the present study explored potential epigenetic mechanisms linking PM exposure to changes in autonomic function and ventricular repolarization by estimating associations between DNAm at cg19004594, cg24102420, and cg12124767 with

HRV and QT. Although DNAm at these CpG sites were associated with higher sub-chronic exposures to PM_{10} and $PM_{2.5-10}$—both herein and in a prior methylome-wide association study in the same population[489] —there was little evidence of DNAm-HRV, DNAm-QT, or as described above, PM-HRV or PM-QT association. Therefore, the study's mediation analyses yielded null results in this population.

Having said that, the results from this study may have been affected by missing data, participant attrition, outcome or exposure measurement error, and dependence on monthly mean PM concentrations. Potential for bias related to missingness and attrition was nevertheless reduced using conventional epidemiologic tools: multivariate imputation and inverse-probability weights. Time-domain measures of HRV and QT also are valid and reliable, even when based on resting, supine, ten-second, standard twelve-lead ECGs.[195,287] Furthermore, the accuracy of the study's geocoding[353,354] and validity of its PM estimation[355,359,406] have been demonstrated. Finally, shorter duration PM exposures may be more relevant to studies of cardiac autonomic function as measured by brief ECG recordings, but unlike monthly mean PM concentrations, they were not associated with DNAm in prior work.[489]

On the basis of the above, we therefore conclude that sub-chronic exposures to coarser particulates may not exert appreciable or epigenetically mediated effects on cardiac autonomic function and ventricular repolarization. Nevertheless, future investigation of the mechanisms underlying shorter duration exposures to finer particulates or non-electrocardiographic outcomes in relatively susceptible populations is warranted, given the preceding discussion. Such investigation may provide insight into epigenetic mechanisms linking PM with cardiovascular

disease, the existence of which may help substantiate the biological plausibility and causality of associations being considered by U.S. Environmental Protection Agency as it sets National Ambient Air Quality Standards for PM under the Clean Air Act.

F. Tables and Figures

Table 7-1. Characteristics of n_{HRV} = 72,820 / n_{QT} = 69,587 study participants at baseline, Women's Health Initiative Clinical Trials (1993-2005), Women's Health Initiative Myocardial Ischemia and Migraine Study (1993-2005), and Atherosclerosis Risk in Communities study (1986-1998)

Characteristic	Heart rate variability				QT Interval		
	WHI CT SV & ARIC V1 & WHI MIMS n = 72,820	WHI CT SV n = 55,906	WHI MIMS n = 2,196	ARIC V1 n = 14,718	WHI CT SV & ARIC V1 n = 69,857	WHI CT SV n = 55,651	ARIC V1 n = 14,206
Age (years), mean (SD)	61 (8)	63 (7)	65 (7)	54 (5.7)	61 (8)	63 (7)	54 (5.7)
Male, n (%)	6,585 (9)	0 (0)	0 (0)	6,585 (45)	6,383 (9)	0 (0)	6,383 (45)
Race / ethnicity, n (%)							
American Indian or Alaskan Native	245 (0)	237 (0)	8 (0)	0 (0)	230 (0)	230 (0)	0 (0)
Asian or Pacific islander	523 (1)	502 (1)	21 (1)	0 (0)	508 (1)	508 (1)	0 (0)
Black or African American	9,327 (13)	5,242 (9)	159 (7)	3,926 (27)	8,855 (13)	5,128 (9)	3,727 (26)
Hispanic/Latino	2,464 (3)	2,399 (4)	65 (3)	--[a]	2,391 (3.4)	2,391 (4)	--[a]
Other	526 (1)	500 (1)	26 (1)	0 (0)	494 (1)	494 (1)	0 (0)
White (not of Hispanic origin) or European American	59,611 (82)	46,906 (84)	1,913 (87)	10,792 (73)	57,259 (82)	46,780 (84)	10,479 (74)
More than high school, n (%)	50,546 (70)	42,297 (76)	1,772 (81)	6,477 (44)	48,546 (70)	42,213 (76)	6,333 (45)
Smoking status, n (%)							
Never	35,560 (49)	28,266 (51)	1,160 (53)	6,134 (42)	34,050 (49)	28,084 (51)	5,966 (42)
Former	28,305 (39)	22,656 (41)	903 (42)	4,746 (32)	27,124 (39)	22,582 (41)	4,542 (32)
Current	8,275 (12)	4,350 (8)	101 (5)	3,824 (26)	8,049 (12)	4,365 (8)	3,684 (26)
Alcohol use, n (%)							
Never	9,537 (13)	5,661 (10)	233 (11)	3,643 (25)	9,120 (13)	5,606 (10)	3,514 (25)
Former	13,291 (18)	10,075 (18)	446 (21)	2,770 (19)	12,394 (18)	9,795 (18)	2,599 (18)
Current	49,465 (68)	39,720 (72)	1,497 (69)	8,248 (56)	47,845 (69)	39,806 (72)	8,039 (57)
Physical activity (MET-hours/week), mean (SD)	10.6 (12.6)	10.6 (12.5)	13.7 (14.0)	10.2 (12.8)	10.6 (12.6)	10.7 (12.5)	10.3 (12.8)

Characteristic	Heart rate variability				QT Interval		
	WHI CT SV & ARIC V1 & WHI MIMS n = 72,820	WHI CT SV n = 55,906	WHI MIMS n = 2,196	ARIC V1 n = 14,718	WHI CT SV & ARIC V1 n = 69,857	WHI CT SV n = 55,651	ARIC V1 n = 14,206
Body mass index (kg/m^2), mean (SD)	28.6 (5.8)	28.9 (5.9)	27.2 (5.7)	27.7 (5.4)	28.6 (5.7)	28.9 (5.8)	27.5 (5.2)
Clinical characteristics, n (%)							
Hypertension	30,570 (42)	25,612 (46)	1,033 (47)	3,920 (27)	28,184 (40)	24,861 (45)	3,323 (23)
Hyperlipidemia	10,332 (15)	6,794 (12)	407 (19)	3,640 (25)	10,056 (14)	6,576 (12)	3,480 (25)
Diabetes	4,428 (6)	3,491 (6)	142 (7)	805 (6)	3,940 (6)	3,245 (6)	695 (5)
Chronic lung disease	6,820 (9)	5,309 (10)	203 (9)	1308 (9)	6,370 (9)	5,234 (9)	1,136 (8)
Coronary heart disease	4,353 (6)	3,375 (6)	160 (7)	818 (6)	3,585 (5)	2,951 (5)	634 (5)
Congestive heart failure	1,939 (3)	1,161 (2)	61 (3)	717 (5)	0 (0)	0 (0)	0 (0)
ECG traits (ms), mean (SD)							
RR	925 (127)[b] / 802 (94)[c]	925 (137)[b]	802 (94)[c]	928 (142)[b]	926 (138)[b]	925 (137)[b]	929 (141)[b]
SDNN	20 (16)[b] / 116 (32)[c]	20 (16)[b]	116 (32)[c]	22 (16)[b]	20 (16)[b]	20 (16)[b]	22 (16)[b]
RMSSD	22 (20)[b]	22 (21)[b]	--	24 (20)[b]	22 (21)[b]	22 (20.7)[b]	24 (20)[b]
QT	403 (30)[b]	402 (31)[b]	--	409 (28)[b]	403 (30)[b]	401 (30.3)[b]	408 (27)[b]
PM (μg/m3)							
PM_{10}, 28 days	29.9 (8.2)	27.5 (6.4)	30.5 (7.2)	39.1 (7.5)	29.8 (8.2)	27.5 (6.4)	39.1 (7.5)
PM_{10}, 1 month	21.4 (6.9)	20.5 (6.6)	24.4 (6.9)	25.1 (7.0)	21.3 (6.9)	20.5 (6.6)	25.1 (7.0)
$PM_{2.5-10}$, 1 month	8.7 (4.7)	8.6 (4.8)	6.3 (5.5)	10.0 (3.4)	8.8 (4.6)	8.59 (4.8)	10.0 (3.4)

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CT, clinical trials; METS, metabolic equivalent; MIMS, Myocardial Ischemia and Migraine Study; PM, particulate matter; PM10, PM < 10 μm in diameter; PM2.5-10, PM > 2.5 and < 10 μm in diameter; QT, QT interval; RMSSD, root mean square of successive differences between RR intervals; RR, RR interval; SD, standard deviation; SDNN, SD of normally conducted RR intervals; SV, screening visit; V1, visits 1; WHI, Women's Health Initiative

[a]ARIC recruitment and data collection occurred before the National Instutite of Health required collection of information about Hispanic/Latino ethnicity

[b]Based on 10-second ECGs in WHI CT and ARIC participants

[c]Based on 24-hour ECGs in WHI MIMS participants

Table 7-2. Stratified and meta-analyzed changesa in heart rate variability and QT interval duration per 10 μg/m^3 increase in PM concentrations among n_{HRV} = 82,107 / n_{QT} = 76,711 study participants, Women's Health Initiative (1993-2005) and Atherosclerosis Risk in Communities study (1986-1998)

Exposure	Subpopulation	RR			SDNN			RMSSD			QT		
		Δ %	95% CI	$P_{Cochran's\ Q}$	Δ %	95% CI	$P_{Cochran's\ Q}$	Δ %	95% CI	$P_{Cochran's\ Q}$	Δ ms	95% CI	$P_{Cochran's\ Q}$
PM_{10}, 28 days	ARIC	0.2	-0.2, 0.7		-0.5	-3.1, 2.3		-1.5	-4.1, 1.1		-0.1	-0.4, 0.1	
	WHI CT	0.0	-0.3, 0.2		-0.1	-0.8, 0.6		0.2	-0.6, 1.0		-0.2	-0.5, 0.1	
	WHI MIMS	0.0	-0.9, 0.8		1.1	-1.3, 3.5		--	--	--	--	--	--
	Pooled	**0.0**	**-0.2, 0.2**	**0.66**	**-0.0**	**-0.7, 0.6**	**0.62**	**0.0**	**-0.7, 0.8**	**0.23**	**-0.2**	**-0.3, 0.0**	**0.80**
PM_{10}, 1 month	ARIC	-0.2	-0.8, 0.4		1.0	-2.0, 4.1		-0.8	-3.7, 2.3		-0.5	-0.8, -0.2	
	WHI CT	0.0	-0.2, 0.2		0.0	-0.8, 0.8		0.0	-1.0, 1.0		-0.2	-0.5, 0.1	
	WHI MIMS	-0.8	-1.6, 0.0		-1.3	-4.5, 1.9		--	--	--	--	--	--
	Pooled	**-0.0**	**-0.2, 0.1**	**0.14**	**0.0**	**-0.8, 0.8**	**0.58**	**-0.1**	**-1.0, 0.9**	**0.64**	**-0.4**	**-0.6, -0.1**	**0.16**
$PM_{2.5-10}$, 1 month	ARIC	-0.6	-1.6, 0.4		1.1	-4.3, 6.7		-0.7	-6.0, 4.9		-0.3	-0.8, 0.2	
	WHI CT	0.2	-0.2, 0.6		1.1	-0.1, 2.3		0.6	-0.8, 2.0		0.1	-0.4, 0.6	
	WHI MIMS	-1.2	-2.1, -0.2		0.3	-2.5, 3.1		--	--	--	--	--	--
	Pooled	**-0.1**	**-0.5, 0.3**	**0.02**	**1.0**	**-0.1, 2.0**	**0.88**	**0.5**	**-0.9, 1.9**	**0.64**	**-0.1**	**-0.5, 0.3**	**0.28**

Abbreviations: Δ, change; ARIC, Atherosclerosis Risk in Communities; CI, confidence intervals; CT, clinical trials; MIMS, Myocardial Ischemia and Migraine Study; PM, particulate matter; PM10, PM < 10 μm in diameter; PM2.5, PM < 2.5 μm in diameter; PM2.5-10, PM > 2.5 and < 10 μm in diameter; QT, QT interval; RMSSD, root mean square of successive differences between RR intervals; RR, RR interval; SDNN, SD of normally conducted RR intervals; WHI, Women's Health Initiative

[a]Model 4: Adjusted for race/ethnicity, age, gender (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, individual-level education, neighborhood socioeconomic status, smoking status, alcohol use, body mass index, physical activity, hypertension, hyperlipidemia, diabetes, coronary heart disease, and coronary heart disease (in HRV analyses only), and RR interval (in QT analyses only)

Table 7-3. Meta-analyzed changesa in DNA methylation per 10 μg/m^3 increase in PM concentrations among n_{HRV} = 7,169 / n_{QT} = 6,895 study participants, Women's Health Initiative (1993-2005) and Atherosclerosis Risk in Communities study (1986-1998)

Exposure	CpG	Participants with HRV data (n = 7,169)				Participants with QT data (n = 6,895)			
		Δ %	95% CI	*P*	$P_{Cochran's\ Q}$	Δ (ms)	95% CI	*P*	$P_{Cochran's\ Q}$
PM_{10}, 28 days	cg19004594	0.2	0.1, 0.3	9.0E-04	0.16	0.2	0.1, 0.3	3.1E-04	0.25
PM_{10}, 1 month	cg24102420	-0.4	-0.6, -0.2	1.7E-04	0.82	-0.3	-0.5, -0.1	1.0E-03	0.74
$PM_{2.5-10}$, 1 month	cg12124767	-0.3	-0.5, -0.0	2.1E-02	0.51	-0.3	-0.5, -0.0	2.3E-02	0.38

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CI, confidence intervals; CpG, Cytosine-phosphate-Guanine site; PM, particulate matter; PM10, PM < 10 μm in diameter; PM2.5, PM < 2.5 μm in diameter; PM2.5-10, PM > 2.5 and < 10 μm in diameter; QT, QT interval; WHI, Women's Health Initiative

[a]Adjusted for race/ethnicity, age, gender (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, individual-level education, neighborhood socioeconomic status, smoking status, alcohol use, body mass index, physical activity, hypertension, hyperlipidemia, diabetes, coronary heart disease, and congestive heart failure (in the heart rate variability subset only)

Table 7-4. Meta-analyzed changesa in heart rate variability and QT interval duration per 10 percentage point increase in DNA methylation among n_{HRV} = 7,169 / n_{QT} = 6,895 study participants, Women's Health Initiative (1993-2005) and Atherosclerosis Risk in Communities study (1986-1998)

CpG	RR				RMSSD				SDNN				QT			
	Δ %	95% CI	*P*	$P_{Cochran's\ Q}$	Δ %	95% CI	*P*	$P_{Cochran's\ Q}$	Δ %	95% CI	*P*	$P_{Cochran's\ Q}$	Δ ms	95% CI	*P*	$P_{Cochran's\ Q}$
cg19004594	0.5	-0.8, 1.8	0.43	0.93	3.2	-2.7, 9.4	0.30	0.43	2.3	-3.3, 8.3	0.42	0.45	-0.7	-2.9, 1.4	0.41	0.14
cg24102420	0.0	-0.9, 0.9	0.98	0.75	2.0	-2.2, 6.4	0.35	0.74	3.9	-0.2, 8.2	0.06	0.88	-0.9	-2.0, 0.2	0.09	0.47
cg12124767	0.0	-0.9, 1.0	0.97	0.67	-3.0	-7.2, 1.5	0.19	0.97	-0.7	-4.9, 3.6	0.75	0.81	0.4	-0.8, 1.6	0.51	0.68

Abbreviations: Δ, change; ARIC, Atherosclerosis Risk in Communities; CI, confidence intervals; Cytosine-phosphate-Guanine site; PM, particulate matter; PM10, PM < 10 μm in diameter; PM2.5, PM < 2.5 μm in diameter; PM2.5-10, PM > 2.5 and < 10 μm in diameter; QT, QT interval; RMSSD, root mean square of successive differences between RR intervals; RR, RR interval; SDNN, SD of normally conducted RR intervals; WHI, Women's Health Initiative

[a]Model 4: adjusted for race/ethnicity, age, gender (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, individual-level education, neighborhood socioeconomic status, smoking status, alcohol use, body mass index, physical activity, hypertension, hyperlipidemia, diabetes, coronary heart disease, and coronary heart disease (in HRV analyses only), and RR interval (in QT analyses only)

Table 7-5. Analyses investigating the mediation of PM-HRV and PM-QT associations by DNA methylation among n_{HRV} = 7,169 / n_{QT} = 6,895 study participants, Women's Health Initiative (1993-2005) and Atherosclerosis Risk in Communities study (1986-1998)

			Natural direct effect			**Natural indirect effect**			**Proportion mediated[d]**
Exposure	CpG	ECG[a]	Estimate[a]	95% CI	*P*	Estimate[a]	95% CI	*P*	%
PM_{10}, 28 days	cg19004594		0.9	-0.2, 0.4	0.58	0.00	-0.02, 0.02	0.79	0
PM_{10}, 1 month	cg24102420	RR[b]	0.6	-0.2, 0.3	0.61	0.00	-0.02, 0.03	0.86	0
$PM_{2.5-10}$, 1 month	cg12124767		0.2	-0.2, 0.7	0.30	0.01	-0.04, 0.05	0.74	3
PM_{10}, 28 days	cg19004594		-0.1	-4.0, 3.9	0.95	0.00	-0.28, 0.29	0.98	--
PM_{10}, 1 month	cg24102420	SDNN[b]	3.9	0.1, 7.8	0.04	-0.10	-0.49, 0.29	0.61	--
$PM_{2.5-10}$, 1 month	cg12124767		3.6	-2.0, 9.5	0.21	0.08	-0.21, 0.36	0.60	2
PM_{10}, 28 days	cg19004594		0.6	-3.4, 4.7	0.78	0.03	-0.27, 0.32	0.86	5
PM_{10}, 1 month	cg24102420	RMSSD[b]	4.7	0.7, 8.8	0.02	-0.09	-0.48, 0.30	0.66	--
$PM_{2.5-10}$, 1 month	cg12124767		4.3	-1.7, 10.7	0.16	0.12	-0.20, 0.46	0.46	3
PM_{10}, 28 days	cg19004594		-0.5	-1.5, 0.5	0.32	0.00	-0.07, 0.08	0.90	--
PM_{10}, 1 month	cg24102420	QT[c]	-0.1	-1.0, 0.9	0.88	-0.01	-0.11, 0.09	0.86	12
$PM_{2.5-10}$, 1 month	cg12124767		0.4	-1.0, 1.8	0.55	-0.02	-0.13, 0.10	0.78	--

Abbreviations: ARIC, Atherosclerosis Risk in Communities; CI, confidence intervals; CpG, Cytosine-phosphate-Guanine site; PM, particulate matter; PM10, PM < 10 μm in diameter; PM2.5, PM < 2.5 μm in diameter; PM2.5-10, PM > 2.5 and < 10 μm in diameter; RMSSD, root mean square of successive differences between RR intervals; SDNN, SD of normally conducted RR intervals; WHI, Women's Health Initiative

[a]Model 4: adjusted for race/ethnicity, age, gender (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, individual-level education, neighborhood socioeconomic status, smoking status, alcohol use, body mass index, physical activity, hypertension, hyperlipidemia, diabetes, coronary heart disease, and coronary heart disease (in HRV analyses only), and RR interval (in QT analyses only)

[b]Unit of Estimate is % change (% Δ)

[c]Unit of Estimare is millisecond (ms)

[p]roportion mediated not estimated when the indirect effect and direct effects were oppositely signed

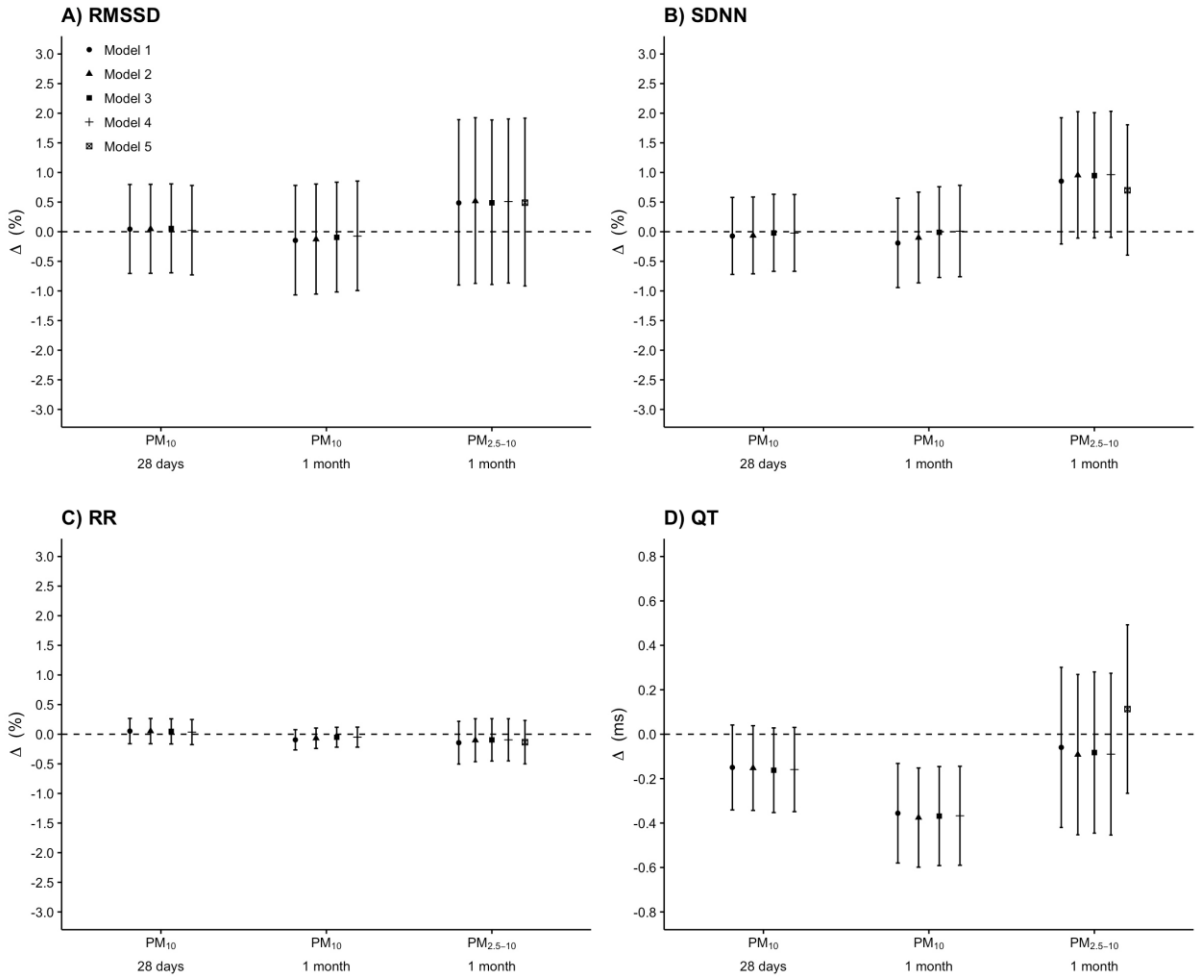

Figure 7-1. Pooled, adjusted changes in heart rate variability (Δ, %) and QT interval duration (Δ, ms) per 10 μg/m^3 increase in PM concentrations among n_{HRV} = 82,107 / n_{QT} = 76,711 study participants, Women's Health Initiative (1993-2005) and Atherosclerosis Risk in Communities study (1986-1998). Model 1 adjusted for race/ethnicity, age, sex (in ARIC), randomly assigned treatment group (in WHI), mean temperature, mean dew point, mean barometric pressure, season, and RR interval duration (for QT analyses). Model 2 adjusted for all covariates in Model 1 plus individual-level education and neighborhood socioeconomic status. Model 3 adjusted for all covariates in Model 2 plus smoking status, alcohol use, body mass index, and physical activity. Model 4 adjusted for all covariates in Model 3 plus coronary heart disease, diabetes, hyperlipidemia, hypertension, chronic lung disease, and congestive heart failure (in HRV analyses only). For only $PM_{2.5-10}$ analyses, Model 5 adjusted for all covariates in Model 4 plus 1-month mean concentrations of $PM_{2.5}$

G. Supplement

G1. Mediation methods

Mediation analyses involved three steps: 1) estimating exposure-mediator (PM-DNAm) associations, 2) estimating mediator-outcome (DNAm-ECG measure) associations, and 3) using mediation methods to estimate the natural direct effect (NDE) i.e. effect of PM on the ECG measure independent of DNAm; and the natural indirect effect (NIE), i.e. mediated effect of PM on the ECG measure through DNAm; where the sum of NDE and NIE is the TE. Then, the proportion mediated (%) was calculated as the NIE divided by the TE.

