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Epigenetically mediated electrocardiographic manifestations of sub-chronic exposures to ambient particulate matter air pollution in the Women's Health Initiative and Atherosclerosis Risk in Communities Study

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RG: conceptualization, methodology, software, formal analysis, writing – original draft, writing – review & editing, visualization
AB: software, methodology, writing – review & editing
KMH: methodology, writing – review & editing
AEJ: conceptualization, methodology, writing – review & editing, supervision
JDS: data curation, writing – review & editing
DL: data curation, writing – review & editing
JDY: data curation, writing – review & editing
SME: conceptualization, methodology, writing – review & editing, supervision
DS: resources, writing – review & editing
KMJ: data curation, writing – review & editing
PB: resources, data curation, writing – review & editing
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LH: resources, writing – review & editing, funding acquisition
AAB: resources, writing – review & editing, funding acquisition
EAW: conceptualization, methodology, resources, writing – review & editing, visualization, supervision, project administration, funding acquisition

Conflicts of interest

No authors have declared any potential conflicts of interest

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abstract

Background: 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.

Materials and methods: We estimated associations between monthly mean concentrations of PM < 10μm and 2.5-10μm in diameter ($PM_{10;}$ $PM_{2.5-10}$) with time-domain measures of heart rate

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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 PM, electrocardiographic and covariable 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.

Results: We found little evidence of PM-HRV association, PM-QT association, or mediation by DNAm.

Conclusions: 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. Further investigation in better-powered studies is warranted, with additional focus on shorter duration exposures to finer particulates and non-electrocardiographic outcomes among relatively susceptible populations.

Keywords

particulate matter; DNA methylation; heart rate variability; QT interval duration; mediation

1. Introduction

Exposure to ambient particulate matter (PM) air pollution has been consistently associated with increases in cardiovascular disease (CVD) risk (Brook et al., 2004; Brook et al., 2010; Miller et al., 2007). For example, short-duration exposures to PM have been associated with decreased heart rate variability (HRV) (Liao et al., 2004; Pieters et al., 2012; Whitsel et al., 2009) and increased QT interval duration (QT) (Liao et al., 2010; Mordukhovich et al., 2016; Van Hee et al., 2011), both of which are established cardiovascular disease risk factors (Dekker et al., 2000; Dekker et al., 2004; Goldberg et al., 1991; Liao et al., 1997; Rautaharju et al., 2006; Schouten et al., 1991; Tsuji et al., 1996; Zhang et al., 2011). 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}; $PM_{2.5-10}$) 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 (Baccarelli et al., 2010; Bollati and Baccarelli, 2010; Panni et al., 2016; Plusquin et al., 2017; Zhong et al., 2016). Indeed, we recently discovered that DNAm was associated with higher monthly mean PM_{10} and $PM_{2.5-10}$ concentrations at

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three PM-sensitive CpG sites annotated to neurological, pulmonary, endocrine, and / or cardiovascular disease-related genes (*MATN4*; *ARPP21*; *CFTR*) that can affect cardiac electrophysiology (Gondalia et al., 2019). 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 $PM_{2.5-10}$ 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.

2. Material and Methods

2.1. Study populations

Figure 1 describes the study populations. The WHI is a multicenter, prospective study of risk factors for cardiovascular disease, breast / colorectal cancer, and osteoporotic fractures (Anderson et al., 2003; NIH, 1998). 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 (Anderson et al., 2003; NIH, 1998) 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 visits at 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* (Smoller et al., 2003) (MIMS, n = 3,369), an ancillary study of WHI OS participants enrolled by ten clinical centers (SV or AV3; 1997-2000).

Three WHI CT subpopulations contributed DNAm data to the present study: *Epigenetic Mechanisms of PM-Mediated CVD Risk* (WHI-EMPC; n = 2,200) (Whitsel), *Broad Agency Announcement 23* (WHI-BAA23; n = 1,546) (Assimes et al.), and *Ancillary Study 311* (WHI-AS311; n = 405) (Bhatti). WHI-EMPC is a 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*

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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 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 (ARIC, 1989). Participants were selected as a community-stratified probability sample of 15,792 mostly 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.