1) Estimating PM-DNAm associations

In each subpopulation, covariate-adjusted, multi-level, linear, mixed-effects models were used to estimate DNAm-PM associations. In WHI-EMPC, three-level, longitudinal models had a random intercept for examination at the participant level, a random intercept and slope and for PM at the WHI center level, and a random intercept for chip, as given by

$$DNAm_{ijk} = \beta_0 + \beta_1 PM_{ijk} + \beta_2 Z_{ijk} + b_{0k}^{C} + b_{1k}^{C} PM_{ijk} + b_{0jk}^{P} + b_{0ijk}^{E} + \varepsilon_{ijk}^{E}. \quad (25)$$

In WHI-BAA23, and WHI-AS311, two-level cross-sectional models had a random intercept and slope for PM at the WHI center level and a random intercept for plate and chip, as given by

$$DNAm_{ik} = \beta_0 + \beta_1 PM_{ik} + \beta_2 Z_{ik} + b_{0k}^{C} + b_{1k}^{C} PM_{ik} + b_{0ik}^{E} + \varepsilon_{ik}^{E}. \quad (26)$$

In ARIC-AA and ARIC-EA, one-level cross-sectional models had a random intercept for plate and chip, as given by

$$DNAm_{i} = \beta_0 + \beta_1 PM_{i} + \beta_2 Z_{i} + b_{0i}^{E} + \varepsilon_{i}^{E}. \quad (27)$$

Above, i, j and k denote the i^{th} examination of the j^{th} participant in the k^{th} center; $DNAm$ is the site-specific beta (i.e. DNAm) value from cg19004594, cg24102420, or cg12124767; β_0 is the intercept; PM is 28-day or 1-month mean PM_{10} or 1-month mean $PM_{2.5\text{-}10}$; and Z is a vector of covariates. The terms $(b_0^C, b_1^C) \sim N(O, G)$ are a random intercept and a random slope for PM at the center level, $(b_0^P) \sim N(O, G)$ is a random intercept for examination at the participant level, and $(b_0^E) \sim N(O, G)$ are random intercepts for technical covariates and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level. Measures of association (β_1) and their 95% confidence intervals ($\beta_1 \pm 1.96\ x\ standard\ error$) were reported as an absolute percentage change in DNAm ($\Delta, \%$) per 10 μg/m³ increase in PM. Stratum-specific results were combined using fixed-effects, inverse-variance weighted meta-analysis. Homogeneity of associations was assessed using Cochran's Q test statistic.[374]

2) Estimating DNAm-ECG measure associations

In each subpopulation, covariate-adjusted, linear mixed-effects models were used to estimate DNAm-ECG measure associations. In WHI-EMPC, two-level longitudinal models had a random intercept for examination at the participant level and a random intercept for chip, as given by

$$ECG_{ij} = \beta_0 + \beta_1 DNAm_{ij} + \beta_2 Z_{ij} + b_{0j}^P + b_{0ij}^E + \varepsilon_{ij}^E. \quad (28)$$

In WHI-BAA23, WHI-AS311, ARIC-AA, and ARIC-EA, one-level cross-sectional models of DNAm-HRV associations had a random intercept for plate and chip, as given by

$$ECG_i = \beta_0 + \beta_1 DNAm_i + \beta_2 Z_i + b_{0i}^E + \varepsilon_i^E. \quad (29)$$

where i and j denote the i^{th} examination (level 1) of the j^{th} participant (level 2); ECG is the QT interval or the log-transformed measure of RR, RMSSD, or SDNN from a 10-second ECG; β_0 is the intercept; $DNAm$ is the beta value at cg19004594, cg24102420, or cg12124767; and Z is a vector of covariates. The term $(b_0^P) \sim N(O, G)$ is a random intercept for examination at the participant level, $(b_0^E) \sim N(O, G)$ represents random intercepts for technical variables plate and/or chip, and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level. The measures of association (β_1) and 95% CIs $(\beta_1 \pm 1.96 Standard\ Error\ [SE])$ were reported as millisecond changes (Δ, ms) in QT interval duration and percent changes $(\Delta, \%)$ in HRV per 10% increase in DNAm.

3) Estimating NDE, NIE, TE, and proportion mediated

For each CpG site associated with at least one ECG trait and PM exposure after Bonferroni correction ($P < 0.016$; $P_{Cochran's\ Q} < 0.10$), mediation methods[180,392,393] were used to decompose the total effect (TE) between PM and the ECG measure into the NDE and DIE. The mediation effect estimation required models 1-3 (above) as well as models 6-8 described below:

In each subpopulation, covariate-adjusted, linear mixed-effects models were used to estimate adjusted PM-ECG measure and DNAm-ECG measure associations. PM x DNAm interactions were also assessed, while none were statistically significant at $P < 0.016$; the interaction terms were nonetheless included in mediation models. In WHI-EMPC, three-level longitudinal models had a random intercept for examination at the participant level, a random intercept and slope and for PM at the WHI center level, and a random intercept for chip, as given by

$$(30) \qquad ECG_{ijk} = \theta_0 + \theta_1 PM_{ijk} + \theta_2 DNAm_{ijk} + \theta_3 PM_{ijk} x DNAm_{ijk} + \theta_4 Z_{ijk} + b_{0k}^C + b_{1k}^C PM_{ijk} + b_{0jk}^P + b_{0ijk}^E + \varepsilon_{ijk}^E.$$

In WHI-BAA23 CT, and WHI-AS311 CT, two-level cross-sectional models had a random intercept and slope for PM at the WHI center level and a random intercept for plate and chip, as given by

$$(31) \qquad ECG_{ik} = \theta_0 + \theta_1 PM_{ik} + \theta_2 DNAm_{ik} + \theta_3 PM_{ik} x DNAm_{ik} + \theta_4 Z_{ik} + b_{0k}^{C} + b_{1k}^{C} PM_{ik} + b_{0ik}^{E} + \varepsilon_{ik}^{E}.$$

In ARIC-AA and ARIC-EA, one-level cross-sectional models had a random intercept for plate and chip, as given by

$$(32) \qquad ECG_i = \theta_0 + \theta_1 PM_i + \theta_2 DNAm_i + \theta_3 PM_i x DNAm_i + \theta_4 Z_i + b_{0i}^{E} + \in_{ik}^{E}.$$

where i, j and k denote the i^{th} examination (level 1) of the j^{th} participant (level 2) in the k^{th} center (level 3); ECG is the QT interval or the log-transformed measure of RR, RMSSD, or SDNN from a 10-second ECG; β_0 is the intercept; $DNAm$ is DNAm at a relevant CpG site; PM is 28-day or 1-month mean PM_{10} or $PM_{2.5\text{-}10}$; $PMxDNAm$ is the PM-DNAm interaction term; and Z is a vector of covariates. The terms $(b_0^C, b_1^C) \sim N(O, G)$ are a random intercept and a random slope for PM at center level, $(b_0^P) \sim N(O, G)$ is a random intercept for examination at the participant level, $(b_0^E) \sim N(O, G)$ represents random intercepts for technical covariates plate and/or chip, and $\varepsilon^E \sim (O, \sigma^2)$ is the random error at the examination level.

The NDE and NIE were estimated for a 10 μg/m^3 increase PM exposure using

$$(33) \qquad NDE = 10[\theta_1 + \theta_3(\beta_0 + \beta_2 Z)],$$

$$(34) \qquad NIE = 10(\beta_1\theta_2 + 10\beta_1\theta_3), \text{ and}$$

$$(35) \qquad TE = NDE + NIE$$

where β_1 denotes the PM coefficients in models of PM-DNAm associations (from equations 25, 26, and 27); and θ_1,θ_2, and θ_3 are coefficients for PM, $DNAm$, and $PMxDNAm$ interaction term (from equations 31, 31, and 32).

Bootstrapping was implemented to estimate standard errors and 95% CIs for the NDE and NIE estimates. [24-26] Finally, if the NDE and NIE were both positive or both negative (i.e. had the same signs), the proportion mediated (%) was estimated by dividing the NIE by the TE.[180,394] When the NDE and NIE have opposite signs, or when the total effect is small, the proportion mediated can be unstable and interpretable, with values greater than one or less than zero.[394,395]

Table 7-S1. Characteristics of n_{HRV} = 7,169 / n_{QT} = 6,895 study participants with DNA methylation data, Women's Health Initiative (1993-2005) and Atherosclerosis Risk in Communities study (1990-1995)

Characteristic	Heart rate variability					
	WHI & ARIC n = 7,169	WHI-EMPC[a] n = 1,980	WHI-AS311 n = 308	WHI-BAA23 n = 1,331	ARIC-AA n = 2,514	ARIC-EA n = 1,036
Age (years), mean (SD)	61 (7)	64 (7)	64 (7)	65 (7)	56 (6)	60 (5)
Male, n (%)	1,342 (19)	0 (0)	0 (0)	0 (0)	910 (36)	432 (42)
Race / ethnicity, n (%)						
Black or African American	3,390 (47)	560 (28)	0 (0)	316 (24)	2,514 (100)	0 (0)
Hispanic/Latino	510 (7)	318 (16)	0 (0)	192 (14)	--[c]	--[c]
White (not of Hispanic origin) or European American	3,269 (46)	1,102 (56)	308 (100)	823 (62)	0 (0)	1,036 (100)
More than high school, n (%)	3,905 (55)	1,403 (72)	66 (22)	425 (32)	1,526 (61)	485 (47)
Smoking status, n (%)						
Never	3,408 (48)	1,012 (52)	127 (42)	709 (54)	1,122 (45)	438 (42)
Former	2,541 (35)	771 (40)	148 (49)	478 (36)	750 (30)	394 (38)
Current	1,135 (16)	154 (8)	28 (9)	126 (10)	624 (25)	203 (20)
Alcohol use, n (%)						
Never	1,662 (23)	238 (12)	32 (10)	196 (15)	879 (35)	317 (31)
Former	1,859 (26)	561 (29)	53 (17)	300 (23)	794 (32)	151 (15)
Current	3,593 (50)	1,151 (59)	223 (72)	829 (63)	823 (33)	567 (55)
Physical activity (MET-hours/week), mean (SD)	12.4 (12.7)	9.7 (11.7)	10.8 (12.7)	10.0 (12.7)	12.7 (11.3)	20.2 (14.0)
Body mass index (kg/m^2), mean (SD)	29.3 (6.0)	29.7 (6.0)	28.5 (5.6)	29.9 (6.0)	30.1 (6.2)	26.2 (4.4)
Clinical characteristics, n (%)						
Hypertension	3,069 (43)	999 (51)	143 (46)	726 (55)	1,002 (40)	199 (19)
Hyperlipidemia	1,341 (19)	300 (15)	38 (12)	196 (15)	572 (23)	235 (23)
Diabetes	776 (11)	182 (9)	17 (6)	161 (12)	383 (15)	33 (3)
Chronic lung disease	701 (10)	194 (10)	27 (9)	146 (11)	199 (8)	135 (13)
Coronary heart disease	486 (7)	128 (7)	24 (8)	90 (7)	183 (7)	61 (6)
Congestive heart failure	341 (5)	56 (3)	10 (3)	14 (1)	222 (9)	39 (4)
ECG traits (ms), mean (SD)						
RR	925 (142)	925 (140)	924 (128)	910 (139)	924 (148)	948 (137)
RMSSD	23 (22)	23 (24)	22 (18)	21 (20)	26 (22)	20 (16)
SDNN	20 (16)	20 (18)	20 (14)	18 (14)	22 (18)	19 (14)
QT	406 (30)	402 (31)	402 (27)	401 (31)	411 (31)	413 (26)
PM (μg/m3)						
PM_{10}, 28 days	27.4 (6.2)	27.5 (6.2)	26.6 (6.0)	27.5 (6.3)	34.8 (6.3)	34.4 (5.8)
PM_{10}, 1 month	20.3 (6.1)	20.6 (6.5)	19.4 (5.5)	20.4 (6.1)	20.4 (4.5)	23.2 (5.2)
$PM_{2.5-10}$, 1 month	8.4 (4.5)	6.9 (5.2)	7.9 (4.2)	8.5 (4.5)	7.3 (2.1)	7.8 (2.4)

Characteristic	QT interval					
	WHI & ARIC n = 6,895	WHI-EMPC[b] n = 1,872	WHI-AS311 n = 300	WHI-BAA23 n = 1,339	ARIC-AA n = 2,365	ARIC-EA n = 1,019
Age (years), mean (SD)	61 (7)	63 (7)	64 (7)	65 (7)	56 (6)	60 (5)
Male, n (%)	1,300 (19)	0 (0)	0 (0)	0 (0)	880 (37)	420 (41)
Race / ethnicity, n (%)						
Black or African American	3,198 (46)	515 (28)	0 (0)	318 (24)	2,365 (100)	0 (0.0)
Hispanic/Latino	501 (7)	310 (17)	0 (0)	191 (14)	--[c]	--[c]
White (not of Hispanic origin) or European American	3,196 (46)	1,047 (56)	300 (100)	830 (62)	0 (0.0)	1,019 (100)
More than high school, n (%)	3,697 (54)	1,328 (72)	64 (22)	427 (32)	1,408 (60)	470 (46)
Smoking status, n (%)						
Never	3,274 (48)	949 (52)	124 (42)	707 (54)	1,060 (45)	434 (43)
Former	2,446 (36)	732 (40)	142 (48)	484 (37)	698 (30)	390 (38)
Current	1,098 (16)	151 (8)	30 (10)	131 (10)	591 (25)	195 (19)
Alcohol use, n (%)						
Never	1,576 (23)	222 (12)	30 (10)	193 (15)	822 (35)	309 (30)
Former	1,733 (25)	514 (28)	50 (17)	299 (22)	724 (31)	146 (14)
Current	3,536 (51)	1,107 (59)	220 (73)	842 (63)	803 (34)	564 (55)
Physical activity (MET-hours/week), mean (SD)	12.6 (12.8)	9.9 (11.9)	10.8 (12.7)	10.0 (12.4)	13.1 (11.5)	20.4 (14.1)
Body mass index (kg/m^2), mean (SD)	29.2 (5.9)	29.6 (5.9)	28.6 (5.7)	29.9 (6.0)	29.9 (6.1)	26.1 (4.4)
Clinical characteristics, n (%)						
Hypertension	2,804 (41)	911 (49)	131 (44)	725 (54)	859 (36)	178 (18)
Hyperlipidemia	1,249 (18)	267 (14)	40 (13)	198 (15)	519 (22)	225 (22)
Diabetes	687 (10)	157 (8)	16 (5)	162 (12)	323 (14)	29 (3)
Chronic lung disease	631 (9)	179 (10)	28 (9)	146 (11)	151 (6)	127 (13)
Coronary heart disease	374 (5)	101 (5)	19 (6)	88 (7)	120 (5)	46 (5)
Congestive heart failure	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ECG traits (ms), mean (SD)						
RR	927 (142)	927 (141)	923 (129)	910 (140)	928 (148)	950 (137)
RMSSD	23 (22)	23 (24)	22 (19)	21 (20)	26 (22)	20 (16)
SDNN	20 (17)	21 (18)	20 (14)	18 (14)	22 (18)	19 (14)
QT	405 (30)	401 (31)	400 (26)	400 (31)	410 (30)	412 (26)
PM (μg/m3)						
PM_{10}, 28 days	31.0 (7.2)	27.5 (6.2)	26.7 (6.0)	27.4 (6.3)	34.8 (6.3)	34.4 (5.8)
PM_{10}, 1 month	20.8 (5.7)	20.6 (6.5)	19.4 (5.6)	20.4 (6.2)	20.4 (4.6)	23.1 (5.2)
$PM_{2.5\text{-}10}$, 1 month	7.5 (3.9)	7.0 (5.2)	7.8 (4.2)	8.4 (4.6)	7.3 (2.1)	7.9 (2.5)

Abbreviations: AA, African Americans; ARIC, Atherosclerosis Risk in Communities; AS311, Ancillary Study 311; BAA23, Broad Agency Award 23; EA, European Americans; ECG, electrocardiography; EMPC, Epigenetic Mechanisms of Particulate Matter-Mediated CVD Risk; PM, particulate matter; PM10, PM < 10 μm in diameter; PM2.5-10, PM > 2.5 and < 10 μm in diameter; RMSSD, root mean square of successive differences between RR intervals; SD,

standard deviation; SDNN, SD of normally conducted RR intervals; WHI, Women's Health Initiative
[a]At the 1st visit. Methylation & HRV data also were available among 186 WHI-EMPC participants @ the 2nd visit
[b]At the 1st visit. Methylation & QT data also were available among 178 WHI-EMPC participants @ the 2nd visit
[c]ARIC recruitment and data collection occurred before the National Instutite of Health required collection of information about Hispanic/Latino ethnicity

CHAPTER 8. DISCUSSION AND CONCLUSION

Ambient particulate matter (PM) air pollution is a modifiable exposure that has been consistently associated with cardiovascular disease (CVD), partly through changes in autonomic function and ventricular repolarization, as measured by heart rate variability (HRV) and QT interval duration (QT) on the electrocardiogram. However, the molecular mechanisms underlying PM-associated cardiac autonomic dysfunction and QT prolongation are not well understood. Therefore, a series of well-powered analyses evaluating PM associations with leukocyte count, proportions, and DNA methylation were conducted. Additionally, epigenetic mediation of PM-associated changes in HRV and QT was analyzed to examine the biological plausibility and causality of PM-CVD associations being considered by the U.S. Environmental Protection Agency (EPA) as it sets National Ambient Air Quality Standards (NAAQS) for PM.

The analyses provided evidence of PM-associated health effects, including associations of mid- to longer-duration (i.e. monthly to yearly) mean $PM_{2.5}$ concentrations with higher leukocyte counts, higher granulocyte proportions, and lower CD8+ T cell proportions. While shorter-duration (i.e. daily to weekly) mean PM_{10} concentrations were inversely associated with leukocyte counts – potentially due to relatively acute, PM-induced leukocyte sequestration from peripheral blood – the latter associations were modest. Additionally, methylome-wide association analyses identified three significant CpG sites (cg19004594, cg24102420, and cg12124767) at which higher monthly mean PM_{10} and $PM_{2.5-10}$ concentrations were associated with leukocyte DNAm. Each of the three sites was annotated to a neurological, pulmonary, endocrine, or cardiovascular disease-related gene (*MATN4*, *ARPP21* and *CFTR*), potentially

linking PM exposure to poor health through epigenetic mechanisms. Although monthly mean concentrations of these coarser particulates were associated with DNAm within biologically intriguing genes, neither they nor methylation at cg19004594, cg24102420, or cg12124767 were appreciably associated with HRV and QT. As such, mediation analyses of PM-DNAm-HRV and PM-DNAm-QT associations yielded null results in this study.

Collectively, the results of this study provide insight into PM-related inflammatory processes while generating hypotheses regarding methylomic pathways that may help explain the established relationship between PM and CVD risk. Although DNAm at the discovered CpG sites was not associated with gene expression in whole blood, examining gene expression in myocardial, pulmonary, and neural tissues – those arguably more relevant to cardiovascular and respiratory health – is warranted in future research aimed at elucidating molecular underpinnings of PM-associated CVD risk.

Similarly, monthly mean concentrations of coarser particulates were not associated with HRV or QT, but their associations with shorter duration, finer particulates in susceptible (e.g. diabetic or hypertensive) populations have been observed in previous studies. Future research therefore should consider short-duration exposures to ambient $PM_{2.5}$ and PM_{10} in such populations when investigating the environmental and epigenetic determinants of ten-second, resting, standard twelve-lead electrocardiographic measures.

Finally, future research should consider methylome-wide association, causal association, and mediation analyses that adjust for leukocyte composition to avoid spurious associations of PM with DNAm through its inflammatory, rather than methylomic effects. Mediation analyses may otherwise be biased or uninformative if leukocyte composition confounds what is in effect a multiply mediated PM-DNAm-outcome association, thereby complicating its decomposition. As

DNAm and other leukocyte-derived genomic biomarkers become more common, causal modeling would benefit from thoughtful consideration of PM-leukocyte composition-DNAm-outcome associations when attempting to elucidate the complex pathways of exposure-induced disease.

The culmination of this dissertation – an innovative attempt to examine epigenetically mediated electrocardiographic effects of PM using multi-center, longitudinal data in racially, ethnically and environmentally diverse populations of U.S. women and men – provides further understanding of these pathways. Specifically, it identified inflammatory effects of and putative epigenetic mechanisms by which PM may affect health at concentrations below EPA NAAQS, while simultaneously informing causal and mediation methods at the junction of epigenetics, environmental and cardiovascular epidemiology. Its findings have implications for the next PM Integrated Science Assessment, a comprehensive evaluation of relevant literature that provides the scientific basis for policy-relevant decision-making and standard setting under the Clean Air Act.

REFERENCES

1. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M, Gillespie C, Isasi CR, Jiménez MC, Jordan LC, Judd SE, Lackland D, Lichtman JH, Lisabeth L, Liu S, Longenecker CT, Mackey RH, Matsushita K, Mozaffarian D, Mussolino ME, Nasir K, Neumar RW, Palaniappan L, Pandey DK, Thiagarajan RR, Reeves MJ, Ritchey M, Rodriguez CJ, Roth GA, Rosamond WD, Sasson C, Towfighi A, Tsao CW, Turner MB, Virani SS, Voeks JH, Willey JZ, Wilkins JT, Wu JH, Alger HM, Wong SS, Muntner P. Heart Disease and Stroke Statistics—2017 Update: A Report From the American Heart Association. *Circulation* 2017;**135**(10):e146-e603.

2. Anderson JO, Thundiyil JG, Stolbach A. Clearing the Air: A Review of the Effects of Particulate Matter Air Pollution on Human Health. *Journal of Medical Toxicology* 2012;**8**(2):166-175.

3. Lepeule J, Laden F, Dockery D, Schwartz J. Chronic exposure to fine particles and mortality: an extended follow-up of the Harvard Six Cities study from 1974 to 2009. *Environ Health Perspect* 2012;**120**(7):965-70.

4. Pope CAI, Ezzati M, Dockery DW. Fine-Particulate Air Pollution and Life Expectancy in the United States. *New England Journal of Medicine* 2009;**360**(4):376-386.

5. Cohen AJ, Brauer M, Burnett R, Anderson HR, Frostad J, Estep K, Balakrishnan K, Brunekreef B, Dandona L, Dandona R, Feigin V, Freedman G, Hubbell B, Jobling A, Kan H, Knibbs L, Liu Y, Martin R, Morawska L, Pope CA, III, Shin H, Straif K, Shaddick G, Thomas M, van Dingenen R, van Donkelaar A, Vos T, Murray CJL, Forouzanfar MH. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015. *The Lancet* 2017;**389**(10082):1907-1918.

6. Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, Luepker R, Mittleman M, Samet J, Smith SC, Tager I. Air Pollution and Cardiovascular Disease. *Circulation* 2004;**109**(21):2655.

7. Brook RD, Rajagopalan S, Pope CA, Brook JR, Bhatnagar A, Diez-Roux AV, Holguin F, Hong Y, Luepker RV, Mittleman MA, Peters A, Siscovick D, Smith SC, Whitsel L, Kaufman JD. Particulate Matter Air Pollution and Cardiovascular Disease. *Circulation* 2010;**121**(21):2331.

8. Fuzzi S, Baltensperger U, Carslaw K, Decesari S, Denier Van Der Gon H, Facchini M, Fowler D, Koren I, Langford B, Lohmann U. Particulate matter, air quality and climate: lessons learned and future needs. *Atmospheric chemistry and physics* 2015;**15**(14):8217-8299.

9. Philip S, Martin RV, van Donkelaar A, Lo JW-H, Wang Y, Chen D, Zhang L, Kasibhatla PS, Wang S, Zhang Q, Lu Z, Streets DG, Bittman S, Macdonald DJ. Global Chemical Composition of Ambient Fine Particulate Matter for Exposure Assessment. *Environmental Science & Technology* 2014;**48**(22):13060-13068.

10. Integrated science assessment for particulate matter. *US Environmental Protection Agency Washington, DC* 2009.

11. EPA. What are the Air Quality Standards for PM? https://www3.epa.gov/region1/airquality/pm-aq-standards.html Accessed 04/20, 2018.

12. Miller KA, Siscovick DS, Sheppard L, Shepherd K, Sullivan JH, Anderson GL, Kaufman JD. Long-Term Exposure to Air Pollution and Incidence of Cardiovascular Events in Women. *New England Journal of Medicine* 2007;**356**(5):447-458.

13. Di Q, Wang Y, Zanobetti A, Wang Y, Koutrakis P, Choirat C, Dominici F, Schwartz JD. Air Pollution and Mortality in the Medicare Population. *New England Journal of Medicine* 2017;**376**(26):2513-2522.

14. Puett RC, Schwartz J, Hart JE, Yanosky JD, Speizer FE, Suh H, Paciorek CJ, Neas LM, Laden F. Chronic Particulate Exposure, Mortality, and Coronary Heart Disease in the Nurses' Health Study. *American Journal of Epidemiology* 2008;**168**(10):1161-1168.

15. Cesaroni G, Forastiere F, Stafoggia M, Andersen ZJ, Badaloni C, Beelen R, Caracciolo B, de Faire U, Erbel R, Eriksen KT, Fratiglioni L, Galassi C, Hampel R, Heier M, Hennig F, Hilding A, Hoffmann B, Houthuijs D, Jöckel K-H, Korek M, Lanki T, Leander K, Magnusson PKE, Migliore E, Ostenson C-G, Overvad K, Pedersen NL, J JP, Penell J, Pershagen G, Pyko A, Raaschou-Nielsen O, Ranzi A, Ricceri F, Sacerdote C, Salomaa V, Swart W, Turunen AW, Vineis P, Weinmayr G, Wolf K, de Hoogh K, Hoek G, Brunekreef B, Peters A. Long term exposure to ambient air pollution and incidence of acute coronary events: prospective cohort study and meta-analysis in 11 European cohorts from the ESCAPE Project. *BMJ : British Medical Journal* 2014;**348**.

16. Zanobetti A, Schwartz J. The effect of particulate air pollution on emergency admissions for myocardial infarction: a multicity case-crossover analysis. *Environmental health perspectives* 2005;**113**(8):978.

17. Pope CA, Muhlestein JB, May HT, Renlund DG, Anderson JL, Horne BD. Ischemic Heart Disease Events Triggered by Short-Term Exposure to Fine Particulate Air Pollution. *Circulation* 2006;**114**(23):2443.

18. Peters A, Dockery DW, Muller JE, Mittleman MA. Increased Particulate Air Pollution and the Triggering of Myocardial Infarction. *Circulation* 2001;**103**(23):2810.

19. Peters A, von Klot S, Heier M, Trentinaglia I, Hörmann A, Wichmann HE, Löwel H. Exposure to Traffic and the Onset of Myocardial Infarction. *New England Journal of Medicine* 2004;**351**(17):1721-1730.

20. Sullivan J, Sheppard L, Schreuder A, Ishikawa N, Siscovick D, Kaufman J. Relation between short-term fine-particulate matter exposure and onset of myocardial infarction. *Epidemiology* 2005;**16**(1):41-48.

21. Dominici F, Peng RD, Bell ML, Pham L, McDermott A, Zeger SL. Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. *JAMA* 2006;**295**.

22. Shah ASV, Langrish JP, Nair H, McAllister DA, Hunter AL, Donaldson K, Newby DE, Mills NL. Global association of air pollution and heart failure: a systematic review and meta-analysis. *The Lancet* 2013;**382**(9897):1039-1048.

23. Peters A, Liu E, Verrier RL, Schwartz J, Gold DR, Mittleman M, Baliff J, Oh JA, Allen G, Monahan K, Dockery DW. Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 2000;**11**(1):11-7.

24. Dockery DW, Luttmann-Gibson H, Rich DQ, Link MS, Mittleman MA, Gold DR, Koutrakis P, Schwartz JD, Verrier RL. Association of Air Pollution with Increased Incidence of Ventricular Tachyarrhythmias Recorded by Implanted Cardioverter Defibrillators. *Environmental Health Perspectives* 2005;**113**(6):670-674.

25. Rich DQ, Schwartz J, Mittleman MA, Link M, Luttmann-Gibson H, Catalano PJ, Speizer FE, Dockery DW. Association of Short-term Ambient Air Pollution Concentrations and Ventricular Arrhythmias. *American Journal of Epidemiology* 2005;**161**(12):1123-1132.

26. Rich DQ, Mittleman MA, Link MS, Schwartz J, Luttmann-Gibson H, Catalano PJ, Speizer FE, Gold DR, Dockery DW. Increased Risk of Paroxysmal Atrial Fibrillation Episodes Associated with Acute Increases in Ambient Air Pollution. *Environmental Health Perspectives* 2006;**114**(1):120-123.

27. Sarnat SE, Suh HH, Coull BA, Schwartz J, Stone PH, Gold DR. Ambient particulate air pollution and cardiac arrhythmia in a panel of older adults in Steubenville, Ohio. *Occupational and Environmental Medicine* 2006;**63**(10):700.

28. He F, Shaffer ML, Rodriguez-Colon S, Yanosky JD, Bixler E, Cascio WE, Liao D. Acute Effects of Fine Particulate Air Pollution on Cardiac Arrhythmia: The APACR Study. *Environmental Health Perspectives* 2011;**119**(7):927-932.

29. Rich DQ, Kim MH, Turner JR, Mittleman MA, Schwartz J, Catalano PJ, Dockery DW. Association of ventricular arrhythmias detected by implantable cardioverter defibrillator and ambient air pollutants in the St Louis, Missouri metropolitan area. *Occupational and Environmental Medicine* 2006;**63**(9):591.

30. Ljungman PL, Berglind N, Holmgren C, Gadler F, Edvardsson N, Pershagen G, Rosenqvist M, Sjögren B, Bellander T. Rapid effects of air pollution on ventricular arrhythmias. *Eur Heart J* 2008;**29**.

31. Folino F, Buja G, Zanotto G, Marras E, Allocca G, Vaccari D, Gasparini G, Bertaglia E, Zoppo F, Calzolari V, Suh RN, Ignatiuk B, Lanera C, Benassi A, Gregori D, Iliceto S. Association between air pollution and ventricular arrhythmias in high-risk patients (ARIA study): a multicentre longitudinal study. *The Lancet Planetary Health* 2017;**1**(2):e58-e64.

32. Vedal S, Rich K, Brauer M, White R, Petkau J. Air Pollution and Cardiac Arrhythmias in Patients with Implantable Cardioverter Defibrillators. *Inhalation Toxicology* 2004;**16**(6-7):353-362.

33. Metzger KB, Klein M, Flanders WD, Peel JL, Mulholland JA, Langberg JJ, Tolbert PE. Ambient air pollution and cardiac arrhythmias in patients with implantable defibrillators. *Epidemiology* 2007;**18**(5):585-592.

34. Watkins A, Danilewitz M, Kusha M, Massé S, Urch B, Quadros K, Spears D, Farid T, Nanthakumar K. Air Pollution and Arrhythmic Risk: The Smog Is Yet to Clear. *Canadian Journal of Cardiology* 2013;**29**(6):734-741.

35. Anderson HR, Armstrong B, Hajat S, Harrison R, Monk V, Poloniecki J, Timmis A, Wilkinson P. Air pollution and activation of implantable cardioverter defibrillators in London. *Epidemiology* 2010;**21**(3):405-413.

36. Liao D, Whitsel EA, Duan Y, Lin H-M, Quibrera PM, Smith R, Peuquet DJ, Prineas RJ, Zhang Z-M, Anderson G. Ambient Particulate Air Pollution and Ectopy—The Environmental Epidemiology of Arrhythmogenesis in Women's Health Initiative Study, 1999–2004. *Journal of Toxicology and Environmental Health, Part A* 2008;**72**(1):30-38.

37. Berger A, Zareba W, Schneider A, Rückerl R, Ibald-Mulli A, Cyrys J, Wichmann H-E, Peters A. Runs of ventricular and supraventricular tachycardia triggered by air pollution in patients with coronary heart disease. *Journal of occupational and environmental medicine* 2006;**48**(11):1149-1158.

38. Ebelt ST, Wilson WE, Brauer M. Exposure to ambient and nonambient components of particulate matter: a comparison of health effects. *Epidemiology* 2005;**16**(3):396-405.

39. Yang H-J, Liu X, Qu C, Shi S-B, Liang J-J, Yang B. Main air pollutants and ventricular arrhythmias in patients with implantable cardioverter-defibrillators: A systematic review and meta-analysis. *Chronic Diseases and Translational Medicine* 2017;**3**(4):242-251.

40. Rich KE, Petkau J, Vedal S, Brauer M. A Case-Crossover Analysis of Particulate Air Pollution and Cardiac Arrhythmia in Patients with Implantable Cardioverter Defibrillators. *Inhalation Toxicology* 2004;**16**(6-7):363-372.

41. O'Neal WT, Soliman EZ, Efird JT, Howard VJ, Howard G, McClure LA. Fine particulate air pollution and premature ventricular contractions: The REasons for Geographic And Racial Differences in Stroke (REGARDS) Study. *Environmental Research* 2017;**154**:115-119.

42. O'Neal WT, Soliman EZ, Efird JT, Judd SE, Howard VJ, Howard G, McClure LA. Fine particulate air pollution and premature atrial contractions: The REasons for Geographic And Racial Differences in Stroke study. *J Expos Sci Environ Epidemiol* 2017;**27**(3):271-275.

43. Pappano AJ. *Cardiovascular physiology [electronic resource]*. Mosby physiology monograph series. Philadelphia, PA: Elsevier/Mosby, 2013.

44. Hulstaert F, Hannet I, Deneys V, Munhyeshuli V, Reichert T, De Bruyere M, Strauss K. Age-Related Changes in Human Blood Lymphocyte Subpopulations: II. Varying Kinetics of Percentage and Absolute Count Measurements. *Clinical Immunology and Immunopathology* 1994;**70**(2):152-158.

45. Ross R. Atherosclerosis — An Inflammatory Disease. *New England Journal of Medicine* 1999;**340**(2):115-126.

46. Madjid M, Awan I, Willerson JT, Casscells SW. Leukocyte count and coronary heart disease: Implications for risk assessment. *Journal of the American College of Cardiology* 2004;**44**(10):1945-1956.

47. Tan WC, Qiu D, Liam BL, Ng TP, Lee SH, van Eeden SF, D'Yachkova Y, Hogg JC. The Human Bone Marrow Response to Acute Air Pollution Caused by Forest Fires. *American Journal of Respiratory and Critical Care Medicine* 2000;**161**(4):1213-1217.

48. Lowe GD, Machado SG, Krol WF, Barton BA, Forbes CD. White blood cell count and haematocrit as predictors of coronary recurrence after myocardial infarction. *Thromb Haemost* 1985;**54**(3):700-3.

49. Hajj-Ali R, Zareba W, Ezzeddine R, Moss AJ. Relation of the leukocyte count to recurrent cardiac events in stable patients after acute myocardial infarction. *The American Journal of Cardiology* 2001;**88**(11):1221-1224.

50. Cannon CP, McCabe CH, Wilcox RG, Bentley JH, Braunwald E. Association of white blood cell count with increased mortality in acute myocardial infarction and unstable angina pectoris. *American Journal of Cardiology* 2001;**87**(5):636-639.

51. Barron HV, Cannon CP, Murphy SA, Braunwald E, Gibson CM. Association Between White Blood Cell Count, Epicardial Blood Flow, Myocardial Perfusion, and Clinical Outcomes in the Setting of Acute Myocardial Infarction. *Circulation* 2000;**102**(19):2329.

52. Barron HV, Harr SD, Radford MJ, Wang Y, Krumholz HM. The association between white blood cell count and acute myocardial infarction mortality in patients ≥65 years of age: findings from the cooperative cardiovascular project. *Journal of the American College of Cardiology* 2001;**38**(6):1654-1661.

53. Mueller C, Neumann FJ, Perruchoud AP, Buettner HJ. White blood cell count and long term mortality after non-ST elevation acute coronary syndrome treated with very early revascularisation. *Heart* 2003;**89**(4):389-392.

54. Furman MI, Becker RC, Yarzebski J, Savegeau J, Gore JM, Goldberg RJ. Effect of Elevated Leukocyte Count on In-Hospital Mortality Following Acute Myocardial Infarction*. *The American Journal of Cardiology* 1996;**78**(8):945-948.

55. Haim M, Boyko V, Goldbourt U, Battler A, Behar S. Predictive value of elevated white blood cell count in patients with preexisting coronary heart disease: The bezafibrate infarction prevention study. *Archives of Internal Medicine* 2004;**164**(4):433-439.

56. Gillum RF, Ingram DD, Makuc DM. White blood cell count, coronary heart disease, and death: The NHANES I Epidemiologic Follow-up Study. *American Heart Journal* 1993;**125**(3):855-863.

57. Brown DW, Giles WH, Croft JB. White blood cell count: An independent predictor of coronary heart disease mortality among a national cohort. *Journal of Clinical Epidemiology* 2001;**54**(3):316-322.

58. Kannel WB, Anderson K, Wilson PF. White blood cell count and cardiovascular disease: Insights from the framingham study. *JAMA* 1992;**267**(9):1253-1256.

59. De Labry LO, Campion EW, Glynn RJ, Vokonas PS. White blood cell count as a predictor of mortality: Results over 18 years from the normative aging study. *Journal of Clinical Epidemiology* 1990;**43**(2):153-157.

60. Lee CD, Folsom AR, Nieto FJ, Chambless LE, Shahar E, Wolfe DA. White Blood Cell Count and Incidence of Coronary Heart Disease and Ischemic Stroke and Mortality from Cardiovascular Disease in African-American and White Men and Women: Atherosclerosis Risk in Communities Study. *American Journal of Epidemiology* 2001;**154**(8):758-764.

61. Kabat GC, Kim MY, Manson JE, Lessin L, Lin J, Wassertheil-Smoller S, Rohan TE. White Blood Cell Count and Total and Cause-Specific Mortality in the Women's Health Initiative. *Am J Epidemiol* 2017:1-10.

62. Margolis KL, Manson JE, Greenland P, et al. Leukocyte count as a predictor of cardiovascular events and mortality in postmenopausal women: The women's health initiative observational study. *Archives of Internal Medicine* 2005;**165**(5):500-508.

63. Ruggiero C, Metter EJ, Cherubini A, Maggio M, Sen R, Najjar SS, Windham GB, Ble A, Senin U, Ferrucci L. White Blood Cell Count and Mortality in the Baltimore Longitudinal Study of Aging. *Journal of the American College of Cardiology* 2007;**49**(18):1841-1850.

64. Shankar A, Mitchell P, Rochtchina E, Wang JJ. The association between circulating white blood cell count, triglyceride level and cardiovascular and all-cause mortality: Population-based cohort study. *Atherosclerosis* 2007;**192**(1):177-183.

65. Jee SH, Park JY, Kim H-S, Lee TY, Samet JM. White Blood Cell Count and Risk for All-Cause, Cardiovascular, and Cancer Mortality in a Cohort of Koreans. *American Journal of Epidemiology* 2005;**162**(11):1062-1069.

66. Tamakoshi K, Toyoshima H, Yatsuya H, Matsushita K, Okamura T, Hayakawa T, Okayama A, Ueshima H. White blood cell count and risk of all-cause and cardiovascular mortality in nationwide sample of Japanese--results from the NIPPON DATA90. *Circ J* 2007;**71**(4):479-85.

67. Weijenberg MP, Feskens EJM, Kromhout D. White Blood Cell Count and the Risk of Coronary Heart Disease and All-Cause Mortality in Elderly Men. *Arteriosclerosis, Thrombosis, and Vascular Biology* 1996;**16**(4):499.

68. Phillips AN, Neaton JD, Cook DG, Grimm RH, Shaper AG. Leukocyte Count and Risk of Major Coronary Heart Disease Events. *American Journal of Epidemiology* 1992;**136**(1):59-70.

69. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, c-reactive protein, albumin, or leukocyte count with coronary heart disease: Meta-analyses of prospective studies. *JAMA* 1998;**279**(18):1477-1482.

70. Engström G, Melander O, Hedblad B. Leukocyte Count and Incidence of Hospitalizations Due to Heart Failure. *Circulation: Heart Failure* 2009;**2**(3):217.

71. Rienstra M, Sun JX, Magnani JW, Sinner MF, Lubitz SA, Sullivan LM, Ellinor PT, Benjamin EJ. White Blood Cell Count and Risk of Incident Atrial Fibrillation (From the Framingham Heart Study). *The American Journal of Cardiology* 2012;**109**(4):533-537.

72. Shankar A, Klein BEK, Klein R. Relationship between white blood cell count and incident hypertension*. *American Journal of Hypertension* 2004;**17**(3):233-239.

73. Gillum RF, Mussolino ME. White blood cell count and hypertension incidence. The nhanes i epidemiologic follow-up study. *Journal of Clinical Epidemiology* 1994;**47**(8):911-919.

74. Twig G, Afek A, Shamiss A, Derazne E, Tzur D, Gordon B, Tirosh A. White Blood Cells Count and Incidence of Type 2 Diabetes in Young Men. *Diabetes Care* 2013;**36**(2):276-282.

75. Gkrania-Klotsas E, Ye Z, Cooper AJ, Sharp SJ, Luben R, Biggs ML, Chen L-K, Gokulakrishnan K, Hanefeld M, Ingelsson E, Lai W-A, Lin S-Y, Lind L, Lohsoonthorn V, Mohan V, Muscari A, Nilsson G, Ohrvik J, Chao Qiang J, Jenny NS, Tamakoshi K, Temelkova-Kurktschiev T, Wang Y-Y, Yajnik CS, Zoli M, Khaw K-T, Forouhi NG, Wareham NJ, Langenberg C. Differential White Blood Cell Count and Type 2 Diabetes: Systematic Review and Meta-Analysis of Cross-Sectional and Prospective Studies. *PLOS ONE* 2010;**5**(10):e13405.

76. Bash LD, Erlinger TP, Coresh J, Marsh-Manzi J, Folsom AR, Astor BC. Inflammation, Hemostasis, and the Risk of Kidney Function Decline in the Atherosclerosis Risk in Communities (ARIC) Study. *American Journal of Kidney Diseases* 2009;**53**(4):596-605.

77. Erlinger TP, Tarver-Carr ME, Powe NR, Appel LJ, Coresh J, Eberhardt MS, Brancati FL. Leukocytosis, hypoalbuminemia, and the risk for chronic kidney disease in US adults. *American Journal of Kidney Diseases* 2003;**42**(2):256-263.

78. Wannamethee SG, Lowe GDO, Shaper AG, Rumley A, Lennon L, Whincup PH. Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease. *European Heart Journal* 2005;**26**(17):1765-1773.

79. Wannamethee SG, Lowe GDO, Whincup PH, Rumley A, Walker M, Lennon L. Physical Activity and Hemostatic and Inflammatory Variables in Elderly Men. *Circulation* 2002;**105**(15):1785.

80. Guasti L, Dentali F, Castiglioni L, Maroni L, Marino F, Squizzato A, Ageno W, Gianni M, Gaudio G, Grandi AM, Cosentino M, Venco A. Neutrophils and clinical outcomes in patients with acute coronary syndromes and/or cardiac revascularisation. A systematic review on more than 34,000 subjects. *Thromb Haemost* 2011;**106**(4):591-9.

81. Wheeler JG, Mussolino ME, Gillum RF, Danesh J. Associations between differential leucocyte count and incident coronary heart disease: 1764 incident cases from seven prospective studies of 30 374 individuals. *European Heart Journal* 2004;**25**(15):1287-1292.

82. Huang ZS, Chien KL, Yang CY, Wang CH, Chang TC, Chen CJ. Peripheral differential leukocyte counts and subsequent mortality from all diseases, cancers, and cardiovascular diseases in Taiwanese. *J Formos Med Assoc* 2003;**102**(11):775-81.

83. Olivares R, Ducimetière P, Claude JR. Monocyte Count: A Risk Factor for Coronary Heart Disease? *American Journal of Epidemiology* 1993;**137**(1):49-53.

84. van Eeden SF, Yeung A, Quinlam K, Hogg JC. Systemic response to ambient particulate matter: relevance to chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 2005;**2**(1):61-7.

85. Salvi S, Blomberg A, Rudell B, Kelly F, SandstrÖM T, Holgate ST, Frew A. Acute Inflammatory Responses in the Airways and Peripheral Blood After Short-Term Exposure to Diesel Exhaust in Healthy Human Volunteers. *American Journal of Respiratory and Critical Care Medicine* 1999;**159**(3):702-709.

86. Mills NL, Törnqvist H, Robinson SD, Gonzalez M, Darnley K, MacNee W, Boon NA, Donaldson K, Blomberg A, Sandstrom T, Newby DE. Diesel Exhaust Inhalation Causes Vascular Dysfunction and Impaired Endogenous Fibrinolysis. *Circulation* 2005;**112**(25):3930.

87. Mills NL, Törnqvist H, Gonzalez MC, Vink E, Robinson SD, Söderberg S, Boon NA, Donaldson K, Sandström T, Blomberg A, Newby DE. Ischemic and Thrombotic Effects of Dilute Diesel-Exhaust Inhalation in Men with Coronary Heart Disease. *New England Journal of Medicine* 2007;**357**(11):1075-1082.

88. Törnqvist H, Mills NL, Gonzalez M, Miller MR, Robinson SD, Megson IL, MacNee W, Donaldson K, Söderberg S, Newby DE, Sandström T, Blomberg A. Persistent Endothelial Dysfunction in Humans after Diesel Exhaust Inhalation. *American Journal of Respiratory and Critical Care Medicine* 2007;**176**(4):395-400.

89. Pope CA, Hansen ML, Long RW, Nielsen KR, Eatough NL, Wilson WE, Eatough DJ. Ambient particulate air pollution, heart rate variability, and blood markers of inflammation in a panel of elderly subjects. *Environmental Health Perspectives* 2004;**112**(3):339-345.