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 (Mosley et al., 2005), all at Visits 2 (1990-1992) or 3 (1993-1995).

2.2. Heart rate variability and QT interval duration measurement

In the WHI CT and ARIC, ten-second, resting, supine, standard twelve-lead ECGs (1987; 1994) 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 (Schroeder et al., 2004; Vaidean et al., 2005). 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.

2.3. 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) (EPA, 2017). Daily mean PM_{10} concentrations ($\mu g/m^3$) were spatially estimated at all geocoded participant addresses (Whitsel et al., 2006; Whitsel et al., 2004) 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, henceforth called spatial monthly means. Validity of the PM_{10} exposure estimation was given by the average prediction error (PE) and the standardized prediction

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error (SPE) near 0; the root mean square standardized (RMSS) near 1; and root mean square prediction errors (RMS) near the mathematically calculated standard error (SE).

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 (Yanosky et al., 2014). Monthly mean concentrations of $PM_{2.5-10}$ concentrations were defined as the differences between PM_{10} and $PM_{2.5}$ concentrations, henceforth called spatiotemporal monthly means. A five- to ten-set, out-of-sample cross-validation of the estimates suggested that the model performed well (Pearson R^2=0.68–0.77).

2.4. 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 (Teschendorff et al., 2013), and in WHI-EMPC, ComBat-adjusted for stage and plate using empirical Bayes methods (Johnson et al., 2007). The reliability of the resulting data has been described (Bose et al., 2014). 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 (Houseman et al., 2012). Analyses focused on DNAm at three CpG sites previously identified as PM-sensitive: cg19004594, cg24102420, and cg12124767 (Gondalia et al., 2019).

2.5. 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 (Roux et al., 2001), 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), and season (using sine/cosine functions) (Stolwijk et al., 1999). 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; self-reported history of physician diagnosis; or in ARIC, fasting glucose ≥ 126 mg/dL or non-fasting glucose ≥ 200 mg/dl), hyperlipidemia (anti-hyperlipidemic

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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; 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).

2.6. 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.

2.7. Multiple imputation

To avoid potential for selection bias in complete-data analyses when data are missing at random (Hernan et al., 2004), multivariate imputation by chained equations (MICE) (Azur et al., 2011; Stuart et al., 2009) was used to impute missing PM, electrocardiographic and model covariable data (range: 0.1% - 6.0%), excluding DNAm and related covariables, in ten multiply-imputed datasets. Binary and categorical data were imputed using the logistic and discriminant functions whereas interval-scale data were imputed using predictive means matching. Model results from the multiply-imputed datasets were pooled using Rubin's rules (Barnard and Rubin, 1999; Rubin, 1987).

2.8. Attrition weights

Stabilized inverse probability of attrition weights for each participant were calculated at each examination as a function of PM, electrocardiographic and model covariables, 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 (Howe et al., 2016).

2.9. 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

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random intercept for examination at the participant level and a random intercept and slope for PM at the clinical center level, as given by

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

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

$$ECG_{ij} = \beta_0 + \beta_1 PM_{ij} + \beta_3 Z_{ij} + b_{0j}^{P} + \varepsilon_{ij}^{E} \quad (2)$$

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

$$ECG_{ik} = \beta_0 + \beta_1 PM_{ik} + \beta_3 Z_{ik} + b_{0k}^{C} + b_{1k}^{C} PM_{ik} + \varepsilon_{ik}^{E} \quad (3)$$

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 the spatial or spatiotemporal monthly mean PM_{10} or spatiotemporal monthly 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 (Δ, %) in log-transformed HRV analyses, per 10 μg/m^3 increase in PM, where

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

Subpopulation-specific measures of Δ and their 95% CIs were combined in fixed-effects inverse variance-weighted meta-analyses (DerSimonian and Laird, 1986) after testing homogeneity of associations ($P_{Cochran's\ Q} < 0.10$) (Cochran, 1954).