90. Brook RD, Urch B, Dvonch JT, Bard RL, Speck M, Keeler G, Morishita M, Marsik FJ, Kamal AS, Kaciroti N, Harkema J, Corey P, Silverman F, Gold DR, Wellenius G, Mittleman MA, Rajagopalan S, Brook JR. Insights Into the Mechanisms and Mediators of the Effects of Air Pollution Exposure on Blood Pressure and Vascular Function in Healthy Humans. *Hypertension* 2009;**54**(3):659.

91. Dubowsky SD, Suh H, Schwartz J, Coull BA, Gold DR. Diabetes, Obesity, and Hypertension May Enhance Associations between Air Pollution and Markers of Systemic Inflammation. *Environmental Health Perspectives* 2006;**114**(7):992-998.

92. Gong H, Linn WS, Terrell SL, Anderson KR, Clark KW, Sioutas C, Cascio WE, Alexis N, Devlin RB. Exposures of Elderly Volunteers with and without Chronic Obstructive Pulmonary Disease (COPD) to Concentrated Ambient Fine Particulate Pollution. *Inhalation Toxicology* 2004;**16**(11-12):731-744.

93. Ghio AJ, Hall A, Bassett MA, Cascio WE, Devlin RB. Exposure to Concentrated Ambient Air Particles Alters Hematologic Indices in Humans. *Inhalation Toxicology* 2003;**15**(14):1465-1478.

94. Emmerechts J, Jacobs L, Van Kerckhoven S, Loyen S, Mathieu C, Fierens F, Nemery B, Nawrot TS, Hoylaerts MF. Air pollution-associated procoagulant changes: the role of circulating microvesicles. *Journal of Thrombosis and Haemostasis* 2012;**10**(1):96-106.

95. Huang W-H, Yen T-H, Chan M-J, Su Y-J. Impact of Environmental Particulate Matter and Peritoneal Dialysis-related Infection in Patients Undergoing Peritoneal Dialysis. *Medicine* 2014;**93**(25):e149.

96. Jacobs L, Emmerechts J, Mathieu C, Hoylaerts MF, Fierens F, Hoet PH, Nemery B, Nawrot TS. Air Pollution–Related Prothrombotic Changes in Persons with Diabetes. *Environmental Health Perspectives* 2010;**118**(2):191-196.

97. Mittal H, Roberts L, Fuller GW, O'Driscoll S, Dick MC, Height SE, Thein SL, Rees DC. The effects of air quality on haematological and clinical parameters in children with sickle cell anaemia. *Annals of Hematology* 2009;**88**(6):529-533.

98. Seaton A, Soutar A, Crawford V, Elton R, McNerlan S, Cherrie J, Watt M, Agius R, Stout R. Particulate air pollution and the blood. *Thorax* 1999;**54**(11):1027.

99. Steinvil A, Kordova-Biezuner L, Shapira I, Berliner S, Rogowski O. Short-term exposure to air pollution and inflammation-sensitive biomarkers. *Environmental Research* 2008;**106**(1):51-61.

100. Liao D, Heiss G, Chinchilli VM, Duan Y, Folsom AR, Lin H-M, Salomaa V. Association of criteria pollutants with plasma hemostatic//inflammatory markers: a population-based study. *J Expo Anal Environ Epidemiol* 2004;**15**(4):319-328.

101. Schwartz J. Air pollution and blood markers of cardiovascular risk. *Environmental Health Perspectives* 2001;**109**(Suppl 3):405-409.

102. Chen J-C, Schwartz J. Metabolic Syndrome and Inflammatory Responses to Long-Term Particulate Air Pollutants. *Environmental Health Perspectives* 2008;**116**(5):612-617.

103. Viehmann A, Hertel S, Fuks K, Eisele L, Moebus S, Möhlenkamp S, Nonnemacher M, Jakobs H, Erbel R, Jöckel K-H, Hoffmann B. Long-term residential exposure to urban air pollution, and repeated measures of systemic blood markers of inflammation and coagulation. *Occupational and Environmental Medicine* 2015;**72**(9):656.

104. Chuang K-J, Yan Y-H, Chiu S-Y, Cheng T-J. Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. *Occupational and Environmental Medicine* 2010;**68**(1):64.

105. Bollati V, Baccarelli A. Environmental epigenetics. *Heredity* 2010;**105**(1):105-112.

106. Neidhart M. *DNA methylation and complex human disease*. Amsterdam: Elsevier, 2016.

107. Clouaire T, Stancheva I. Methyl-CpG binding proteins: specialized transcriptional repressors or structural components of chromatin? *Cellular and Molecular Life Sciences* 2008;**65**(10):1509-1522.

108. Zhong J, Agha G, Baccarelli AA. The Role of DNA Methylation in Cardiovascular Risk and Disease. *Methodological Aspects, Study Design, and Data Analysis for Epidemiological Studies* 2016;**118**(1):119-131.

109. Baccarelli A, Rienstra M, Benjamin EJ. Cardiovascular Epigenetics. *Circulation: Cardiovascular Genetics* 2010;**3**(6):567.

110. Turunen MP, Aavik E, Ylä-Herttuala S. Epigenetics and atherosclerosis. *Biochimica et Biophysica Acta (BBA) - General Subjects* 2009;**1790**(9):886-891.

111. Lund G, Andersson L, Lauria M, Lindholm M, Fraga MF, Villar-Garea A, Ballestar E, Esteller M, Zaina S. DNA Methylation Polymorphisms Precede Any Histological Sign of Atherosclerosis in Mice Lacking Apolipoprotein E. *Journal of Biological Chemistry* 2004;**279**(28):29147-29154.

112. Chen Z, Karaplis AC, Ackerman SL, Pogribny IP, Melnyk S, Lussier-Cacan S, Chen MF, Pai A, John SWM, Smith RS, Bottiglieri T, Bagley P, Selhub J, Rudnicki MA, James SJ, Rozen R. Mice deficient in methylenetetrahydrofolate reductase exhibit hyperhomocysteinemia and decreased methylation capacity, with neuropathology and aortic lipid deposition. *Human Molecular Genetics* 2001;**10**(5):433-444.

113. Movassagh M, Choy M-K, Goddard M, Bennett MR, Down TA, Foo RSY. Differential DNA Methylation Correlates with Differential Expression of Angiogenic Factors in Human Heart Failure. *PLOS ONE* 2010;**5**(1):e8564.

114. Makar KW, Wilson CB. DNA Methylation Is a Nonredundant Repressor of the Th2 Effector Program. *The Journal of Immunology* 2004;**173**(7):4402-4406.

115. McCullough SD, Dhingra R, Fortin MC, Diaz-Sanchez D. Air Pollution and the Epigenome: A Model Relationship for the Exploration of Toxicoepigenetics. *Current Opinion in Toxicology* 2017.

116. Ma B, Wilker EH, Willis-Owen SAG, Byun H-M, Wong KCC, Motta V, Baccarelli AA, Schwartz J, Cookson WOCM, Khabbaz K, Mittleman MA, Moffatt MF, Liang L. Predicting DNA methylation level across human tissues. *Nucleic Acids Research* 2014;**42**(6):3515-3528.

117. Byun H-M, Siegmund KD, Pan F, Weisenberger DJ, Kanel G, Laird PW, Yang AS. Epigenetic profiling of somatic tissues from human autopsy specimens identifies tissue- and individual-specific DNA methylation patterns. *Human Molecular Genetics* 2009;**18**(24):4808-4817.

118. Fan S, Zhang X. CpG island methylation pattern in different human tissues and its correlation with gene expression. *Biochemical and Biophysical Research Communications* 2009;**383**(4):421-425.

119. Stenvinkel P, Karimi M, Johansson S, Axelsson J, Suliman M, Lindholm B, Heimbürger O, Barany P, Alvestrand A, Nordfors L, Qureshi AR, Ekström TJ, Schalling M. Impact of inflammation on epigenetic DNA methylation – a novel risk factor for cardiovascular disease? *Journal of Internal Medicine* 2007;**261**(5):488-499.

120. Friso S, Pizzolo F, Choi S-W, Guarini P, Castagna A, Ravagnani V, Carletto A, Pattini P, Corrocher R, Olivieri O. Epigenetic control of 11 beta-hydroxysteroid dehydrogenase 2 gene promoter is related to human hypertension. *Atherosclerosis* 2008;**199**(2):323-327.

121. Baccarelli A, Wright R, Bollati V, Litonjua A, Zanobetti A, Tarantini L, Sparrow D, Vokonas P, Schwartz J. Ischemic heart disease and stroke in relation to blood DNA methylation. *Epidemiology* 2010;**21**(6):819-28.

122. Talens RP, Jukema JW, Trompet S, Kremer D, Westendorp RGJ, Lumey LH, Sattar N, Putter H, Slagboom PE, Heijmans BT. Hypermethylation at loci sensitive to the prenatal environment is associated with increased incidence of myocardial infarction. *International Journal of Epidemiology* 2012;**41**(1):106-115.

123. Kim M, Long TI, Arakawa K, Wang R, Yu MC, Laird PW. DNA Methylation as a Biomarker for Cardiovascular Disease Risk. *PLOS ONE* 2010;**5**(3):e9692.

124. Boks MP, Derks EM, Weisenberger DJ, Strengman E, Janson E, Sommer IE, Kahn RS, Ophoff RA. The Relationship of DNA Methylation with Age, Gender and Genotype in Twins and Healthy Controls. *PLOS ONE* 2009;**4**(8):e6767.

125. Bollati V, Schwartz J, Wright R, Litonjua A, Tarantini L, Suh H, Sparrow D, Vokonas P, Baccarelli A. Decline in genomic DNA methylation through aging in a cohort of elderly subjects. *Mechanisms of Ageing and Development* 2009;**130**(4):234-239.

126. Teschendorff AE, Menon U, Gentry-Maharaj A, Ramus SJ, Weisenberger DJ, Shen H, Campan M, Noushmehr H, Bell CG, Maxwell AP, Savage DA, Mueller-Holzner E, Marth C, Kocjan G, Gayther SA, Jones A, Beck S, Wagner W, Laird PW, Jacobs IJ, Widschwendter M. Age-dependent DNA methylation of genes that are suppressed in stem cells is a hallmark of cancer. *Genome Research* 2010;**20**(4):440-446.

127. Bell JT, Tsai P-C, Yang T-P, Pidsley R, Nisbet J, Glass D, Mangino M, Zhai G, Zhang F, Valdes A, Shin S-Y, Dempster EL, Murray RM, Grundberg E, Hedman AK, Nica A, Small KS, The Mu TC, Dermitzakis ET, McCarthy MI, Mill J, Spector TD, Deloukas P. Epigenome-Wide Scans Identify Differentially Methylated Regions for Age and Age-Related Phenotypes in a Healthy Ageing Population. *PLOS Genetics* 2012;**8**(4):e1002629.

128. Christensen BC, Houseman EA, Marsit CJ, Zheng S, Wrensch MR, Wiemels JL, Nelson HH, Karagas MR, Padbury JF, Bueno R, Sugarbaker DJ, Yeh R-F, Wiencke JK, Kelsey KT. Aging and Environmental Exposures Alter Tissue-Specific DNA Methylation Dependent upon CpG Island Context. *PLOS Genetics* 2009;**5**(8):e1000602.

129. Horvath S, Zhang Y, Langfelder P, Kahn R, Boks M, van Eijk K, van den Berg L, Ophoff RA. Aging effects on DNA methylation modules in human brain and blood tissue. *Genome Biol* 2012;**13**.

130. Zhang FF, Cardarelli R, Carroll J, Fulda KG, Kaur M, Gonzalez K. Significant differences in global genomic DNA methylation by gender and race/ethnicity in peripheral blood. *Epigenetics* 2011;**6**.

131. El-Maarri O, Becker T, Junen J, Manzoor SS, Diaz-Lacava A, Schwaab R, Wienker T, Oldenburg J. Gender specific differences in levels of DNA methylation at selected loci from human total blood: a tendency toward higher methylation levels in males. *Human Genetics* 2007;**122**(5):505-514.

132. Terry MB, Ferris JS, Pilsner R, Flom JD, Tehranifar P, Santella RM, Gamble MV, Susser E. Genomic DNA Methylation among Women in a Multiethnic New York City Birth Cohort. *Cancer Epidemiology Biomarkers & Prevention* 2008;**17**(9):2306.

133. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, Slagboom PE, Lumey LH. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proceedings of the National Academy of Sciences* 2008;**105**(44):17046-17049.

134. Lumey LH, Stein AD, Kahn HS, van der Pal-de Bruin KM, Blauw GJ, Zybert PA, Susser ES. Cohort Profile: The Dutch Hunger Winter Families Study. *International Journal of Epidemiology* 2007;**36**(6):1196-1204.

135. Hoyo C, Murtha AP, Schildkraut JM, Jirtle RL, Demark-Wahnefried W, Forman MR, Iversen ES, Kurtzberg J, Overcash F, Huang Z, Murphy SK. Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. *Epigenetics* 2011;**6**(7):928-36.

136. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, Lindemans J, Siebel C, Steegers EA, Slagboom PE, Heijmans BT. Periconceptional Maternal Folic Acid Use of 400 µg per Day Is Related to Increased Methylation of the IGF2 Gene in the Very Young Child. *PLOS ONE* 2009;**4**(11):e7845.

137. Ba Y, Yu H, Liu F, Geng X, Zhu C, Zhu Q, Zheng T, Ma S, Wang G, Li Z, Zhang Y. Relationship of folate, vitamin B12 and methylation of insulin-like growth factor-II in maternal and cord blood. *Eur J Clin Nutr* 2011;**65**(4):480-485.

138. Lim U, Song M-A. Dietary and Lifestyle Factors of DNA Methylation. In: Dumitrescu RG, Verma M, eds. *Cancer Epigenetics: Methods and Protocols*. Totowa, NJ: Humana Press, 2012;359-376.

139. Zhang FF, Morabia A, Carroll J, Gonzalez K, Fulda K, Kaur M, Vishwanatha JK, Santella RM, Cardarelli R. Dietary Patterns Are Associated with Levels of Global Genomic DNA Methylation in a Cancer-Free Population. *The Journal of Nutrition* 2011;**141**(6):1165-1171.

140. Joehanes R, Just AC, Marioni RE, Pilling LC, Reynolds LM, Mandaviya PR, Guan W, Xu T, Elks CE, Aslibekyan S, Moreno-Macias H, Smith JA, Brody JA, Dhingra R, Yousefi P, Pankow JS, Kunze S, Shah S, McRae AF, Lohman K, Sha J, Absher DM, Ferrucci L, Zhao W, Demerath EW, Bressler J, Grove ML, Huan T, Liu C, Mendelson MM, Yao C, Kiel DP, Peters A, Wang-Sattler R, Visscher PM, Wray NR, Starr JM, Ding J, Rodriguez CJ, Wareham NJ, Irvin MR, Zhi D, Barrdahl M, Vineis P, Ambatipudi S, Uitterlinden AG, Hofman A, Schwartz J, Colicino E, Hou L, Vokonas PS, Hernandez DG, Singleton AB, Bandinelli S, Turner ST, Ware EB, Smith AK, Klengel T, Binder EB, Psaty BM, Taylor KD, Gharib SA, Swenson BR, Liang L, DeMeo DL, Connor GT, Herceg Z, Ressler KJ, Conneely KN, Sotoodehnia N, Kardia SLR, Melzer D, Baccarelli AA, van Meurs JBJ, Romieu I, Arnett DK, Ong KK, Liu Y, Waldenberger M, Deary IJ, Fornage M, Levy D, London SJ. Epigenetic Signatures of Cigarette Smoking. *Circulation: Cardiovascular Genetics* 2016.

141. Breton CV, Byun HM, Wenten M, Pan F, Yang A, Gilliland FD. Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. *Am J Respir Crit Care Med* 2009;**180**(5):462-7.

142. Breitling Lutz P, Yang R, Korn B, Burwinkel B, Brenner H. Tobacco-Smoking-Related Differential DNA Methylation: 27K Discovery and Replication. *The American Journal of Human Genetics* 2011;**88**(4):450-457.

143. Joubert BR, Haberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, Huang Z, Hoyo C, Midttun O, Cupul-Uicab LA, Ueland PM, Wu MC, Nystad W, Bell DA, Peddada SD, London SJ. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. *Environ Health Perspect* 2012;**120**(10):1425-31.

144. Allione A, Marcon F, Fiorito G, Guarrera S, Siniscalchi E, Zijno A, Crebelli R, Matullo G. Novel Epigenetic Changes Unveiled by Monozygotic Twins Discordant for Smoking Habits. *PLOS ONE* 2015;**10**(6):e0128265.

145. Wan ES, Qiu W, Baccarelli A, Carey VJ, Bacherman H, Rennard SI, Agusti A, Anderson W, Lomas DA, DeMeo DL. Cigarette smoking behaviors and time since quitting are associated with differential DNA methylation across the human genome. *Human Molecular Genetics* 2012;**21**(13):3073-3082.

146. Zhang Y, Yang R, Burwinkel B, Breitling LP, Brenner H. F2RL3 methylation as a biomarker of current and lifetime smoking exposures. *Environ Health Perspect* 2014;**122**(2):131-7.

147. Zeilinger S, Kühnel B, Klopp N, Baurecht H, Kleinschmidt A, Gieger C, Weidinger S, Lattka E, Adamski J, Peters A, Strauch K, Waldenberger M, Illig T. Tobacco Smoking Leads to Extensive Genome-Wide Changes in DNA Methylation. *PLOS ONE* 2013;**8**(5):e63812.

148. Lee MK, Hong Y, Kim S-Y, London SJ, Kim WJ. DNA methylation and smoking in Korean adults: epigenome-wide association study. *Clinical Epigenetics* 2016;**8**(1):103.

149. Zaghlool SB, Al-Shafai M, Al Muftah WA, Kumar P, Falchi M, Suhre K. Association of DNA methylation with age, gender, and smoking in an Arab population. *Clin Epigenetics* 2015;**7**.

150. Shenker NS, Polidoro S, van Veldhoven K, Sacerdote C, Ricceri F, Birrell MA, Belvisi MG, Brown R, Vineis P, Flanagan JM. Epigenome-wide association study in the European Prospective Investigation into Cancer and Nutrition (EPIC-Turin) identifies novel genetic loci associated with smoking. *Human Molecular Genetics* 2013;**22**(5):843-851.

151. Harlid S, Xu Z, Panduri V, Sandler DP, Taylor JA. CpG sites associated with cigarette smoking: analysis of epigenome-wide data from the Sister Study. *Environ Health Perspect* 2014;**122**(7):673-8.

152. Rönn T, Volkov P, Davegårdh C, Dayeh T, Hall E, Olsson AH, Nilsson E, Tornberg Å, Dekker Nitert M, Eriksson K-F, Jones HA, Groop L, Ling C. A Six Months Exercise Intervention Influences the Genome-wide DNA Methylation Pattern in Human Adipose Tissue. *PLOS Genetics* 2013;**9**(6):e1003572.

153. Fan T, Fang SC, Cavallari JM, Barnett IJ, Wang Z, Su L, Byun H-M, Lin X, Baccarelli AA, Christiani DC. Heart rate variability and DNA methylation levels are altered after short-term metal fume exposure among occupational welders: a repeated-measures panel study. *BMC Public Health* 2014;**14**(1):1279.

154. Chen R, Meng X, Zhao A, Wang C, Yang C, Li H, Cai J, Zhao Z, Kan H. DNA hypomethylation and its mediation in the effects of fine particulate air pollution on cardiovascular biomarkers: A randomized crossover trial. *Environment International* 2016;**94**:614-619.

155. Bellavia A, Urch B, Speck M, Brook RD, Scott JA, Albetti B, Behbod B, North M, Valeri L, Bertazzi PA, Silverman F, Gold D, A. Baccarelli A. DNA Hypomethylation, Ambient Particulate Matter, and Increased Blood Pressure: Findings From Controlled Human Exposure Experiments. *Journal of the American Heart Association* 2013;**2**(3).

156. Tarantini L, Bonzini M, Apostoli P, Pegoraro V, Bollati V, Marinelli B, Cantone L, Rizzo G, Hou L, Schwartz J, Bertazzi PA, Baccarelli A. Effects of Particulate Matter on Genomic DNA Methylation Content and iNOS Promoter Methylation. *Environmental Health Perspectives* 2009;**117**(2):217-222.

157. De Prins S, Koppen G, Jacobs G, Dons E, Van de Mieroop E, Nelen V, Fierens F, Int Panis L, De Boever P, Cox B, Nawrot TS, Schoeters G. Influence of ambient air pollution on global DNA methylation in healthy adults: A seasonal follow-up. *Environment International* 2013;**59**:418-424.

158. Wang C, Chen R, Cai J, Shi J, Yang C, Tse LA, Li H, Lin Z, Meng X, Liu C, Niu Y, Xia Y, Zhao Z, Kan H. Personal exposure to fine particulate matter and blood pressure: A role of angiotensin converting enzyme and its DNA methylation. *Environment International* 2016;**94**:661-666.

159. Sanchez-Guerra M, Zheng Y, Osorio-Yanez C, Zhong J, Chervona Y, Wang S, Chang D, McCracken JP, Diaz A, Bertazzi PA, Koutrakis P, Kang CM, Zhang X, Zhang W, Byun HM, Schwartz J, Hou L, Baccarelli AA. Effects of particulate matter exposure on blood 5-hydroxymethylation: results from the Beijing truck driver air pollution study. *Epigenetics* 2015;**10**(7):633-42.

160. Guo L, Byun H-M, Zhong J, Motta V, Barupal J, Zheng Y, Dou C, Zhang F, McCracken JP, Diaz A, Marco S-G, Colicino S, Schwartz J, Wang S, Hou L, Baccarelli AA. Effects of short-term exposure to inhalable particulate matter on DNA methylation of tandem repeats. *Environmental and Molecular Mutagenesis* 2014;**55**(4):322-335.

161. Kile ML, Fang S, Baccarelli AA, Tarantini L, Cavallari J, Christiani DC. A panel study of occupational exposure to fine particulate matter and changes in DNA methylation over a single workday and years worked in boilermaker welders. *Environ Health* 2013;**12**.

162. Chen R, Qiao L, Li H, Zhao Y, Zhang Y, Xu W, Wang C, Wang H, Zhao Z, Xu X, Hu H, Kan H. Fine Particulate Matter Constituents, Nitric Oxide Synthase DNA Methylation and Exhaled Nitric Oxide. *Environmental Science & Technology* 2015;**49**(19):11859-11865.

163. Tarantini L, Bonzini M, Tripodi A, Angelici L, Nordio F, Cantone L, Apostoli P, Bertazzi PA, Baccarelli AA. Blood hypomethylation of inflammatory genes mediates the effects of metal-rich airborne pollutants on blood coagulation. *Occup Environ Med* 2013;**70**.

164. Bind MA, Coull BA, Peters A, Baccarelli AA, Tarantini L, Cantone L, Vokonas PS, Koutrakis P, Schwartz JD. Beyond the Mean: Quantile Regression to Explore the Association of Air Pollution with Gene-Specific Methylation in the Normative Aging Study. *Environ Health Perspect* 2015;**123**(8):759-65.

165. Cantone L, Iodice S, Tarantini L, Albetti B, Restelli I, Vigna L, Bonzini M, Pesatori AC, Bollati V. Particulate matter exposure is associated with inflammatory gene methylation in obese subjects. *Environmental Research* 2017;**152**:478-484.

166. Bell B, Rose CL, Damon A. The Normative Aging Study: An Interdisciplinary and Longitudinal Study of Health and Aging. *Aging and Human Development* 1972;**3**(1):5-17.

167. Baccarelli A, Wright RO, Bollati V, Tarantini L, Litonjua AA, Suh HH, Zanobetti A, Sparrow D, Vokonas PS, Schwartz J. Rapid DNA Methylation Changes after Exposure to Traffic Particles. *American Journal of Respiratory and Critical Care Medicine* 2009;**179**(7):572-578.

168. Madrigano J, Baccarelli A, Mittleman MA, Wright RO, Sparrow D, Vokonas PS, Tarantini L, Schwartz J. Prolonged Exposure to Particulate Pollution, Genes Associated with Glutathione Pathways, and DNA Methylation in a Cohort of Older Men. *Environmental Health Perspectives* 2011;**119**(7):977-982.

169. Madrigano J, Baccarelli A, Mittleman MA, Sparrow D, Spiro IIIA, Vokonas PS, Cantone L, Kubzansky L, Schwartz J. Air Pollution and DNA Methylation: Interaction by Psychological Factors in the VA Normative Aging Study. *American Journal of Epidemiology* 2012;**176**(3):224-232.

170. Peng C, Bind MC, Colicino E, Kloog I, Byun HM, Cantone L, Trevisi L, Zhong J, Brennan K, Dereix AE, Vokonas PS, Coull BA, Schwartz JD, Baccarelli AA. Particulate Air Pollution and Fasting Blood Glucose in Nondiabetic Individuals: Associations and Epigenetic Mediation in the Normative Aging Study, 2000-2011. *Environ Health Perspect* 2016;**124**(11):1715-1721.

171. Chi GC, Liu Y, MacDonald JW, Barr RG, Donohue KM, Hensley MD, Hou L, McCall CE, Reynolds LM, Siscovick DS, Kaufman JD. Long-term outdoor air pollution and DNA methylation in circulating monocytes: results from the Multi-Ethnic Study of Atherosclerosis (MESA). *Environmental Health* 2016;**15**(1):119.

172. de FCLAJ, van der Plaat DA, de Jong K, van Diemen CC, Postma DS, Nedeljkovic I, van Duijn CM, Amin N, la Bastide-van Gemert S, de Vries M, Ward-Caviness CK, Wolf K, Waldenberger M, Peters A, Stolk RP, Brunekreef B, Boezen HM, Vonk JM. Long-term Air Pollution Exposure, Genome-wide DNA Methylation and Lung Function in the LifeLines Cohort Study. *Environ Health Perspect* 2018;**126**(2):027004.

173. Panni T, Mehta AJ, Schwartz JD, Baccarelli AA, Just AC, Wolf K. A Genome-Wide Analysis of DNA Methylation and Fine Particulate Matter Air Pollution in Three Study Populations: KORA F3, KORA F4, and the Normative Aging Study. *Environ Health Perspect* 2016.

174. Plusquin M, Guida F, Polidoro S, Vermeulen R, Raaschou-Nielsen O, Campanella G, Hoek G, Kyrtopoulos SA, Georgiadis P, Naccarati A, Sacerdote C, Krogh V, Bas Bueno-de-Mesquita H, Monique Verschuren WM, Sayols-Baixeras S, Panni T, Peters A, Hebels DGAJ, Kleinjans J, Vineis P, Chadeau-Hyam M. DNA methylation and exposure to ambient air pollution in two prospective cohorts. *Environment International* 2017;**108**:127-136.

175. Nelson HH, Marsit CJ, Kelsey KT. Global Methylation in Exposure Biology and Translational Medical Science. *Environmental Health Perspectives* 2011;**119**(11):1528-1533.

176. Colhoun HM, McKeigue PM, Smith GD. Problems of reporting genetic associations with complex outcomes. *The Lancet* 2003;**361**(9360):865-872.

177. Sullivan PF. Spurious Genetic Associations. *Biological Psychiatry* 2007;**61**(10):1121-1126.

178. Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol* 2014;**15**.

179. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, Wiencke JK, Kelsey KT. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC bioinformatics* 2012;**13**(1):1.

180. VanderWeele T. *Explanation in Causal Inference: Methods for Mediation and Interaction* Oxford University Press, Incorporated, 2015.

181. Vanderweele TJ, Vansteelandt S, Robins JM. Effect decomposition in the presence of an exposure-induced mediator-outcome confounder. *Epidemiology* 2014;**25**(2):300-6.

182. Pacing ETFotESoCtNASo. Heart Rate Variability. *Circulation* 1996;**93**(5):1043.

183. van Ravenswaaij-Arts CA, Kollee LA, Hopman JW, Stoelinga GA, van Geijn HP. Heart rate variability. *Annals of Internal Medicine* 1993;**118**(6):436-447.

184. Berntson GG, Thomas Bigger J, Eckberg DL, Grossman P, Kaufmann PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH, Van Der Molen MW. Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology* 1997;**34**(6):623-648.

185. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *International Journal of Cardiology* 2010;**141**(2):122-131.

186. Mathias CJ, Bannister SR. Autonomic FailureA Textbook of Clinical Disorders of the Autonomic Nervous System. Oxford, UK: 'Oxford University Press', 2013.

187. Gordan R, Gwathmey JK, Xie L-H. Autonomic and endocrine control of cardiovascular function. *World Journal of Cardiology* 2015;**7**(4):204-214.

188. Sperelakis N. *Heart physiology and pathophysiology [electronic resource]*. San Diego, Calif. ; London: Academic Press, 2001.

189. Klabunde R. Sinoatrial Node Action Potentials. http://www.cvphysiology.com/Arrhythmias/A004 Accessed 06/10, 2017.

190. Nolte IM, Munoz ML, Tragante V, Amare AT, Jansen R, Vaez A, von der Heyde B, Avery CL, Bis JC, Dierckx B, van Dongen J, Gogarten SM, Goyette P, Hernesniemi J, Huikari V, Hwang S-J, Jaju D, Kerr KF, Kluttig A, Krijthe BP, Kumar J, van der Laan SW, Lyytikäinen L-P, Maihofer AX, Minassian A, van der Most PJ, Müller-Nurasyid M, Nivard M, Salvi E, Stewart JD, Thayer JF, Verweij N, Wong A, Zabaneh D, Zafarmand MH, Abdellaoui A, Albarwani S, Albert C, Alonso A, Ashar F, Auvinen J, Axelsson T, Baker DG, de Bakker PIW, Barcella M, Bayoumi R, Bieringa RJ, Boomsma D, Boucher G, Britton AR, Christophersen I, Dietrich A, Ehret GB, Ellinor PT, Eskola M, Felix JF, Floras JS, Franco OH, Friberg P, Gademan MGJ, Geyer MA, Giedraitis V, Hartman CA, Hemerich D, Hofman A, Hottenga J-J, Huikuri H, Hutri-Kähönen N, Jouven X, Junttila J, Juonala M, Kiviniemi AM, Kors JA, Kumari M, Kuznetsova T, Laurie CC, Lefrandt JD, Li Y, Li Y, Liao D, Limacher MC, Lin HJ, Lindgren CM, Lubitz SA, Mahajan A, McKnight B, zu Schwabedissen HM, Milaneschi Y, Mononen N, Morris AP, Nalls MA, Navis G, Neijts M, Nikus K, North KE, O'Connor DT, Ormel J, Perz S, Peters A, Psaty BM, et al. Genetic loci associated with heart rate variability and their effects on cardiac disease risk. 2017;**8**:15805.

191. Kerr KF, Avery CL, Lin HJ, Raffield LM, Zhang QS, Browning BL, Browning SR, Conomos MP, Gogarten SM, Laurie CC, Sofer T, Thornton TA, Hohensee C, Jackson RD, Kooperberg C, Li Y, Méndez-Giráldez R, Perez MV, Peters U, Reiner AP, Zhang Z-M, Yao J, Sotoodehnia N, Taylor KD, Guo X, Lange LA, Soliman EZ, Wilson JG, Rotter JI, Heckbert SR, Jain D, Whitsel EA. Genome-wide Association Study of Heart Rate and Its Variability in Hispanic/Latino Cohorts. *Heart Rhythm* 2017.

192. den Hoed M, Eijgelsheim M, Esko T, Brundel BJJM, Peal DS, Evans DM, Nolte IM, Segre AV, Holm H, Handsaker RE, Westra H-J, Johnson T, Isaacs A, Yang J, Lundby A, Zhao JH, Kim YJ, Go MJ, Almgren P, Bochud M, Boucher G, Cornelis MC, Gudbjartsson D, Hadley D, van der Harst P, Hayward C, den Heijer M, Igl W, Jackson AU, Kutalik Z, Luan Ja, Kemp JP, Kristiansson K, Ladenvall C, Lorentzon M, Montasser ME, Njajou OT, O'Reilly PF, Padmanabhan S, St. Pourcain B, Rankinen T, Salo P, Tanaka T, Timpson NJ, Vitart V, Waite L, Wheeler W, Zhang W, Draisma HHM, Feitosa MF, Kerr KF, Lind PA, Mihailov E, Onland-Moret NC, Song C, Weedon MN, Xie W, Yengo L, Absher D, Albert CM, Alonso A, Arking DE, de Bakker PIW, Balkau B, Barlassina C, Benaglio P, Bis JC, Bouatia-Naji N, Brage S, Chanock SJ, Chines PS, Chung M, Darbar D, Dina C, Dorr M, Elliott P, Felix SB, Fischer K, Fuchsberger C, de Geus EJC, Goyette P, Gudnason V, Harris TB, Hartikainen A-L, Havulinna AS, Heckbert SR, Hicks AA, Hofman A, Holewijn S, Hoogstra-Berends F, Hottenga J-J, Jensen MK, Johansson A, Junttila J, Kaab S, Kanon B, Ketkar S, Khaw K-T, Knowles JW, Kooner AS, et al. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. *Nat Genet* 2013;**45**(6):621-631.

193. McCraty R, Shaffer F. Heart Rate Variability: New Perspectives on Physiological Mechanisms, Assessment of Self-regulatory Capacity, and Health risk. *Global Advances in Health and Medicine* 2015;**4**(1):46-61.

194. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 1981;**213**(4504):220.

195. Schroeder EB, Whitsel EA, Evans GW, Prineas RJ, Chambless LE, Heiss G. Repeatability of heart rate variability measures. *Journal of Electrocardiology* 2004;**37**(3):163-172.

196. ARIC. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. *Am J Epidemiol* 1989;**129**(4):687-702.

197. NIH. Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. *Control Clin Trials* 1998;**19**(1):61-109.

198. Nussinovitch U, Elishkevitz KP, Katz K, Nussinovitch M, Segev S, Volovitz B, Nussinovitch N. Reliability of Ultra-Short ECG Indices for Heart Rate Variability. *Annals of Noninvasive Electrocardiology* 2011;**16**(2):117-122.

199. Munoz ML, van Roon A, Riese H, Thio C, Oostenbroek E, Westrik I, de Geus EJC, Gansevoort R, Lefrandt J, Nolte IM, Snieder H. Validity of (Ultra-)Short Recordings for Heart Rate Variability Measurements. *PLOS ONE* 2015;**10**(9):e0138921.

200. Park SK, Auchincloss AH, O'Neill MS, Prineas R, Correa JC, Keeler J, Barr RG, Kaufman JD, Diez Roux AV. Particulate Air Pollution, Metabolic Syndrome, and Heart Rate Variability: The Multi-Ethnic Study of Atherosclerosis (MESA). *Environmental Health Perspectives* 2010;**118**(10):1406-1411.

201. Bruyne MCd, Kors JA, Hoes AW, Klootwijk P, Dekker JM, Hofman A, van Bemmel JH, Grobbee DE. Both Decreased and Increased Heart Rate Variability on the Standard 10-Second Electrocardiogram Predict Cardiac Mortality in the ElderlyThe Rotterdam Study. *American Journal of Epidemiology* 1999;**150**(12):1282-1288.

202. Dekker JM, Crow RS, Folsom AR, Hannan PJ, Liao D, Swenne CA, Schouten EG. Low Heart Rate Variability in a 2-Minute Rhythm Strip Predicts Risk of Coronary Heart Disease and Mortality From Several Causes. *Circulation* 2000;**102**(11):1239.

203. Gerritsen J, Dekker JM, TenVoorde BJ, Kostense PJ, Heine RJ, Bouter LM, Heethaar RM, Stehouwer CDA. Impaired Autonomic Function Is Associated With Increased Mortality, Especially in Subjects With Diabetes, Hypertension, or a History of Cardiovascular Disease. *Diabetes Care* 2001;**24**(10):1793.

204. Dekker JM, Schouten EG, Klootwijk P, Pool J, Swenne CA, Kromhout D. Heart Rate Variability from Short Electrocardiographic Recordings Predicts Mortality from All Causes in Middle-aged and Elderly MenThe Zutphen Study. *American Journal of Epidemiology* 1997;**145**(10):899-908.

205. Tsuji H, Venditti FJ, Manders ES, Evans JC, Larson MG, Feldman CL, Levy D. Reduced heart rate variability and mortality risk in an elderly cohort. The Framingham Heart Study. *Circulation* 1994;**90**(2):878.

206. Bigger JT, Fleiss JL, Steinman RC, Rolnitzky LM, Kleiger RE, Rottman JN. Frequency domain measures of heart period variability and mortality after myocardial infarction. *Circulation* 1992;**85**(1):164.

207. Kleiger RE, Miller JP, Bigger JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *The American Journal of Cardiology* 1987;**59**(4):256-262.

208. Malik M, Farrell T, Camm AJ. Circadian rhythm of heart rate variability after acute myocardial infarction and its influence on the prognostic value of heart rate variability. *American Journal of Cardiology* 1990;**66**(15):1049-1054.

209. Lombardi F, Sandrone G, Pernpruner S, Sala R, Garimoldi M, Cerutti S, Baselli G, Pagani M, Malliani A. Heart rate variability as an index of sympathovagal interaction after acute myocardial infarction. *The American Journal of Cardiology* 1987;**60**(16):1239-1245.

210. Vaishnav S, Stevenson R, Marchant B, Lagi K, Ranjadayalan K, Timmis AD. Relation between heart rate variability early after acute myocardial infarction and long-term mortality. *The American Journal of Cardiology* 1994;**73**(9):653-657.

211. Stein PK, Domitrovich PP, Huikuri HV, Kleiger RE, Cast I. Traditional and Nonlinear Heart Rate Variability Are Each Independently Associated with Mortality after Myocardial Infarction. *Journal of Cardiovascular Electrophysiology* 2005;**16**(1):13-20.

212. Rovere MTL, Bigger JT, Jr., Marcus FI, Mortara A, Schwartz PJ. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. *The Lancet* 1998;**351**(9101):478-484.

213. Liao D, Carnethon M, Evans GW, Cascio WE, Heiss G. Lower Heart Rate Variability Is Associated With the Development of Coronary Heart Disease in Individuals With Diabetes. *Diabetes* 2002;**51**(12):3524.

214. Nolan J, Batin PD, Andrews R, Lindsay SJ, Brooksby P, Mullen M, Baig W, Flapan AD, Cowley A, Prescott RJ, Neilson JMM, Fox KAA. Prospective Study of Heart Rate Variability and Mortality in Chronic Heart Failure. *Circulation* 1998;**98**(15):1510.

215. La Rovere MT, Pinna GD, Maestri R, Mortara A, Capomolla S, Febo O, Ferrari R, Franchini M, Gnemmi M, Opasich C, Riccardi PG, Traversi E, Cobelli F. Short-Term Heart Rate Variability Strongly Predicts Sudden Cardiac Death in Chronic Heart Failure Patients. *Circulation* 2003;**107**(4):565.

216. Mäkikallio TH, Huikuri HV, Hintze U, Videbæk J, Mitrani RD, Castellanos A, Myerburg RJ, Møller M. Fractal analysis and time- and frequency-domain measures of heart rate variability as predictors of mortality in patients with heart failure. *The American Journal of Cardiology* 2001;**87**(2):178-182.

217. Liao D, Cai J, Brancati FL, Folsom A, Barnes RW, Tyroler HA, Heiss G. Association of vagal tone with serum insulin, glucose, and diabetes mellitus — The ARIC Study. *Diabetes Research and Clinical Practice* 1995;**30**(3):211-221.

218. Singh JP, Larson MG, O'Donnell CJ, Wilson PF, Tsuji H, Lloyd-Jones DM, Levy D. Association of hyperglycemia with reduced heart rate variability (The Framingham Heart Study). *American Journal of Cardiology* 2000;**86**(3):309-312.

219. Schroeder EB, Chambless LE, Liao D, Prineas RJ, Evans GW, Rosamond WD, Heiss G. Diabetes, Glucose, Insulin, and Heart Rate Variability. *Diabetes Care* 2005;**28**(3):668.

220. Wulsin LR, Horn PS, Perry JL, Massaro JM, D'Agostino RB. Autonomic Imbalance as a Predictor of Metabolic Risks, Cardiovascular Disease, Diabetes, and Mortality. *The Journal of Clinical Endocrinology & Metabolism* 2015;**100**(6):2443-2448.

221. Christensen JH, Toft E, Christensen MS, Schmidt EB. Heart rate variability and plasma lipids in men with and without ischaemic heart disease. *Atherosclerosis* 1999;**145**(1):181-186.

222. Kupari M, Virolainen J, Koskinen P, Tikkanen MJ. Short-term heart rate variability and factors modifying the risk of coronary artery disease in a population sample. *The American Journal of Cardiology* 1993;**72**(12):897-903.

223. Licht CMM, de Geus EJC, Penninx BWJH. Dysregulation of the Autonomic Nervous System Predicts the Development of the Metabolic Syndrome. *The Journal of Clinical Endocrinology & Metabolism* 2013;**98**(6):2484-2493.

224. Schroeder EB, Liao D, Chambless LE, Prineas RJ, Evans GW, Heiss G. Hypertension, Blood Pressure, and Heart Rate Variability. *Hypertension* 2003;**42**(6):1106.

225. Singh JP, Larson MG, Tsuji H, Evans JC, O'Donnell CJ, Levy D. Reduced Heart Rate Variability and New-Onset Hypertension. *Hypertension* 1998;**32**(2):293.

226. Liao D, Cai J, Rosamond WD, Barnes RW, Hutchinson RG, Whitsel EA, Rautaharju P, Heiss G. Cardiac Autonomic Function and Incident Coronary Heart Disease: A Population-based Case-Cohort StudyThe ARIC Study. *American Journal of Epidemiology* 1997;**145**(8):696-706.

227. Tsuji H, Larson MG, Venditti FJ, Manders ES, Evans JC, Feldman CL, Levy D. Impact of Reduced Heart Rate Variability on Risk for Cardiac Events. *Circulation* 1996;**94**(11):2850.

228. Shah SA, Kambur T, Chan C, Herrington DM, Liu K, Shah SJ. Relation of Short-Term Heart Rate Variability to Incident Heart Failure (from the Multi-Ethnic Study of Atherosclerosis). *The American Journal of Cardiology* 2013;**112**(4):533-540.

229. Rossy LA, Thayer JF. Fitness and Gender-Related Differences in Heart Period Variability. *Psychosomatic Medicine* 1998;**60**(6):773-781.

230. Carter JB, Banister EW, Blaber AP. Effect of Endurance Exercise on Autonomic Control of Heart Rate. *Sports Medicine* 2003;**33**(1):33-46.

231. Antelmi I, De Paula RS, Shinzato AR, Peres CA, Mansur AJ, Grupi CJ. Influence of age, gender, body mass index, and functional capacity on heart rate variability in a cohort of subjects without heart disease. *American Journal of Cardiology* 2004;**93**(3):381-385.

232. Seals DR, Chase PB. Influence of physical training on heart rate variability and baroreflex circulatory control. *Journal of Applied Physiology* 1989;**66**(4):1886.

233. Rennie KL, Hemingway H, Kumari M, Brunner E, Malik M, Marmot M. Effects of Moderate and Vigorous Physical Activity on Heart Rate Variability in a British Study of Civil Servants. *American Journal of Epidemiology* 2003;**158**(2):135-143.

234. Sloan RP, Shapiro PA, DeMeersman RE, Bagiella E, Brondolo EN, McKinley PS, Slavov I, Fang Y, Myers MM. The Effect of Aerobic Training and Cardiac Autonomic Regulation in Young Adults. *American Journal of Public Health* 2009;**99**(5):921-928.

235. Hayano J, Yamada M, Sakakibara Y, Fujinami T, Yokoyama K, Watanabe Y, Takata K. Short- and long-term effects of cigarette smoking on heart rate variability. *American Journal of Cardiology* 1990;**65**(1):84-88.

236. Kobayashi F, Watanabe T, Akamatsu Y, Furui H, Tomita T, Ohashi R, Hayano J. Acute effects of cigarette smoking on the heart rate variability of taxi drivers during work. *Scandinavian Journal of Work, Environment & Health* 2005(5):360-366.

237. Minami J, Ishimitsu T, Matsuoka H. Effects of Smoking Cessation on Blood Pressure and Heart Rate Variability in Habitual Smokers. *Hypertension* 1999;**33**(1):586.

238. Yotsukura M, Koide Y, Fujii K, Tomono Y, Katayama A, Ando H, Suzuki J, Ishikawa K. Heart rate variability during the first month of smoking cessation. *American Heart Journal* 1998;**135**(6):1004-1009.

239. Pieters N, Plusquin M, Cox B, Kicinski M, Vangronsveld J, Nawrot TS. An epidemiological appraisal of the association between heart rate variability and particulate air pollution: a meta-analysis. *Heart* 2012;**98**(15):1127-35.

240. Pope Iii CA, Verrier RL, Lovett EG, Larson AC, Raizenne ME, Kanner RE, Schwartz J, Villegas GM, Gold DR, Dockery DW. Heart rate variability associated with particulate air pollution. *American Heart Journal* 1999;**138**(5):890-899.

241. Whitsel EA, Quibrera PM, Christ SL, Liao D, Prineas RJ, Anderson GL, Heiss G. Heart Rate Variability, Ambient Particulate Matter Air Pollution, and Glucose Homeostasis: The Environmental Epidemiology of Arrhythmogenesis in the Women's Health Initiative. *American Journal of Epidemiology* 2009;**169**(6):693-703.

242. Liao D, Duan Y, Whitsel EA, Zheng Z-j, Heiss G, Chinchilli VM, Lin H-M. Association of Higher Levels of Ambient Criteria Pollutants with Impaired Cardiac Autonomic Control: A Population-based Study. *American Journal of Epidemiology* 2004;**159**(8):768-777.

243. Graff DW, Cascio WE, Rappold A, Zhou H, Huang Y-CT, Devlin RB. Exposure to Concentrated Coarse Air Pollution Particles Causes Mild Cardiopulmonary Effects in Healthy Young Adults. *Environmental Health Perspectives* 2009;**117**(7):1089-1094.

244. Yeatts K, Svendsen E, Creason J, Alexis N, Herbst M, Scott J, Kupper L, Williams R, Neas L, Cascio W, Devlin RB, Peden DB. Coarse Particulate Matter (PM(2.5–10)) Affects Heart Rate Variability, Blood Lipids, and Circulating Eosinophils in Adults with Asthma. *Environmental Health Perspectives* 2007;**115**(5):709-714.

245. Lipsett MJ, Tsai FC, Roger L, Woo M, Ostro BD. Coarse Particles and Heart Rate Variability among Older Adults with Coronary Artery Disease in the Coachella Valley, California. *Environmental Health Perspectives* 2006;**114**(8):1215-1220.

246. Gong H, Jr., Linn WS, Terrell SL, Clark KW, Geller MD, Anderson KR, Cascio WE, Sioutas C. Altered heart-rate variability in asthmatic and healthy volunteers exposed to concentrated ambient coarse particles. *Inhal Toxicol* 2004;**16**(6-7):335-43.