All PM-HRV and PM-QT models adjusted for race/ethnicity, age, sex (in ARIC), randomly assigned treatment group (in WHI), study visit, monthly mean temperature (°C), monthly mean dew point (°C), monthly 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 spatiotemporal monthly mean $PM_{2.5}$ concentrations.

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2.10. 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 ($P_{Cochran's\ Q} < 0.10$) (Cochran, 1954).

A detailed description of the mediation analysis is reported in the Supplement. Briefly, associations of the spatial monthly mean PM_{10}), spatiotemporal monthly mean PM_{10}, and spatiotemporal monthly 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 (Δ, %) in DNAm per 10 μg/m^3 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 (Δ, %) 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 (Bauer et al., 2006; Bind et al., 2016; VanderWeele, 2015) 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 (MacKinnon et al., 2006; Valeri and VanderWeele, 2013). Causal mediation analyses do not require an observed association between the exposure and outcome, as there may be instances of exposure-mediator and mediator-outcome associations yielding NDE and NIE estimations with opposite signs (Fairchild and McDaniel, 2017; MacKinnon et al., 2006). Because this "inconsistent" mediation may still explain underlying mechanisms between the exposure and outcome, mediation analyses herein were conducted regardless of observed PM-HRV and PM-QT associations.

Mediation models relied on Model 4 adjustments described above 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).

3. 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 1). Participants with DNAm data (n_{HRV}

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= 7,169; n_{QT}= 6,895; Table 2) 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 was high in the WHI MIMS subpopulation with ECGs recorded using 24-hour Holter monitors. QT was relatively high in the ARIC subpopulations. In all subpopulations, spatiotemporal 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 μg/m^3 (EPA, 2017).

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 2A-C). For example in Model 4, SDNN was 1.0 ms (−0.1, 2.0) higher per 10 μg/m^3 increase in the spatiotemporal monthly mean $PM_{2.5-10}$ concentration (Table 3), but the estimate fell to 0.7 ms (−0.4, 1.8) after adjusting for the spatiotemporal monthly 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 μg/m^3 increase in spatiotemporal monthly mean 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 μg/m^3 increase in the spatial and spatiotemporal monthly mean PM_{10} concentrations (Figure 2D; Table 3). 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 μg/m^3 increase in the spatial monthly mean PM_{10}, spatiotemporal monthly mean PM_{10}, and spatiotemporal monthly mean $PM_{2.5-10}$ concentrations, respectively, (Table 4). 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 α= 0.016; however, SDNN was 3.9% (−0.2%, 8.2%; P = 0.06) higher and QT was −0.9 ms (−2.0, 0.2; P = 0.09) lower per 10% increase in DNAm at cg24102420 (Table 5). 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 6).

4. 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 (Gondalia et al., 2019). Despite that motivation, we found little evidence of PM-HRV association, PM-QT association, or mediation by DNAm in the present study. In lieu of greater power, the findings preliminarily suggest that sub-chronic exposures to coarser particulates may not exert appreciable or epigenetically mediated effects on cardiac autonomic function or ventricular repolarization.

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The lack of an observed PM-HRV association in this context is at odds with evidence of inverse 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 μg/m^3 increase in 2-hour to 3-day mean $PM_{2.5}$ or PM_{10}) concentrations (Pieters et al., 2012).Two, small-scale controlled exposure panel studies of shorter duration $PM_{2.5-10}$-HRV associations also found similarly inverse associations (Gong et al., 2004; Graff et al., 2009). 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 inverse to slightly positive associations with HRV (Adhikari et al., 2016; Mordukhovich et al., 2015; Park et al., 2010). 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 (Adhikari et al., 2016), 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 (Adam et al., 2012). 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 (Liao et al., 1999) as well as middle-aged adults with hypertension (Liao et al., 2004), diabetes (Whitsel et al., 2009), or metabolic syndrome (Park et al., 2010). Other susceptible groups have been identified in small-scale studies of $PM_{2.5-10}$, including elderly adults (Chang et al., 2007; Lipsett et al., 2006) and populations with asthma (Yeatts et al., 2007) or coronary heart disease (Lipsett et al., 2006).