247. Brook RD, Bard RL, Morishita M, Dvonch JT, Wang L, Yang H-y, Spino C, Mukherjee B, Kaplan MJ, Yalavarthi S, Oral EA, Ajluni N, Sun Q, Brook JR, Harkema J, Rajagopalan S. Hemodynamic, Autonomic, and Vascular Effects of Exposure to Coarse Particulate Matter Air Pollution from a Rural Location. *Environmental Health Perspectives* 2014;**122**(6):624-630.

248. Holliday KM, Avery CL, Poole C, McGraw K, Williams R, Liao D, Smith RL, Whitsel EA. Estimating Personal Exposures from Ambient Air Pollution Measures: Using Meta-Analysis to Assess Measurement Error. *Epidemiology* 2014;**25**(1):35-43.

249. Adhikari R, D'Souza J, Soliman EZ, Burke GL, Daviglus ML, Jacobs DR, Jr., Park SK, Sheppard L, Thorne PS, Kaufman JD, Larson TV, Adar SD. Long-term Coarse Particulate Matter Exposure and Heart Rate Variability in the Multi-ethnic Study of Atherosclerosis. *Epidemiology* 2016;**27**(3).

250. Adam M, Felber Dietrich D, Schaffner E, Carballo D, Barthélémy J-C, Gaspoz J-M, Tsai M-Y, Rapp R, Phuleria HC, Schindler C, Schwartz J, Künzli N, Probst-Hensch NM. Long-term exposure to traffic-related PM10 and decreased heart rate variability: Is the association restricted to subjects taking ACE inhibitors? *Environment International* 2012;**48**:9-16.

251. Liao D, Creason J, Shy C, Williams R, Watts R, Zweidinger R. Daily variation of particulate air pollution and poor cardiac autonomic control in the elderly. *Environmental Health Perspectives* 1999;**107**(7):521-525.

252. Holguín F, Téllez-Rojo MM, Hernández M, Cortez M, Chow JC, Watson JG, Mannino D, Romieu I. Air Pollution and Heart Rate Variability Among the Elderly in Mexico City. *Epidemiology* 2003;**14**(5):521-527.

253. Park SK, O'Neill MS, Vokonas PS, Sparrow D, Schwartz J. Effects of Air Pollution on Heart Rate Variability: The VA Normative Aging Study. *Environmental Health Perspectives* 2005;**113**(3):304-309.

254. Schwartz J, Litonjua A, Suh H, Verrier M, Zanobetti A, Syring M, Nearing B, Verrier R, Stone P, MacCallum G, Speizer F, Gold D. Traffic related pollution and heart rate variability in a panel of elderly subjects. *Thorax* 2005;**60**(6):455-461.

255. Luttmann-Gibson H, Suh HH, Coull BA, Dockery DW, Sarnat SE, Schwartz J, Stone PH, Gold DR. Short-Term Effects of Air Pollution on Heart Rate Variability in Senior Adults in Steubenville, Ohio. *Journal of Occupational and Environmental Medicine* 2006;**48**(8):780-788.

256. Creason J, Neas L, Walsh D, Williams RON, Sheldon L, Liao D, Shy C. Particulate matter and heart rate variability among elderly retirees: the Baltimore 1998 PM study. *J Expo Anal Environ Epidemiol* 2001;**11**(2):116-122.

257. Devlin RB, Ghio AJ, Kehrl H, Sanders G, Cascio W. Elderly humans exposed to concentrated air pollution particles have decreased heart rate variability. *European Respiratory Journal* 2003;**21**(40 suppl):76s.

258. Chuang K-J, Chan C-C, Chen N-T, Su T-C, Lin L-Y. Effects of Particle Size Fractions on Reducing Heart Rate Variability in Cardiac and Hypertensive Patients. *Environmental Health Perspectives* 2005;**113**(12):1693-1697.

259. Timonen KL, Vanninen E, de Hartog J, Ibald-Mulli A, Brunekreef B, Gold DR, Heinrich J, Hoek G, Lanki T, Peters A, Tarkiainen T, Tiittanen P, Kreyling W, Pekkanen J. Effects of ultrafine and fine particulate and gaseous air pollution on cardiac autonomic control in subjects with coronary artery disease: The ULTRA study. *J Expos Sci Environ Epidemiol* 2005;**16**(4):332-341.

260. Zanobetti A, Gold DR, Stone PH, Suh HH, Schwartz J, Coull BA, Speizer FE. Reduction in Heart Rate Variability with Traffic and Air Pollution in Patients with Coronary Artery Disease. *Environmental Health Perspectives* 2010;**118**(3):324-330.

261. Riojas-Rodriguez H, Escamilla-Cejudo JA, Gonzalez-Hermosillo JA, Tellez-Rojo MM, Vallejo M, Santos-Burgoa C, Rojas-Bracho L. Personal PM2.5 and CO exposures and heart rate variability in subjects with known ischemic heart disease in Mexico City. *J Expos Sci Environ Epidemiol* 2005;**16**(2):131-137.

262. Stone PH, Godleski JJ. First steps toward understanding the pathophysiologic link between air pollution and cardiac mortality. *American Heart Journal* 1999;**138**(5):804-807.

263. Chin MT. Basic mechanisms for adverse cardiovascular events associated with air pollution. *Heart* 2015;**101**(4):253.

264. Park SK, O'Neill MS, Wright RO, Hu H, Vokonas PS, Sparrow D, Suh H, Schwartz J. HFE Genotype, Particulate Air Pollution, and Heart Rate Variability. *Circulation* 2006;**114**(25):2798.

265. Schwartz J, Park SK, O'Neill MS, Vokonas PS, Sparrow D, Weiss S, Kelsey K. Glutathione-S-Transferase M1, Obesity, Statins, and Autonomic Effects of Particles. *American Journal of Respiratory and Critical Care Medicine* 2005;**172**(12):1529-1533.

266. Baccarelli A, Cassano PA, Litonjua A, Park SK, Suh H, Sparrow D, Vokonas P, Schwartz J. Cardiac Autonomic Dysfunction: Effects From Particulate Air Pollution and Protection by Dietary Methyl Nutrients and Metabolic Polymorphisms. *Circulation* 2008;**117**(14):1802.

267. Chahine T, Baccarelli A, Litonjua A, Wright RO, Suh H, Gold DR, Sparrow D, Vokonas P, Schwartz J. Particulate Air Pollution, Oxidative Stress Genes, and Heart Rate Variability in an Elderly Cohort. *Environmental Health Perspectives* 2007;**115**(11):1617-1622.

268. Ren C, Baccarelli A, Wilker E, Suh H, Sparrow D, Vokonas P, Wright R, Schwartz J. Lipid and endothelium-related genes, ambient particulate matter, and heart rate variability—the VA Normative Aging Study. *Journal of Epidemiology and Community Health* 2009;**64**(01):49.

269. Romieu I, Téllez-Rojo MM, Lazo M, Manzano-Patiño A, Cortez-Lugo M, Julien P, Bélanger MC, Hernandez-Avila M, Holguin F. Omega-3 Fatty Acid Prevents Heart Rate Variability Reductions Associated with Particulate Matter. *American Journal of Respiratory and Critical Care Medicine* 2005;**172**(12):1534-1540.

270. Min KB, Min JY, Cho SI, Paek D. The relationship between air pollutants and heart-rate variability among community residents in Korea. *Inhal Toxicol* 2008;**20**(4):435-44.

271. Breithardt G, Herion JC, Oto A, Palmer JG. *Myocardial repolarization : from gene to bedside*. Armonk, NY: Futura Pub., 2001.

272. Al-Khatib SM, LaPointe N, Kramer JM, Califf RM. What clinicians should know about the qt interval. *JAMA* 2003;**289**(16):2120-2127.

273. Cutler MJ, Jeyaraj D, Rosenbaum DS. Cardiac electrical remodeling in health and disease. *Trends in Pharmacological Sciences* 2011;**32**(3):174-180.

274. Houghton AR, Roebuck A. *Pocket ECGs for Nurses* Taylor & Francis, 2015.

275. Arking DE, Pulit SL, Crotti L, van der Harst P, Munroe PB, Koopmann TT, Sotoodehnia N, Rossin EJ, Morley M, Wang X, Johnson AD, Lundby A, Gudbjartsson DF, Noseworthy PA, Eijgelsheim M, Bradford Y, Tarasov KV, Dörr M, Müller-Nurasyid M, Lahtinen AM, Nolte IM, Smith AV, Bis JC, Isaacs A, Newhouse SJ, Evans DS, Post WS, Waggott D, Lyytikäinen L-P, Hicks AA, Eisele L, Ellinghaus D, Hayward C, Navarro P, Ulivi S, Tanaka T, Tester DJ, Chatel S, Gustafsson S, Kumari M, Morris RW, Naluai ÅT, Padmanabhan S, Kluttig A, Strohmer B, Panayiotou AG, Torres M, Knoflach M, Hubacek JA, Slowikowski K, Raychaudhuri S, Kumar RD, Harris TB, Launer LJ, Shuldiner AR, Alonso A, Bader JS, Ehret G, Huang H, Kao WHL, Strait JB, Macfarlane PW, Brown M, Caulfield MJ, Samani NJ, Kronenberg F, Willeit J, Consortium CA, Consortium C, Smith JG, Greiser KH, Meyer zu Schwabedissen H, Werdan K, Carella M, Zelante L, Heckbert SR, Psaty BM, Rotter JI, Kolcic I, Polašek O, Wright AF, Griffin M, Daly MJ, Dcct/Edic, Arnar DO, Hólm H, Thorsteinsdottir U, e MC, Denny JC, Roden DM, Zuvich RL, Emilsson V, Plump AS, Larson MG, O'Donnell CJ, Yin X, Bobbo M, D'Adamo AP, Iorio A, Sinagra G, et al. Genetic association study of QT interval highlights role for calcium signaling pathways in myocardial repolarization. *Nature Genetics* 2014;**46**:826.

276. Newton-Cheh C, Eijgelsheim M, Rice KM, de Bakker PIW, Yin X, Estrada K, Bis JC, Marciante K, Rivadeneira F, Noseworthy PA, Sotoodehnia N, Smith NL, Rotter JI, Kors JA, Witteman JCM, Hofman A, Heckbert SR, O'Donnell CJ, Uitterlinden AG, Psaty BM, Lumley T, Larson MG, Ch Stricker BH. Common variants at ten loci influence QT interval duration in the QTGEN Study. *Nature Genetics* 2009;**41**:399.

277. Pfeufer A, Sanna S, Arking DE, Müller M, Gateva V, Fuchsberger C, Ehret GB, Orrú M, Pattaro C, Köttgen A, Perz S, Usala G, Barbalic M, Li M, Pütz B, Scuteri A, Prineas RJ, Sinner MF, Gieger C, Najjar SS, Kao WHL, Mühleisen TW, Dei M, Happle C, Möhlenkamp S, Crisponi L, Erbel R, Jöckel K-H, Naitza S, Steinbeck G, Marroni F, Hicks AA, Lakatta E, Müller-Myhsok B, Pramstaller PP, Wichmann HE, Schlessinger D, Boerwinkle E, Meitinger T, Uda M, Coresh J, Kääb S, Abecasis GR, Chakravarti A. Common variants at ten loci modulate the QT interval duration in the QTSCD Study. *Nature Genetics* 2009;**41**:407.

278. Schwartz PJ, Woosley RL. Predicting the Unpredictable: Drug-Induced QT Prolongation and Torsades de Pointes. *Journal of the American College of Cardiology* 2016;**67**(13):1639.

279. Milne JR, Ward DE, Spurrell RAJ, Camm AJ. The Ventricular Paced QT Interval—The Effects of Rate and Exercise. *Pacing and Clinical Electrophysiology* 1982;**5**(3):352-358.

280. Rautaharju PM, Warren JW, Calhoun HP. Estimation of QT prolongation: A persistent, avoidable error in computer electrocardiography. *Journal of Electrocardiology* 1990;**23**:111-117.

281. Fridericia LS. Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. *Acta Medica Scandinavica* 1920;**53**(1):469-486.

282. Rautaharju PM, Zhang Z-M, Prineas R, Heiss G. Assessment of prolonged QT and JT intervals in ventricular conduction defects. *The American Journal of Cardiology* 2004;**93**(8):1017-1021.

283. Sagie A, Larson MG, Goldberg RJ, Bengtson JR, Levy D. An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study). *The American Journal of Cardiology* 1992;**70**(7):797-801.

284. Hodges M. Bazett's QT correction reviewed: evidence that a linear QT correction for heart rate is better. *J Am Coll Cardiol* 1983;**1**:694.

285. Karjalainen J, Viitasalo M, Mänttäri M, Manninen V. Relation between QT intervals and heart rates from 40 to 120 beats/min in rest electrocardiograms of men and a simple method to adjust QT interval values. *Journal of the American College of Cardiology* 1994;**23**(7):1547-1553.

286. Kors JA, van Herpen G. Measurement error as a source of QT dispersion: a computerised analysis. *Heart* 1998;**80**(5):453.

287. Vaidean GD, Schroeder EB, Whitsel EA, Prineas RJ, Chambless LE, Perhac JS, Heiss G, Rautaharju PM. Short-term repeatability of electrocardiographic spatial T-wave axis and QT interval. *J Electrocardiol* 2005;**38**(2):139-47.

288. Kautzner J, Yi G, Camm AJ, Malik M. Short-and Long-Term Reproducibility of QT, QTc, and QT Dispersion Measurement in Healthy Subjects. *Pacing and Clinical Electrophysiology* 1994;**17**(5):928-937.

289. Dessertenne F. Ventricular tachycardia with 2 variable opposing foci. *Arch Mal Coeur Vaiss* 1966;**59**(2):263-72.

290. Yap YG, Camm AJ. Drug induced QT prolongation and torsades de pointes. *Heart* 2003;**89**(11):1363.

291. Dekker JM, Crow RS, Hannan PJ, Schouten EG, Folsom AR. Heart rate-corrected QT interval prolongation predicts risk of coronary heart disease in black and white middle-aged men and women: The ARIC study. *Journal of the American College of Cardiology* 2004;**43**(4):565-571.

292. Rautaharju PM, Kooperberg C, Larson JC, LaCroix A. Electrocardiographic Abnormalities That Predict Coronary Heart Disease Events and Mortality in Postmenopausal Women. *Circulation* 2006;**113**(4):473.

293. Rautaharju PM, Kooperberg C, Larson JC, LaCroix A. Electrocardiographic Predictors of Incident Congestive Heart Failure and All-Cause Mortality in Postmenopausal Women. *Circulation* 2006;**113**(4):481.

294. Soliman EZ, Howard G, Cushman M, Kissela B, Kleindorfer D, Le A, Judd S, McClure LA, Howard VJ. Prolongation of QTc and Risk of Stroke. *The REGARDS (REasons for Geographic and Racial Differences in Stroke) Study* 2012;**59**(16):1460-1467.

295. Schouten EG, Dekker JM, Meppelink P, Kok FJ, Vandenbroucke JP, Pool J. QT interval prolongation predicts cardiovascular mortality in an apparently healthy population. *Circulation* 1991;**84**(4):1516-23.

296. Zhang Y, Post WS, Blasco-Colmenares E, Dalal D, Tomaselli GF, Guallar E. Electrocardiographic QT interval and mortality: a meta-analysis. *Epidemiology (Cambridge, Mass.)* 2011;**22**(5):660-670.

297. Okin PM, Devereux RB, Howard BV, Fabsitz RR, Lee ET, Welty TK. Assessment of QT Interval and QT Dispersion for Prediction of All-Cause and Cardiovascular Mortality in American Indians. *Circulation* 2000;**101**(1):61-66.

298. Straus SMJM, Kors JA, De Bruin ML, van der Hooft CS, Hofman A, Heeringa J, Deckers JW, Kingma JH, Sturkenboom MCJM, Stricker BHC, Witteman JCM. Prolonged QTc Interval and Risk of Sudden Cardiac Death in a Population of Older Adults. *Journal of the American College of Cardiology* 2006;**47**(2):362.

299. Aerssens J, Paulussen ADC. Pharmacogenomics and acquired long QT syndrome. *Pharmacogenomics* 2005;**6**(3):259-270.

300. Benoit SR, Mendelsohn AB, Nourjah P, Staffa JA, Graham DJ. Risk factors for prolonged QTc among US adults: Third National Health and Nutrition Examination Survey. *European Journal of Cardiovascular Prevention & Rehabilitation* 2005;**12**(4):363-368.

301. Lehmann MH, Hardy S, Archibald D, Quart B, MacNeil DJ. Sex Difference in Risk of Torsade de Pointes With d,l-Sotalol. *Circulation* 1996;**94**(10):2535-2541.

302. Strohmer B, Pichler M, Iglseder B, Paulweber B. Relationship of QT interval duration with carotid intima media thickness in a clinically healthy population undergoing cardiovascular risk screening. *Journal of Internal Medicine* 2005;**257**(3):238-246.

303. Festa A, D'Agostino R, Jr., Rautaharju P, O'Leary DH, Rewers M, Mykkanen L, Haffner SM. Is QT interval a marker of subclinical atherosclerosis in nondiabetic subjects? The Insulin Resistance Atherosclerosis Study (IRAS). *Stroke* 1999;**30**(8):1566-71.

304. Naas AAO, Davidson NC, Thompson C, Cummings F, Ogston SA, Jung RT, Newton RW, Struthers AD. QT and QTc dispersion are accurate predictors of cardiac death in newly diagnosed non-insulin dependent diabetes: cohort study. *BMJ* 1998;**316**(7133):745-746.

305. Whitsel EA, Boyko EJ, Rautaharju PM, Raghunathan TE, Lin D, Pearce RM, Weinmann SA, Siscovick DS. Electrocardiographic QT Interval Prolongation and Risk of Primary Cardiac Arrest in Diabetic Patients. *Diabetes Care* 2005;**28**(8):2045-2047.

306. Whitsel EA, Raghunathan TE, Pearce RM, Lin D, Rautaharju PM, Lemaitre R, Siscovick DS. RR interval variation, the QT interval index and risk of primary cardiac arrest among patients without clinically recognized heart disease. *European Heart Journal* 2001;**22**(2):165-173.

307. Veglio M, Bruno G, Borra M, Macchia G, Bargero G, D'Errico N, Pagano GF, Cavallo-Perin P. Prevalence of increased QT interval duration and dispersion in type 2 diabetic patients and its relationship with coronary heart disease: a population-based cohort. *Journal of Internal Medicine* 2002;**251**(4):317-324.

308. Dekker JM, Feskens EJ, Schouten EG, Klootwijk P, Pool J, Kromhout D. QTc Duration is Associated With Levels of Insulin and Glucose Tolerance: The Zutphen Elderly Study. *Diabetes* 1996;**45**(3):376-380.

309. Marfella R, Nappo F, De Angelis L, Siniscalchi M, Rossi F, Giugliano D. The effect of acute hyperglycaemia on QTc duration in healthy man. *Diabetologia* 2000;**43**(5):571-575.

310. Gastaldelli A, Emdin M, Conforti F, Camastra S, Ferrannini E. Insulin prolongs the QTc interval in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 2000;**279**(6):R2022-R2025.

311. Fauchier L, Maison-Blanche P, Forhan A, D'Hour A, Lépinay P, Tichet J, Vol S, Coumel P, Fauchier JP, Balkau B. Association between heart rate–corrected QT interval and coronary risk factors in 2,894 healthy subjects (the DESIR study). *The American Journal of Cardiology* 2000;**86**(5):557-559.

312. Carella MJ, Mantz SL, Rovner DR, Willis PW, 3rd, Gossain VV, Bouknight RR, Ferenchick GS. Obesity, adiposity, and lengthening of the QT interval: improvement after weight loss. *Int J Obes Relat Metab Disord* 1996;**20**(10):938-42.

313. Frank S, Colliver JA, Frank A. The electrocardiogram in obesity: Statistical analysis of 1,029 patients. *Journal of the American College of Cardiology* 1986;**7**(2):295.

314. Roden DM. Drug-Induced Prolongation of the QT Interval. *New England Journal of Medicine* 2004;**350**(10):1013-1022.

315. Kannankeril P, Roden DM, Darbar D. Drug-Induced Long QT Syndrome. *Pharmacological Reviews* 2010;**62**(4):760.

316. Pasquier M, Pantet O, Hugli O, Pruvot E, Buclin T, Waeber G, Aujesky D. Prevalence and determinants of QT interval prolongation in medical inpatients. *Internal Medicine Journal* 2012;**42**(8):933-940.

317. Goldenberg I, Moss AJ. Long QT Syndrome. *Journal of the American College of Cardiology* 2008;**51**(24):2291.

318. Baja ES, Schwartz JD, Wellenius GA, Coull BA, Zanobetti A, Vokonas PS, Suh HH. Traffic-Related Air Pollution and QT Interval: Modification by Diabetes, Obesity, and Oxidative Stress Gene Polymorphisms in the Normative Aging Study. *Environmental Health Perspectives* 2010;**118**(6):840-846.

319. Mordukhovich I, Kloog I, Coull B, Koutrakis P, Vokonas P, Schwartz J. Association between Particulate Air Pollution and QT Interval Duration in an Elderly Cohort. *Epidemiology (Cambridge, Mass.)* 2016;**27**(2):284-290.

320. Zhang S, Breitner S, Cascio WE, Devlin RB, Neas LM, Diaz-Sanchez D, Kraus WE, Schwartz J, Hauser ER, Peters A, Schneider A. Short-term effects of fine particulate matter and ozone on the cardiac conduction system in patients undergoing cardiac catheterization. *Particle and Fibre Toxicology* 2018;**15**(1):38.

321. Liao D, Shaffer ML, Rodriguez-Colon S, He F, Li X, Wolbrette DL, Yanosky J, Cascio WE. Acute adverse effects of fine particulate air pollution on ventricular repolarization. *Environ Health Perspect* 2010;**118**(7):1010-5.

322. Hampel R, Schneider A, Brüske I, Zareba W, Cyrys J, Rückerl R, Breitner S, Korb H, Sunyer J, Wichmann HE, Peters A. Altered Cardiac Repolarization in Association with Air Pollution and Air Temperature among Myocardial Infarction Survivors. *Environmental Health Perspectives* 2010;**118**(12):1755-1761.

323. Henneberger A, Zareba W, Ibald-Mulli A, Ruckerl R, Cyrys J, Couderc JP, Mykins B, Woelke G, Wichmann HE, Peters A. Repolarization changes induced by air pollution in ischemic heart disease patients. *Environ Health Perspect* 2005;**113**(4):440-6.

324. Schneider A, Neas LM, Graff DW, Herbst MC, Cascio WE, Schmitt MT, Buse JB, Peters A, Devlin RB. Association of cardiac and vascular changes with ambient PM2.5 in diabetic individuals. *Particle and Fibre Toxicology* 2010;**7**(1):14.

325. Van Hee VC, Szpiro AA, Prineas R, Neyer J, Watson K, Siscovick D, Kyun Park S, Kaufman JD. Association of Long-term Air Pollution With Ventricular Conduction and Repolarization Abnormalities. *Epidemiology* 2011;**22**(6):773-780.

326. Simkhovich BZ, Kleinman MT, Kloner RA. Air Pollution and Cardiovascular Injury. *Journal of the American College of Cardiology* 2008;**52**(9):719.

327. Coetzee WA, Ichikawa H, Hearse DJ. Oxidant stress inhibits Na-Ca-exchange current in cardiac myocytes: mediation by sulfhydryl groups? *American Journal of Physiology-Heart and Circulatory Physiology* 1994;**266**(3):H909-H919.

328. Chiamvimonvat N, O'Rourke B, Kamp Timothy J, Kallen Roland G, Hofmann F, Flockerzi V, Marban E. Functional Consequences of Sulfhydryl Modification in the Pore-Forming Subunits of Cardiovascular Ca2+ and Na+ Channels. *Circulation Research* 1995;**76**(3):325-334.

329. Diem R, Hobom M, Grötsch P, Kramer B, Bähr M. Interleukin-1β protects neurons via the interleukin-1 (IL-1) receptor-mediated Akt pathway and by IL-1 receptor-independent decrease of transmembrane currents in vivo. *Molecular and Cellular Neuroscience* 2003;**22**(4):487-500.

330. Chanson M, Berclaz P-Y, Scerri I, Dudez T, Wernke-Dollries K, Pizurki L, Pavirani A, Fiedler MA, Suter S. Regulation of Gap Junctional Communication by a Pro-Inflammatory Cytokine in Cystic Fibrosis Transmembrane Conductance Regulator-Expressing but Not Cystic Fibrosis Airway Cells. *The American Journal of Pathology* 2001;**158**(5):1775-1784.

331. Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* 1992;**257**(5068):387.

332. Wang T, Lang GD, Moreno-Vinasco L, Huang Y, Goonewardena SN, Peng YJ, Svensson EC, Natarajan V, Lang RM, Linares JD, Breysse PN, Geyh AS, Samet JM, Lussier YA, Dudley S, Prabhakar NR, Garcia JGN. Particulate Matter Induces Cardiac Arrhythmias via Dysregulation of Carotid Body Sensitivity and Cardiac Sodium Channels. *American Journal of Respiratory Cell and Molecular Biology* 2012;**46**(4):524-531.

333. Ghelfi E, Rhoden CR, Wellenius GA, Lawrence J, Gonzalez-Flecha B. Cardiac Oxidative Stress and Electrophysiological Changes in Rats Exposed to Concentrated Ambient Particles are Mediated by TRP-Dependent Pulmonary Reflexes. *Toxicological Sciences* 2008;**102**(2):328-336.

334. Graff DW, Cascio WE, Brackhan JA, Devlin RB. Metal particulate matter components affect gene expression and beat frequency of neonatal rat ventricular myocytes. *Environmental Health Perspectives* 2004;**112**(7):792-798.

335. Nelin TD, Joseph AM, Gorr MW, Wold LE. Direct and indirect effects of particulate matter on the cardiovascular system. *Toxicology Letters* 2012;**208**(3):293-299.

336. Lazzerini PE, Laghi-Pasini F, Bertolozzi I, Morozzi G, Lorenzini S, Simpatico A, Selvi E, Bacarelli MR, Finizola F, Vanni F, Lazaro D, Aromolaran A, El Sherif N, Boutjdir M, Capecchi PL. Systemic inflammation as a novel QT-prolonging risk factor in patients with torsades de pointes. *Heart* 2017;**103**(22):1821.

337. Adlan AM, Panoulas VF, Smith JP, Fisher JP, Kitas GD. Association Between Corrected QT Interval and Inflammatory Cytokines in Rheumatoid Arthritis. *The Journal of Rheumatology* 2015;**42**(3):421.

338. Kim E, Joo S, Kim J, Ahn J, Kim J, Kimm K, Shin C. Association between C-reactive protein and QTc interval in middle-aged men and women. *European Journal of Epidemiology* 2006;**21**(9):653.

339. Gondalia R, Avery CL, Napier MD, Mendez-Giraldez R, Stewart JD, Sitlani CM, Li Y, Wilhelmsen KC, Duan Q, Roach J, North KE, Reiner AP, Zhang ZM, Tinker LF, Yanosky JD, Liao D, Whitsel EA. Genome-wide Association Study of Susceptibility to Particulate Matter-Associated QT Prolongation. *Environ Health Perspect* 2017;**125**(6):067002.

340. Kobayashi Y. The regulatory role of nitric oxide in proinflammatory cytokine expression during the induction and resolution of inflammation. *Journal of Leukocyte Biology* 2010;**88**(6):1157-1162.

341. Anderson GL, Manson J, Wallace R, Lund B, Hall D, Davis S, Shumaker S, Wang C-Y, Stein E, Prentice RL. Implementation of the Women's Health Initiative study design. *Annals of epidemiology* 2003;**13**(9):S5-S17.

342. Women's Health Initiative Clinical Coordinating Center. https://www.whi.org/researchers/data/_layouts/15/WopiFrame.aspx?sourcedoc=/researchers/data/WHI Progress Reports/2014 Annual.pdf&action=default Accessed 05/24, 2017.

343. Anderson GL, LaCroix A. W64 - Long Life Study (Long Life Study). https://www.whi.org/studies/SitePages/Long Life Study.aspx Accessed 04/20, 2018.

344. Smoller JW, Pollack MH, Wassertheil-Smoller S, Barton B, Hendrix SL, Jackson RD, Dicken T, Oberman A, Sheps DS. Prevalence and correlates of panic attacks in postmenopausal women: results from an ancillary study to the Women's Health Initiative. *Arch Intern Med* 2003;**163**(17):2041-50.

345. Whitsel EA. AS315 - Epigenetic Mechanisms of PM-mediated CVD Risk. https://projectreporter.nih.gov/project_info_description.cfm?aid=9054857&icde=40216248&ddparam=&ddvalue=&ddsub=&cr=1&csb=default&cs=ASC&pball=. Accessed 04/20, 2018.

346. Assimes T, Tsao P, Absher D, Horvath S. BA23 - Integrative genomics and risk of CHD and related phenotypes in the Women's Health Initiative. https://www.whi.org/researchers/data/WHIStudies/StudySites/BA23/pages/home.aspx Accessed 04/20, 2018.

347. M5 - SNP Health Association Resource (SHARe). https://www.whi.org/researchers/data/WHIStudies/StudySites/M5/pages/home.aspx.

348. W54 – CVD Biomarkers in SHARe (M5) Participants. https://www.whi.org/researchers/data/WHIStudies/StudySites/W54/pages/home.aspx.

349. W58 - CVD Biomarkers in Non-minority HT Participants. https://www.whi.org/researchers/data/WHIStudies/StudySites/W58/pages/home.aspx.

350. W63 - GWAS on WHIMS and Subsample of HT EA Women.

351. Bhatti P. AS311 - DNA Methylation Measured in Prospectively Collected Blood Samples and Risk of Bladder Cancer Among Post-menopausal Women. https://www.whi.org/researchers/data/WHIStudies/StudySites/AS311/Pages/home.aspx Accessed 04/20, 2018.

352. Mosley TH, Knopman DS, Catellier DJ, Bryan N, Hutchinson RG, Grothues CA, Folsom AR, Cooper LS, Burke GL, Liao D, Szklo M. Cerebral MRI findings and cognitive functioning. *Neurology* 2005;**64**(12):2056.

353. Whitsel EA, Rose KM, Wood JL, Henley AC, Liao D, Heiss G. Accuracy and repeatability of commercial geocoding. *Am J Epidemiol* 2004;**160**(10):1023-9.

354. Whitsel EA, Quibrera PM, Smith RL, Catellier DJ, Liao D, Henley AC, Heiss G. Accuracy of commercial geocoding: assessment and implications. *Epidemiol Perspect Innov* 2006;**3**:8.

355. Liao D, Peuquet DJ, Duan Y, Whitsel EA, Dou J, Smith RL, Lin H-M, Chen J-C, Heiss G. GIS approaches for the estimation of residential-level ambient PM concentrations. *Environmental health perspectives* 2006:1374-1380.

356. Cressie NA. Spatial prediction and kriging. *Statistics for Spatial Data, Revised Edition* 1993:105-209.

357. Jian X, Olea RA, Yu Y-S. Semivariogram modeling by weighted least squares. *Computers & Geosciences* 1996;**22**(4):387-397.

358. Gribov A, Krivoruchko K, Ver Hoef J. Modified weighted least squares semivariogram and covariance model fitting algorithm. *Stochastic Modeling & Geostatistics: Principles, Methods and Case Studies* 2000;**2**.

359. Yanosky JD, Paciorek CJ, Laden F, Hart JE, Puett RC, Liao D, Suh HH. Spatio-temporal modeling of particulate air pollution in the conterminous United States using geographic and meteorological predictors. *Environ Health* 2014;**13**:63.

360. Teschendorff AE, Marabita F, Lechner M, Bartlett T, Tegner J, Gomez-Cabrero D, Beck S. A beta-mixture quantile normalization method for correcting probe design bias in Illumina Infinium 450 k DNA methylation data. *Bioinformatics* 2013;**29**(2):189-196.

361. Papp AC, Hatzakis H, Bracey A, Wu KK. ARIC hemostasis study--I. Development of a blood collection and processing system suitable for multicenter hemostatic studies. *Thromb Haemost* 1989;**61**(1):15-9.

362. Koestler DC, Christensen B, Karagas MR, Marsit CJ, Langevin SM, Kelsey KT, Wiencke JK, Houseman EA. Blood-based profiles of DNA methylation predict the underlying distribution of cell types: a validation analysis. *Epigenetics* 2013;**8**(8):816-26.

363. Atherosclerosis Risk in Communities Study (ARIC). Operations manual no. 5: electrocardiography. Version 1.0. *Chapel Hill, NC: ARIC Coordinating Center, School of Public Health, University of North Carolina* 1987.

364. WHI - Volume 2, Section 13 - ECG Procedures. https://biolincc.nhlbi.nih.gov/static/studies/whios/hidden/doc/whi/procedur/13.pdf.

365. Roux AVD, Merkin SS, Arnett D, Chambless L, Massing M, Nieto FJ, Sorlie P, Szklo M, Tyroler HA, Watson RL. Neighborhood of Residence and Incidence of Coronary Heart Disease. *New England Journal of Medicine* 2001;**345**(2):99-106.

366. Stolwijk AM, Straatman H, Zielhuis GA. Studying seasonality by using sine and cosine functions in regression analysis. *Journal of Epidemiology and Community Health* 1999;**53**(4):235-238.

367. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine* 2002;**21**(11):1539-1558.

368. Rice K, Higgins JPT, Lumley T. A re-evaluation of fixed effect(s) meta-analysis. *Journal of the Royal Statistical Society: Series A (Statistics in Society)* 2017:n/a-n/a.

369. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Controlled Clinical Trials* 1986;**7**(3):177-188.

370. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. *Epidemiology* 2004;**15**(5):615-25.

371. Azur MJ, Stuart EA, Frangakis C, Leaf PJ. Multiple imputation by chained equations: what is it and how does it work? *Int J Methods Psychiatr Res* 2011;**20**(1):40-9.

372. Stuart EA, Azur M, Frangakis C, Leaf P. Multiple imputation with large data sets: a case study of the Children's Mental Health Initiative. *Am J Epidemiol* 2009;**169**(9):1133-9.

373. Howe CJ, Cole SR, Lau B, Napravnik S, Eron JJ, Jr. Selection Bias Due to Loss to Follow Up in Cohort Studies. *Epidemiology* 2016;**27**(1):91-7.

374. Cochran WG. The Combination of Estimates from Different Experiments. *Biometrics* 1954;**10**(1):101-129.

375. Aitchison J. The Statistical Analysis of Compositional Data. *Journal of the Royal Statistical Society. Series B (Methodological)* 1982;**44**(2):139-177.

376. Egozcue JJ, Pawlowsky-Glahn V, Mateu-Figueras G, Barceló-Vidal C. Isometric Logratio Transformations for Compositional Data Analysis. *Mathematical Geology* 2003;**35**(3):279-300.

377. Chastin SF, Palarea-Albaladejo J, Dontje ML, Skelton DA. Combined Effects of Time Spent in Physical Activity, Sedentary Behaviors and Sleep on Obesity and Cardio-Metabolic Health Markers: A Novel Compositional Data Analysis Approach. *PLoS One* 2015;**10**(10):e0139984.

378. Fairclough SJ, Dumuid D, Taylor S, Curry W, McGrane B, Stratton G, Maher C, Olds T. Fitness, fatness and the reallocation of time between children's daily movement behaviours: an analysis of compositional data. *Int J Behav Nutr Phys Act* 2017;**14**(1):64.

379. Pawlowsky-Glahn V, Egozcue JJ, Tolosana-Delgado R. Linear models. *Modelling and Analysis of Compositional Data* John Wiley & Sons, Ltd, 2015;149-171.

380. Devlin B, Roeder K, Wasserman L. Genomic control, a new approach to genetic-based association studies. *Theor Popul Biol* 2001;**60**(3):155-66.

381. van Iterson M, van Zwet EW, Heijmans BT. Controlling bias and inflation in epigenome- and transcriptome-wide association studies using the empirical null distribution. *Genome Biol* 2017;**18**(1):19.

382. Cornbleet PJ, Gochman N. Incorrect least-squares regression coefficients in method-comparison analysis. *Clinical Chemistry* 1979;**25**(3):432-438.

383. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, Klemm A, Flicek P, Manolio T, Hindorff L, Parkinson H. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Research* 2014;**42**(D1):D1001-D1006.

384. Lonsdale J, Thomas J, Salvatore M, Phillips R, Lo E, Shad S, Hasz R, Walters G, Garcia F, Young N, Foster B, Moser M, Karasik E, Gillard B, Ramsey K, Sullivan S, Bridge J, Magazine H, Syron J, Fleming J, Siminoff L, Traino H, Mosavel M, Barker L, Jewell S, Rohrer D, Maxim D, Filkins D, Harbach P, Cortadillo E, Berghuis B, Turner L, Hudson E, Feenstra K, Sobin L, Robb J, Branton P, Korzeniewski G, Shive C, Tabor D, Qi L, Groch K, Nampally S, Buia S, Zimmerman A, Smith A, Burges R, Robinson K, Valentino K, Bradbury D, Cosentino M, Diaz-Mayoral N, Kennedy M, Engel T, Williams P, Erickson K, Ardlie K, Winckler W, Getz G, DeLuca D, MacArthur D, Kellis M, Thomson A, Young T, Gelfand E, Donovan M, Meng Y, Grant G, Mash D, Marcus Y, Basile M, Liu J, Zhu J, Tu Z, Cox NJ, Nicolae DL, Gamazon ER, Im HK, Konkashbaev A, Pritchard J, Stevens M, Flutre T, Wen X, Dermitzakis ET, Lappalainen T, Guigo R, Monlong J, Sammeth M, Koller D, Battle A, Mostafavi S, McCarthy M, Rivas M, Maller J, Rusyn I, Nobel A, Wright F, Shabalin A, Feolo M, Sharopova N, et al. The Genotype-Tissue Expression (GTEx) project. *Nature Genetics* 2013;**45**:580.

385. Kennedy EM, Goehring GN, Nichols MH, Robins C, Mehta D, Klengel T, Eskin E, Smith AK, Conneely KN. An integrated -omics analysis of the epigenetic landscape of gene expression in human blood cells. *BMC Genomics* 2018;**19**(1):476.

386. Breeze Charles E, Paul Dirk S, van Dongen J, Butcher Lee M, Ambrose John C, Barrett James E, Lowe R, Rakyan Vardhman K, Iotchkova V, Frontini M, Downes K, Ouwehand Willem H, Laperle J, Jacques P-É, Bourque G, Bergmann Anke K, Siebert R, Vellenga E, Saeed S, Matarese F, Martens Joost HA, Stunnenberg Hendrik G, Teschendorff Andrew E, Herrero J, Birney E, Dunham I, Beck S. eFORGE: A Tool for Identifying Cell Type-Specific Signal in Epigenomic Data. *Cell Reports* 2016;**17**(8):2137-2150.

387. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. *Nature* 2012;**489**(7414):57-74.

388. Bernstein BE, Stamatoyannopoulos JA, Costello JF, Ren B, Milosavljevic A, Meissner A, Kellis M, Marra MA, Beaudet AL, Ecker JR, Farnham PJ, Hirst M, Lander ES, Mikkelsen TS, Thomson JA. The NIH Roadmap Epigenomics Mapping Consortium. *Nat Biotechnol* 2010;**28**(10):1045-8.

389. Stunnenberg HG, Abrignani S, Adams D, de Almeida M, Altucci L, Amin V, Amit I, Antonarakis SE, Aparicio S, Arima T, Arrigoni L, Arts R, Asnafi V, Esteller M, Bae J-B, Bassler K, Beck S, Berkman B, Bernstein BE, Bilenky M, Bird A, Bock C, Boehm B, Bourque G, Breeze CE, Brors B, Bujold D, Burren O, Bussemakers MJ, Butterworth A, Campo E, Carrillo-de-Santa-Pau E, Chadwick L, Chan KM, Chen W, Cheung TH, Chiapperino L, Choi NH, Chung H-R, Clarke L, Connors JM, Cronet P, Danesh J, Dermitzakis M, Drewes G, Durek P, Dyke S, Dylag T, Eaves CJ, Ebert P, Eils R, Eils J, Ennis CA, Enver T, Feingold EA, Felder B, Ferguson-Smith A, Fitzgibbon J, Flicek P, Foo RSY, Fraser P, Frontini M, Furlong E, Gakkhar S, Gasparoni N, Gasparoni G, Geschwind DH, Glažar P, Graf T, Grosveld F, Guan X-Y, Guigo R, Gut IG, Hamann A, Han B-G, Harris RA, Heath S, Helin K, Hengstler JG, Heravi-Moussavi A, Herrup K, Hill S, Hilton JA, Hitz BC, Horsthemke B, Hu M, Hwang J-Y, Ip NY, Ito T, Javierre B-M, Jenko S, Jenuwein T, Joly Y, Jones SJM, Kanai Y, Kang HG, Karsan A, Kiemer AK, Kim SC, Kim B-J, et al. The International Human Epigenome Consortium: A Blueprint for Scientific Collaboration and Discovery. *Cell* 2016;**167**(5):1145-1149.

390. Holle R, Happich M, Löwel H, Wichmann H-E, Group nftMKS. KORA-a research platform for population based health research. *Das Gesundheitswesen* 2005;**67**(S 01):19-25.

391. Wichmann H, Gieger C, Illig T, Group MKS. KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen (Bundesverband der Arzte des Offentlichen Gesundheitsdienstes (Germany))* 2005;**67**:S26.

392. Bauer DJ, Preacher KJ, Gil KM. Conceptualizing and testing random indirect effects and moderated mediation in multilevel models: new procedures and recommendations. *Psychol Methods* 2006;**11**(2):142-63.

393. Bind MA, Vanderweele TJ, Coull BA, Schwartz JD. Causal mediation analysis for longitudinal data with exogenous exposure. *Biostatistics* 2016;**17**(1):122-34.

394. MacKinnon DP, Fairchild AJ, Fritz MS. Mediation Analysis. *Annual Review of Psychology* 2006;**58**(1):593-614.

395. Valeri L, VanderWeele TJ. Mediation analysis allowing for exposure–mediator interactions and causal interpretation: Theoretical assumptions and implementation with SAS and SPSS macros. *Psychological Methods* 2013;**18**(2):137-150.

396. Qiu W, Qiu MW. Package 'powerMediation'. 2017.

397. Dupont WD, Plummer WD. Power and sample size calculations for studies involving linear regression. *Controlled clinical trials* 1998;**19**(6):589-601.

398. Vittinghoff E, Neilands TB. Sample Size for Joint Testing of Indirect Effects. *Prevention Science* 2015;**16**(8):1128-1135.

399. Terashima T, Wiggs B, English D, Hogg JC, van Eeden SF. Phagocytosis of small carbon particles (PM10) by alveolar macrophages stimulates the release of polymorphonuclear leukocytes from bone marrow. *American Journal of Respiratory and Critical Care Medicine* 1997;**155**(4):1441-1447.

400. Pope CA, Bhatnagar A, McCracken JP, Abplanalp W, Conklin DJ, O'Toole T. Exposure to Fine Particulate Air Pollution Is Associated With Endothelial Injury and Systemic Inflammation. *Circulation Research* 2016;**119**(11):1204-1214.

401. Chi GC, Hajat A, Bird CE, Cullen MR, Griffin BA, Miller KA, Shih RA, Stefanick ML, Vedal S, Whitsel EA, Kaufman JD. Individual and Neighborhood Socioeconomic Status and the Association between Air Pollution and Cardiovascular Disease. *Environmental Health Perspectives* 2016;**124**(12):1840-1847.

402. Parker JD, Kravets N, Vaidyanathan A. Particulate Matter Air Pollution Exposure and Heart Disease Mortality Risks by Race and Ethnicity in the United States. *1997 to 2009 National Health Interview Survey With Mortality Follow-Up Through 2011* 2018;**137**(16):1688-1697.

403. Riediker M. Cardiovascular Effects of Fine Particulate Matter Components in Highway Patrol Officers. *Inhalation Toxicology* 2007;**19**(sup1):99-105.

404. Steenhof M, Janssen NAH, Strak M, Hoek G, Gosens I, Mudway IS, Kelly FJ, Harrison RM, Pieters RHH, Cassee FR, Brunekreef B. Air pollution exposure affects circulating white blood cell counts in healthy subjects: the role of particle composition, oxidative potential and gaseous pollutants – the RAPTES project. *Inhalation Toxicology* 2014;**26**(3):141-165.

405. Michaël B, Laure B, Steven A, M. KP, E. CMJ. Mind the cell: Seasonal variation in telomere length mirrors changes in leucocyte profile. *Molecular Ecology* 2017;**26**(20):5603-5613.

406. Liao D, Peuquet DJ, Lin H-M, Duan Y, Whitsel EA, Smith RL, Heiss G. National Kriging Exposure Estimation: Liao et al. Respond. *Environmental Health Perspectives* 2007;**115**(7):A338-A339.

407. Bhaskaran K, Gasparrini A, Hajat S, Smeeth L, Armstrong B. Time series regression studies in environmental epidemiology. *International Journal of Epidemiology* 2013;**42**(4):1187-1195.

408. Dominici F, McDermott A, Zeger SL, Samet JM. On the Use of Generalized Additive Models in Time-Series Studies of Air Pollution and Health. *American Journal of Epidemiology* 2002;**156**(3):193-203.

409. Peng RD, Dominici F, Louis TA. Model choice in time series studies of air pollution and mortality. *Journal of the Royal Statistical Society: Series A (Statistics in Society)* 2006;**169**(2):179-203.

410. Elo IT. Social Class Differentials in Health and Mortality: Patterns and Explanations in Comparative Perspective. *Annual Review of Sociology* 2009;**35**(1):553-572.

411. Hajat A, Hsia C, O'Neill MS. Socioeconomic Disparities and Air Pollution Exposure: a Global Review. *Current Environmental Health Reports* 2015;**2**(4):440-450.

412. Petitti DB, Kipp H. The Leukocyte count: Associations with intensity of smoking and persistence of effect after quitting. *American Journal of Epidemiology* 1986;**123**(1):89-95.

413. Hansen LK, Grimm JRH, Neaton JD. The Relationship of White Blood Cell Count to Other Cardiovascular Risk Factors. *International Journal of Epidemiology* 1990;**19**(4):881-888.

414. Schwartz J, Weiss ST. Host and Environmental Factors Influencing the Peripheral Blood Leukocyte Count. *American Journal of Epidemiology* 1991;**134**(12):1402-1409.

415. Riediker M, Cascio WE, Griggs TR, Herbst MC, Bromberg PA, Neas L, Williams RW, Devlin RB. Particulate Matter Exposure in Cars Is Associated with Cardiovascular Effects in Healthy Young Men. *American Journal of Respiratory and Critical Care Medicine* 2004;**169**(8):934-940.

416. Zhao J, Gao Z, Tian Z, Xie Y, Xin F, Jiang R, Kan H, Song W. The biological effects of individual-level PM_{2.5} exposure on systemic immunity and inflammatory response in traffic policemen. *Occupational and Environmental Medicine* 2013;**70**(6):426.