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 (Henneberger et al., 2005; Liao et al., 2010) to longer duration (Mordukhovich et al., 2016; Van Hee et al., 2011) $PM_{2.5}$ exposures have been consistently associated with higher QT. Notable in this regard is the 7.0 ms per 3.4 μg/m^3 increase in 28-day mean $PM_{2.5}$ in the NAS (Mordukhovich et al., 2016), 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 (Dockery et al., 2005; Ljungman et al., 2008), 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. These CpG sites have biologically plausible links to electrophysiology (Gondalia et al., 2019) through cardiac remodeling (Barallobre-Barreiro et al., 2012) and the proliferation of

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hematopoietic stem cells (Uckelmann et al., 2016) (cg19004594), calmodulin signaling regulation in neural (Rakhilin et al., 2004) and cardiac tissues (Kahr et al., 2011; Kirchhof et al., 2011; Mathar et al., 2013) (cg24102420) and the regulation of chloride channel currents in the myocardium (Duan, 2013) (cg12124767). Although DNAm at these CpG sites was 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 (Gondalia et al., 2019) — 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, dependence on monthly mean PM concentrations, and low power. Potential for bias related to missingness and attrition was nevertheless reduced using conventional epidemiologic tools: multivariate imputation and inverse-probability weights. While longer duration ECGs to measure time-domain measures of HRV and QT are ideal, shorter duration ECGs are conveniently recorded, valid, and reliable, even when based on resting, supine, ten-second, standard twelve-lead ECGs (Schroeder et al., 2004; Vaidean et al., 2005). Although frequency domain measures of HRV capture additional information on autonomic function, they were not uniformly available in the study population. In addition, the accuracy of the study's geocoding (Whitsel et al., 2006; Whitsel et al., 2004), validity of its PM estimation (Liao et al., 2006; Liao et al., 2007; Yanosky et al., 2014), and reliability of DNAm measurements at CpG sites have been demonstrated (Bose et al., 2014). 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 (Gondalia et al., 2019). Finally, investigation into susceptibility of PM-HRV and PM-QT associations by age or comorbidities, and mediation of such associations by DNAm would require additional power.

5. Conclusions

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 the U.S. Environmental Protection Agency as it sets National Ambient Air Quality Standards for PM under the Clean Air Act.

Supplementary Material

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https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf.

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Abbreviations:

AA	African American
AV	annual visit
ARIC	Atherosclerosis Risk in Communities
AS311	Ancillary Study 311
AQS	United States Environmental Protection Agency Air Quality System
BAA23	Broad Agency Award 23
CI	confidence interval
CpG	Cytosine-phosphate-Guanine
CT	Clinical Trial
DNAm	deoxyribonucleic acid methylation
CVD	cardiovascular disease
EA	European American
EMPC	Epigenetic Mechanisms of PM-Mediated CVD Risk
HRV	heart rate variability
MESA	Multi-Ethnic Study of Atherosclerosis

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MICE	multiple imputation by chained equations
MET	metabolic equivalent of task
NAAQS	National Ambient Air Quality Standards
OS	Observational Study
PE	prediction error
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
QT	QT interval duration
RMSS	root mean square standardized
RMSSD	square root of mean squared differences in successive, normally conducted RR intervals
RR	RR interval duration
SD	standard deviation
SDNN	standard deviation of normally conducted RR intervals
SE	standard error
SPE	standardized prediction error
SV	screening visit
WHI	Women's Health Initiative

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- Short-duration exposure to air pollution is associated with lower HRV and higher QT
- Less is known about sub-chronic exposures to coarser particulate matter (PM)
- In WHI and ARIC, we investigated PM-HRV, PM-QT, and mediation by DNAm
- Supporting prior work in the same populations, PM was associated with DNAm
- We found little evidence PM-HRV or PM-QT associations, or mediation by DNAm