417. Gerlofs-Nijland ME, Boere AJF, Leseman DLAC, Dormans JAMA, Sandström T, Salonen RO, van Bree L, Cassee FR. Effects of particulate matter on the pulmonary and vascular system: time course in spontaneously hypertensive rats. *Particle and Fibre Toxicology* 2005;**2**(1):2.

418. Kodavanti UP, Schladweiler MC, Ledbetter AD, Hauser R, Christiani DC, McGee J, Richards JR, Costa DL. Temporal Association Between Pulmonary and Systemic Effects of Particulate Matter in Healthy and Cardiovascular Compromised rats. *Journal of Toxicology and Environmental Health, Part A* 2002;**65**(20):1545-1569.

419. Gordon T, Nadziejko C, Schlesinger R, Chi Chen L. Pulmonary and cardiovascular effects of acute exposure to concentrated ambient particulate matter in rats. *Toxicology Letters* 1998;**96-97**:285-288.

420. Huang J-F, Yang Y, Sepulveda H, Shi W, Hwang I, Peterson PA, Jackson MR, Sprent J, Cai Z. TCR-Mediated Internalization of Peptide-MHC Complexes Acquired by T Cells. *Science* 1999;**286**(5441):952.

421. Perez L, Wolf K, Hennig F, Penell J, Basagaña X, Foraster M, Aguilera I, Agis D, Beelen R, Brunekreef B, Cyrys J, Fuks KB, Adam M, Baldassarre D, Cirach M, Elosua R, Dratva J, Hampel R, Koenig W, Marrugat J, de Faire U, Pershagen G, Probst-Hensch NM, de Nazelle A, Nieuwenhuijsen MJ, Rathmann W, Rivera M, Seissler J, Schindler C, Thiery J, Hoffmann B, Peters A, Künzli N. Air Pollution and Atherosclerosis: A Cross-Sectional Analysis of Four European Cohort Studies in the ESCAPE Study. *Environmental Health Perspectives* 2015;**123**(6):597-605.

422. Brook RD, Rajagopalan S. Particulate Matter Air Pollution and Atherosclerosis. *Current Atherosclerosis Reports* 2010;**12**(5):291-300.

423. Kunzli N, Jerrett M, Mack WJ, Beckerman B, LaBree L, Gilliland F, Thomas D, Peters J, Hodis HN. Ambient air pollution and atherosclerosis in Los Angeles. *Environ Health Perspect* 2005;**113**.

424. Adar SD, Sheppard L, Vedal S, Polak JF, Sampson PD, Diez Roux AV, Budoff M, Jacobs DR, Jr., Barr RG, Watson K, Kaufman JD. Fine Particulate Air Pollution and the Progression of Carotid Intima-Medial Thickness: A Prospective Cohort Study from the Multi-Ethnic Study of Atherosclerosis and Air Pollution. *PLOS Medicine* 2013;**10**(4):e1001430.

425. Diez Roux AV, Auchincloss AH, Franklin TG, Raghunathan T, Barr RG, Kaufman J, Astor B, Keeler J. Long-term Exposure to Ambient Particulate Matter and Prevalence of Subclinical Atherosclerosis in the Multi-Ethnic Study of Atherosclerosis. *American Journal of Epidemiology* 2008;**167**(6):667-675.

426. Terashima T, Wiggs B, English D, Hogg JC, van Eeden SF. The effect of cigarette smoking on the bone marrow. *American Journal of Respiratory and Critical Care Medicine* 1997;**155**(3):1021-1026.

427. van Eeden SF, Tan WC, Suwa T, Mukae H, Terashima T, Fujii T, Qui D, Vincent R, Hogg JC. Cytokines Involved in the Systemic Inflammatory Response Induced by Exposure to Particulate Matter Air Pollutants (PM10). *American Journal of Respiratory and Critical Care Medicine* 2001;**164**(5):826-830.

428. Rückerl R, Ibald-Mulli A, Koenig W, Schneider A, Woelke G, Cyrys J, Heinrich J, Marder V, Frampton M, Wichmann HE, Peters A. Air Pollution and Markers of Inflammation and Coagulation in Patients with Coronary Heart Disease. *American Journal of Respiratory and Critical Care Medicine* 2006;**173**(4):432-441.

429. O'Neill MS, Veves A, Sarnat JA, Zanobetti A, Gold DR, Economides PA, Horton ES, Schwartz J. Air pollution and inflammation in type 2 diabetes: a mechanism for susceptibility. *Occupational and Environmental Medicine* 2007;**64**(6):373-379.

430. Baccarelli A, Zanobetti A, Martinelli I, Grillo P, Hou L, Giacomini S, Bonzini M, Lanzani G, Mannucci PM, Bertazzi PA, Schwartz J. Effects of exposure to air pollution on blood coagulation. *J Thromb Haemost* 2007;**5**(2):252-60.

431. Bind M-A, Baccarelli A, Zanobetti A, Tarantini L, Suh H, Vokonas P, Schwartz J. Air Pollution and Markers of Coagulation, Inflammation, and Endothelial Function: Associations and Epigene-environment Interactions in an Elderly Cohort. *Epidemiology* 2012;**23**(2):332-340.

432. Tsai D-H, Amyai N, Marques-Vidal P, Wang J-L, Riediker M, Mooser V, Paccaud F, Waeber G, Vollenweider P, Bochud M. Effects of particulate matter on inflammatory markers in the general adult population. *Particle and Fibre Toxicology* 2012;**9**:24-24.

433. Terashima T, Klut ME, English D, Hards J, Hogg JC, van Eeden SF. Cigarette Smoking Causes Sequestration of Polymorphonuclear Leukocytes Released from the Bone Marrow in Lung Microvessels. *American Journal of Respiratory Cell and Molecular Biology* 1999;**20**(1):171-177.

434. Yatera K, Hsieh J, Hogg JC, Tranfield E, Suzuki H, Shih C-H, Behzad AR, Vincent R, Eeden SFv. Particulate matter air pollution exposure promotes recruitment of monocytes into atherosclerotic plaques. *American Journal of Physiology-Heart and Circulatory Physiology* 2008;**294**(2):H944-H953.

435. Goto Y, Ishii H, Hogg JC, Shih C-H, Yatera K, Vincent R, van Eeden SF. Particulate Matter Air Pollution Stimulates Monocyte Release from the Bone Marrow. *American Journal of Respiratory and Critical Care Medicine* 2004;**170**(8):891-897.

436. Zeka A, Sullivan JR, Vokonas PS, Sparrow D, Schwartz J. Inflammatory markers and particulate air pollution: characterizing the pathway to disease. *International Journal of Epidemiology* 2006;**35**(5):1347-1354.

437. Greenland S, Pearl J, Robins JM. Causal Diagrams for Epidemiologic Research. *Epidemiology* 1999;**10**(1):37-48.

438. VanderWeele TJ, Vansteelandt S. Mediation Analysis with Multiple Mediators. *Epidemiol Methods* 2014;**2**(1):95-115.

439. Cole SR, Hernán MA. Constructing inverse probability weights for marginal structural models. *American journal of epidemiology* 2008;**168**(6):656-664.

440. Nieto FJ, Szklo M, Folsom AR, Rock R, Mercuri M. Leukocyte count correlates in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Epidemiol* 1992;**136**(5):525-37.

441. Schisterman EF, Cole SR, Platt RW. Overadjustment Bias and Unnecessary Adjustment in Epidemiologic Studies. *Epidemiology (Cambridge, Mass.)* 2009;**20**(4):488-495.

442. Gan WQ, FitzGerald JM, Carlsten C, Sadatsafavi M, Brauer M. Associations of Ambient Air Pollution with Chronic Obstructive Pulmonary Disease Hospitalization and Mortality. *American Journal of Respiratory and Critical Care Medicine* 2013;**187**(7):721-727.

443. Laumbach RJ, Kipen HM. Respiratory health effects of air pollution: Update on biomass smoke and traffic pollution. *Journal of Allergy and Clinical Immunology* 2012;**129**(1):3-11.

444. Pope CA, 3rd, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, Thurston GD. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *Jama* 2002;**287**(9):1132-41.

445. Raaschou-Nielsen O, Andersen ZJ, Beelen R, Samoli E, Stafoggia M, Weinmayr G, Hoffmann B, Fischer P, Nieuwenhuijsen MJ, Brunekreef B, Xun WW, Katsouyanni K, Dimakopoulou K, Sommar J, Forsberg B, Modig L, Oudin A, Oftedal B, Schwarze PE, Nafstad P, De Faire U, Pedersen NL, Östenson C-G, Fratiglioni L, Penell J, Korek M, Pershagen G, Eriksen KT, Sørensen M, Tjønneland A, Ellermann T, Eeftens M, Peeters PH, Meliefste K, Wang M, Bueno-de-Mesquita B, Key TJ, de Hoogh K, Concin H, Nagel G, Vilier A, Grioni S, Krogh V, Tsai M-Y, Ricceri F, Sacerdote C, Galassi C, Migliore E, Ranzi A, Cesaroni G, Badaloni C, Forastiere F, Tamayo I, Amiano P, Dorronsoro M, Trichopoulou A, Bamia C, Vineis P, Hoek G. Air pollution and lung cancer incidence in 17 European cohorts: prospective analyses from the European Study of Cohorts for Air Pollution Effects (ESCAPE). *The Lancet Oncology* 2013;**14**(9):813-822.

446. de F. C. Lichtenfels AJ, van der Plaat DA, de Jong K, van Diemen CC, Postma DS, Nedeljkovic I, van Duijn CM, Amin N, la Bastide-van Gemert S, de Vries M, Ward-Caviness CK, Wolf K, Waldenberger M, Peters A, Stolk RP, Brunekreef B, Boezen HM, Vonk JM. Long-term Air Pollution Exposure, Genome-wide DNA Methylation and Lung Function in the LifeLines Cohort Study. *Environ Health Perspect* 2018;**126**(2):027004.

447. Jordahl KM, Randolph TW, Song X, Sather CL, Tinker LF, Phipps AI, Kelsey KT, White E, Bhatti P. Genome-Wide DNA Methylation in Prediagnostic Blood and Bladder Cancer Risk in the Women's Health Initiative. *Cancer Epidemiology, Biomarkers & Prevention* 2018;**27**(6):689.

448. Lee S-J, Serre Marc L, van Donkelaar A, Martin Randall V, Burnett Richard T, Jerrett M. Comparison of Geostatistical Interpolation and Remote Sensing Techniques for Estimating Long-Term Exposure to Ambient PM2.5 Concentrations across the Continental United States. *Environmental Health Perspectives* 2012;**120**(12):1727-1732.

449. Alexeeff SE, Schwartz J, Kloog I, Chudnovsky A, Koutrakis P, Coull BA. Consequences of kriging and land use regression for PM2.5 predictions in epidemiologic analyses: insights into spatial variability using high-resolution satellite data. *Journal Of Exposure Science And Environmental Epidemiology* 2014;**25**:138.

450. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* 2007;**8**(1):118-27.

451. Andrews SV, Ladd-Acosta C, Feinberg AP, Hansen KD, Fallin MD. "Gap hunting" to characterize clustered probe signals in Illumina methylation array data. *Epigenetics & Chromatin* 2016;**9**(1):56.

452. Bates D. Mixed-effects models in Julia. https://github.com/dmbates/MixedModels.jl Accessed 06/07/2018, 2018.

453. Bezanson J, Edelman A, Karpinski S, Shah VB. Julia: A Fresh Approach to Numerical Computing. *SIAM Review* 2017;**59**(1):65-98.

454. Chen Ya, Lemire M, Choufani S, Butcher DT, Grafodatskaya D, Zanke BW, Gallinger S, Hudson TJ, Weksberg R. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics* 2013;**8**.

455. Barallobre-Barreiro J, Didangelos A, Schoendube FA, Drozdov I, Yin X, Fernández-Caggiano M, Willeit P, Puntmann VO, Aldama-López G, Shah AM, Doménech N, Mayr M. Proteomics Analysis of Cardiac Extracellular Matrix Remodeling in a Porcine Model of Ischemia/Reperfusion Injury. *Circulation* 2012;**125**(6):789-802.

456. Liberda EN, Cuevas AK, Gillespie PA, Grunig G, Qu Q, Chen LC. Exposure to inhaled nickel nanoparticles causes a reduction in number and function of bone marrow endothelial progenitor cells. *Inhalation Toxicology* 2010;**22**(sup2):95-99.

457. Uckelmann H, Blaszkiewicz S, Nicolae C, Haas S, Schnell A, Wurzer S, Wagener R, Aszodi A, Essers MAG. Extracellular matrix protein Matrilin-4 regulates stress-induced HSC proliferation via CXCR4. *The Journal of Experimental Medicine* 2016.

458. Pyo RT, Sui J, Dhume A, Palomeque J, Blaxall BC, Diaz G, Tunstead J, Logothetis DE, Hajjar RJ, Schecter AD. CXCR4 modulates contractility in adult cardiac myocytes. *Journal of Molecular and Cellular Cardiology* 2006;**41**(5):834-844.

459. Rakhilin SV, Olson PA, Nishi A, Starkova NN, Fienberg AA, Nairn AC, Surmeier DJ, Greengard P. A Network of Control Mediated by Regulator of Calcium/Calmodulin-Dependent Signaling. *Science* 2004;**306**(5696):698-701.

460. Kahr PC, Piccini I, Fabritz L, Greber B, Schöler H, Scheld HH, Hoffmeier A, Brown NA, Kirchhof P. Systematic Analysis of Gene Expression Differences between Left and Right Atria in Different Mouse Strains and in Human Atrial Tissue. *PLoS ONE* 2011;**6**(10):e26389.

461. Kirchhof P, Kahr PC, Kaese S, Piccini I, Vokshi I, Scheld H-H, Rotering H, Fortmueller L, Laakmann S, Verheule S, Schotten U, Fabritz L, Brown NA. PITX2c Is Expressed in the Adult Left Atrium, and Reducing Pitx2c Expression Promotes Atrial Fibrillation Inducibility and Complex Changes in Gene Expression. *Circulation: Cardiovascular Genetics* 2011;**4**(2):123-133.

462. Mathar I, Kecskes M, Van Der Mieren G, Jacobs G, Uhl S, Camacho Londoño JE, Flockerzi V, Voets T, Freichel M, Nilius B, Herijgers P, Vennekens R. Increased ß-Adrenergic Inotropy in Ventricular Myocardium from Trpm4^{-/-} Mice. *Circulation Research* 2013.

463. Furney SJ, Simmons A, Breen G, Pedroso I, Lunnon K, Proitsi P, Hodges A, Powell J, Wahlund LO, Kloszewska I, Mecocci P, Soininen H, Tsolaki M, Vellas B, Spenger C, Lathrop M, Shen L, Kim S, Saykin AJ, Weiner MW, Lovestone S. Genome-wide association with MRI atrophy measures as a quantitative trait locus for Alzheimer's disease. *Molecular Psychiatry* 2010;**16**:1130.

464. O'Day DH, Eshak K, Myre MA. Calmodulin Binding Proteins and Alzheimer's Disease. *Journal of Alzheimer's Disease* 2015;**46**(3):553-569.

465. Velayudhan L, Proitsi P, Westman E, Muehlboeck JS, Mecocci P, Vellas B, Tsolaki M, Kloszewska I, Soininen H, Spenger C, Hodges A, Powell J, Lovestone S, Simmons A. Entorhinal cortex thickness predicts cognitive decline in Alzheimer's disease. *J Alzheimers Dis* 2013;**33**(3):755-66.

466. Cacciottolo M, Wang X, Driscoll I, Woodward N, Saffari A, Reyes J, Serre ML, Vizuete W, Sioutas C, Morgan TE, Gatz M, Chui HC, Shumaker SA, Resnick SM, Espeland MA, Finch CE, Chen JC. Particulate air pollutants, APOE alleles and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. *Translational Psychiatry* 2017;**7**:e1022.

467. Rehfeld F, Maticzka D, Grosser S, Knauff P, Eravci M, Vida I, Backofen R, Wulczyn FG. The RNA-binding protein ARPP21 controls dendritic branching by functionally opposing the miRNA it hosts. *Nature Communications* 2018;**9**(1):1235.

468. Cheng L, Lau WKW, Fung TKH, Lau BWM, Chau BKH, Liang Y, Wang Z, So KF, Wang T, Chan CCH, Lee TMC. PM2.5 Exposure Suppresses Dendritic Maturation in Subgranular Zone in Aged Rats. *Neurotoxicity Research* 2017;**32**(1):50-57.

469. Bollati V, Angelici L, Rizzo G, Pergoli L, Rota F, Hoxha M, Nordio F, Bonzini M, Tarantini L, Cantone L, Pesatori AC, Apostoli P, Baccarelli AA, Bertazzi PA. Microvesicle-associated microRNA expression is altered upon particulate matter exposure in healthy workers and in A549 cells. *Journal of Applied Toxicology* 2015;**35**(1):59-67.

470. Adlakha YK, Khanna S, Singh R, Singh VP, Agrawal A, Saini N. Pro-apoptotic miRNA-128-2 modulates ABCA1, ABCG1 and RXRα expression and cholesterol homeostasis. *Cell Death & Disease* 2013;**4**(8):e780.

471. Saint-Criq V, Gray MA. Role of CFTR in epithelial physiology. *Cellular and Molecular Life Sciences* 2017;**74**(1):93-115.

472. Brennan AL, Geddes DM, Gyi KM, Baker EH. Clinical importance of cystic fibrosis-related diabetes. *Journal of Cystic Fibrosis* 2004;**3**(4):209-222.

473. Rasmussen JE, Sheridan JT, Polk W, Davies CM, Tarran R. Cigarette Smoke-induced Ca2+ Release Leads to Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Dysfunction. *Journal of Biological Chemistry* 2014;**289**(11):7671-7681.

474. Rab A, Rowe SM, Raju SV, Bebok Z, Matalon S, Collawn JF. Cigarette smoke and CFTR: implications in the pathogenesis of COPD. *American Journal of Physiology-Lung Cellular and Molecular Physiology* 2013;**305**(8):L530-L541.

475. Duan DD. Phenomics of Cardiac Chloride Channels. *Comprehensive Physiology*, 2013.

476. al-Awqati Q. Regulation of ion channels by ABC transporters that secrete ATP. *Science* 1995;**269**(5225):805-806.

477. Cacciapuoti F, Spiezia R, Bianchi U, Lama D, D'Avino M, Varricchio M. Effectiveness of glibenclamide on myocardial ischemic ventricular arrhythmias in non-insulin-dependent diabetes mellitus. *The American Journal of Cardiology* 1991;**67**(9):843-847.

478. Engler RL, Yellon DM. Sulfonylurea KATP Blockade in Type II Diabetes and Preconditioning in Cardiovascular Disease. *Time for Reconsideration* 1996;**94**(9):2297-2301.

479. Leonard CE, Hennessy S, Han X, Siscovick DS, Flory JH, Deo R. Pro- and Antiarrhythmic Actions of Sulfonylureas: Mechanistic and Clinical Evidence. *Trends in Endocrinology & Metabolism* 2017;**28**(8):561-586.

480. Yamazaki J, Hume JR. Inhibitory Effects of Glibenclamide on Cystic Fibrosis Transmembrane Regulator, Swelling-Activated, and Ca2+-Activated Cl− Channels in Mammalian Cardiac Myocytes. *Circulation Research* 1997;**81**(1):101-109.

481. Najeed SA, Khan IA, Molnar J, Somberg JC. Differential effect of glyburide glibenclamide and metformin on qt dispersion: a potential adenosine triphosphate sensitive k+ channel effect. *American Journal of Cardiology* 2002;**90**(10):1103-1106.

482. Aguet F, Brown AA, Castel SE, Davis JR, He Y, Jo B, Mohammadi P, Park Y, Parsana P, Segrè AV, Strober BJ, Zappala Z, Cummings BB, Gelfand ET, Hadley K, Huang KH, Lek M, Li X, Nedzel JL, Nguyen DY, Noble MS, Sullivan TJ, Tukiainen T, MacArthur DG, Getz G, Addington A, Guan P, Koester S, Little AR, Lockhart NC, Moore HM, Rao A, Struewing JP, Volpi S, Brigham LE, Hasz R, Hunter M, Johns C, Johnson M, Kopen G, Leinweber WF, Lonsdale JT, McDonald A, Mestichelli B, Myer K, Roe B, Salvatore M, Shad S, Thomas JA, Walters G, Washington M, Wheeler J, Bridge J, Foster BA, Gillard BM, Karasik E, Kumar R, Miklos M, Moser MT, Jewell SD, Montroy RG, Rohrer DC, Valley D, Mash DC, Davis DA, Sobin L, Barcus ME, Branton PA, Abell NS, Balliu B, Delaneau O, Frésard L, Gamazon ER, Garrido-Martín D, Gewirtz ADH, Gliner G, Gloudemans MJ, Han B, He AZ, Hormozdiari F, Li X, Liu B, Kang EY, McDowell IC, Ongen H, Palowitch JJ, Peterson CB, Quon G, Ripke S, Saha A, Shabalin AA, Shimko TC, Sul JH, Teran NA, Tsang EK, Zhang H, Zhou Y-H, Bustamante CD, Cox NJ, Guigó R, et al. Genetic effects on gene expression across human tissues. *Nature* 2017;**550**:204.

483. Saffari A, Silver MJ, Zavattari P, Moi L, Columbano A, Meaburn EL, Dudbridge F. Estimation of a significance threshold for epigenome-wide association studies. *Genetic Epidemiology* 2018;**42**(1):20-33.

484. Tsai P-C, Spector TD, Bell JT. Using epigenome-wide association scans of DNA methylation in age-related complex human traits. *Epigenomics* 2012;**4**(5):511-526.

485. Rückert I-M, Heier M, Rathmann W, Baumeister SE, Döring A, Meisinger C. Association between Markers of Fatty Liver Disease and Impaired Glucose Regulation in Men and Women from the General Population: The KORA-F4-Study. *PLoS ONE* 2011;**6**(8):e22932.

486. Wichmann HE, Gieger C, Illig T. KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen* 2005;**67 Suppl 1**:S26-30.

487. Horvath S. DNA methylation age of human tissues and cell types. *Genome biology* 2013;**14**(10):R115.

488. Goldberg RJ, Bengtson J, Chen Z, Anderson KM, Locati E, Levy D. Duration of the QT interval and total and cardiovascular mortality in healthy persons (The Framingham heart study experience). *American Journal of Cardiology* 1991;**67**(1):55-58.

489. Gondalia R, Baldassari Antoine R, Holliday Katelyn M, Méndez-Giráldez R, Justice Anne E, Stewart James D, Liao D, Yanosky Jeffrey D, Jordhal Kristina M, Bhatti P, Horvath S, Assimes Themistocles L, Pankow Jim S, Demerath Ellen W, Guan W, Fornage M, Bressler J, North Kari E, Conneely Karen N, Li Y, Baccarelli Andrea A, Hou L, Whitsel Eric A. Abstract P101: Methylome-wide Association Study Provides Evidence of Particulate Matter Air Pollution-associated Dna Methylation at Cardiovascular Disease-related Genes. *Circulation* 2018;**137**(suppl_1):AP101-AP101.

490. Mordukhovich I, Coull B, Kloog I, Koutrakis P, Vokonas P, Schwartz J. Exposure to sub-chronic and long-term particulate air pollution and heart rate variability in an elderly cohort: the Normative Aging Study. *Environmental Health* 2015;**14**(1):87.

491. Chang L-T, Tang C-S, Pan Y-Z, Chan C-C. Association of Heart Rate Variability of the Elderly with Personal Exposure to PM1, PM1–2.5, and PM2.5–10. *Bulletin of Environmental Contamination and Toxicology* 2007;**79**(5):552-556.