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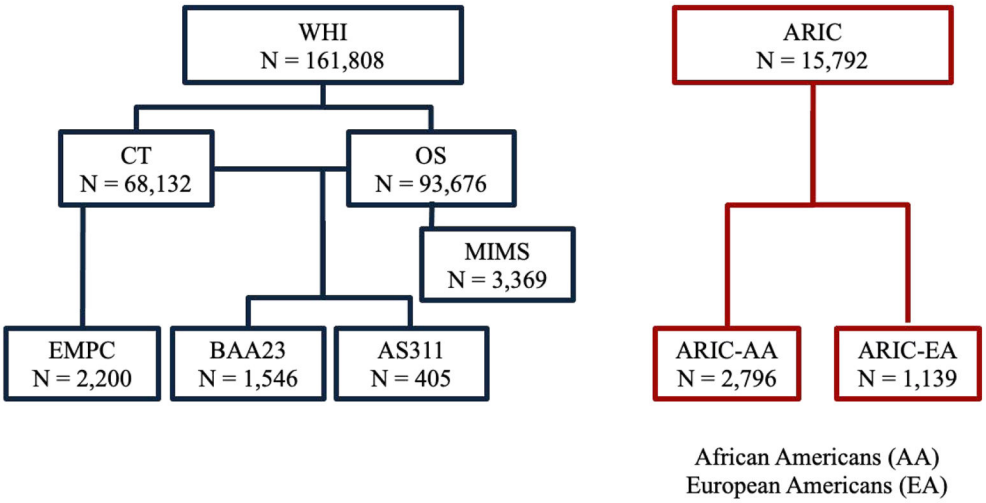

Figure 1.

Diagram of Women's Health Initiative (1993-2002) and Atherosclerosis Risk in Communities study (1986-1998) populations and subpopulations.

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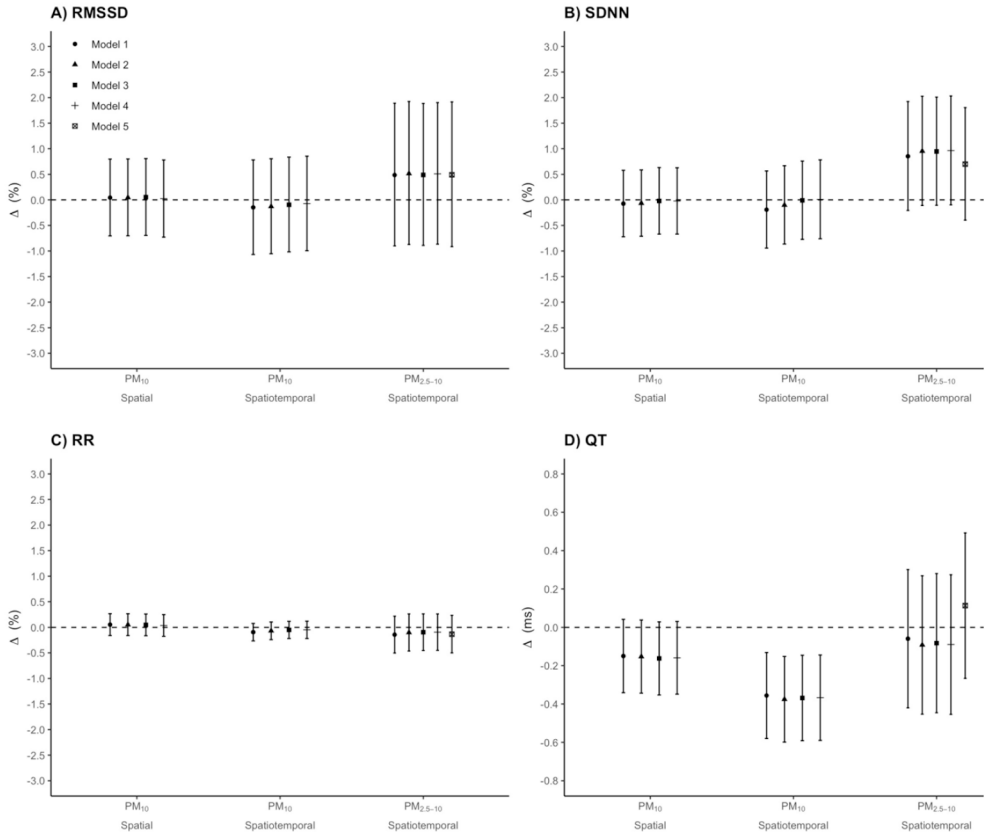

Figure 2.

Pooled, adjusted changes in heart rate variability (Δ, %) and QT interval duration (Δ, ms) per 10 μg/m^3 increase in monthly mean 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 spatiotemporal monthly mean concentrations of $PM_{2.5}$.

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Table 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 (6)	61 (8)	63 (7)	54 (6)
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)	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)
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)							

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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
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 (21)[b]	24 (20)[b]
QT	403 (30)[b]	402 (31)[b]	--	409 (28)[b]	403 (30)[b]	401 (30)[b]	408 (27)[b]
QTc	421 (20)[b]	419 (19)[b]	--	428 (24)[b]	420 (19)[b]	418 (18)[b]	427 (23)[b]
Monthly Mean Exposure (μg/m3)							
PM_{10}, spatial	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}, spatiotemporal	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)
$PM_{2.5-10}$, spatiotemporal	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; PM_{10}, PM < 10 μm in diameter; $PM_{2.5-10}$, PM > 2.5 and < 10 μm in diameter; QT, QT interval; QTc, Bazett's heart rate-corrected QT; 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 Institute 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

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Table 2.

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						QT interval					
	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**	**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)	64 (7)	64 (7)	65 (7)	56 (6)	60 (5)	61 (7)	63 (7)	64 (7)	65 (7)	56 (6)	60 (5)
Male, n (%)	1,342 (19)	0 (0)	0 (0)	0 (0)	910 (36)	432 (42)	1,300 (19)	0 (0)	0 (0)	0 (0)	880 (37)	420 (41)
Race / ethnicity, n (%)												
Black or African American	3,390 (47)	560 (28)	0 (0)	316 (24)	2,514 (100)	0 (0)	3,198 (46)	515 (28)	0 (0)	318 (24)	2,365 (100)	0 (0.0)
Hispanic/Latino	510 (7)	318 (16)	0 (0)	192 (14)	--[c]	--[c]	501 (7)	310 (17)	0 (0)	191 (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)	3,196 (46)	1,047 (56)	300 (100)	830 (62)	0 (0.0)	1,019 (100)
More than high school, n (%)	3,905 (55)	1,403 (72)	66 (22)	425 (32)	1,526 (61)	485 (47)	3,697 (54)	1,328 (72)	64 (22)	427 (32)	1,408 (60)	470 (46)
Smoking status, n (%)												
Never	3,408 (48)	1,012 (52)	127 (42)	709 (54)	1,122 (45)	438 (42)	3,274 (48)	949 (52)	124 (42)	707 (54)	1,060 (45)	434 (43)
Former	2,541 (35)	771 (40)	148 (49)	478 (36)	750 (30)	394 (38)	2,446 (36)	732 (40)	142 (48)	484 (37)	698 (30)	390 (38)
Current	1,135 (16)	154 (8)	28 (9)	126 (10)	624 (25)	203 (20)	1,098 (16)	151 (8)	30 (10)	131 (10)	591 (25)	195 (19)
Alcohol use, n (%)												
Never	1,662 (23)	238 (12)	32 (10)	196 (15)	879 (35)	317 (31)	1,576 (23)	222 (12)	30 (10)	193 (15)	822 (35)	309 (30)
Former	1,859 (26)	561 (29)	53 (17)	300 (23)	794 (32)	151 (15)	1,733 (25)	514 (28)	50 (17)	299 (22)	724 (31)	146 (14)
Current	3,593 (50)	1,151 (59)	223 (72)	829 (63)	823 (33)	567 (55)	3,536 (51)	1,107 (59)	220 (73)	842 (63)	803 (34)	564 (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)	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)	29.3 (6.0)	29.7 (6.0)	28.5 (5.6)	29.9 (6.0)	30.1 (6.2)	26.2 (4.4)	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	3,069 (43)	999 (51)	143 (46)	726 (55)	1,002 (40)	199 (19)	2,804 (41)	911 (49)	131 (44)	725 (54)	859 (36)	178 (18)
Hyperlipidemia	1,341 (19)	300 (15)	38 (12)	196 (15)	572 (23)	235 (23)	1,249 (18)	267 (14)	40 (13)	198 (15)	519 (22)	225 (22)

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Characteristic	Heart rate variability						QT interval					
	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	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
Diabetes	776 (11)	182 (9)	17 (6)	161 (12)	383 (15)	33 (3)	687 (10)	157 (8)	16 (5)	162 (12)	323 (14)	29 (3)
Chronic lung disease	701 (10)	194 (10)	27 (9)	146 (11)	199 (8)	135 (13)	631 (9)	179 (10)	28 (9)	146 (11)	151 (6)	127 (13)
Coronary heart disease	486 (7)	128 (7)	24 (8)	90 (7)	183 (7)	61 (6)	374 (5)	101 (5)	19 (6)	88 (7)	120 (5)	46 (5)
Congestive heart failure	341 (5)	56 (3)	10 (3)	14 (1)	222 (9)	39 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
ECG traits (ms), mean (SD)												
RR	925 (142)	925 (140)	924 (128)	910 (139)	924 (148)	948 (137)	927 (142)	927 (141)	923 (129)	910 (140)	928 (148)	950 (137)
RMSSD	23 (22)	23 (24)	22 (18)	21 (20)	26 (22)	20 (16)	23 (22)	23 (24)	22 (19)	21 (20)	26 (22)	20 (16)
SDNN	20 (16)	20 (18)	20 (14)	18 (14)	22 (18)	19 (14)	20 (17)	21 (18)	20 (14)	18 (14)	22 (18)	19 (14)
QT	406 (30)	402 (31)	402 (27)	401 (31)	411 (31)	413 (26)	405 (30)	401 (31)	400 (26)	400 (31)	410 (30)	412 (26)
QTc	424 (23)	419 (19)	419 (19)	421 (20)	430 (27)	426 (23)	423 (22)	417 (18)	417 (17)	420 (18)	429 (26)	426 (22)
Monthly Mean Exposure (μg/m3)												
$PM_{10,}$ spatial	27.4 (6.2)	27.5 (6.2)	26.6 (6.0)	27.5 (6.3)	34.8 (6.3)	34.4 (5.8)	31.0 (7.2)	27.5 (6.2)	26.7 (6.0)	27.4 (6.3)	34.8 (6.3)	34.4 (5.8)
$PM_{10,}$ spatiotemporal	20.3 (6.1)	20.6 (6.5)	19.4 (5.5)	20.4 (6.1)	20.4 (4.5)	23.2 (5.2)	20.8 (5.7)	20.6 (6.5)	19.4 (5.6)	20.4 (6.2)	20.4 (4.6)	23.1 (5.2)
$PM_{2.5\text{-}10,}$ spatiotemporal	8.4 (4.5)	6.9 (5.2)	7.9 (4.2)	8.5 (4.5)	7.3 (2.1)	7.8 (2.4)	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; PM_{10}, PM < 10 μm in diameter; $PM_{2.5\text{-}10}$, PM > 2.5 and < 10 μm in diameter; QT, QT interval; QTc, Bazett's heart rate-corrected QT; 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

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Table 3.

Stratified and meta-analyzed changes[a] in heart rate variability and QT interval duration per 10 μg/m3 increase in PM concentrations among nHRV = 82,107 / nQT = 76,711 study participants, Women's Health Initiative (1993-2005) and Atherosclerosis Risk in Communities study (1986-1998)

Monthly Mean 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}, spatial	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}, spatiotemporal	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**
$PM_{2.5-10}$, spatiotemporal	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; PM_{10}, PM < 10 μm in diameter; $PM_{2.5-10}$, 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)

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Table 4.

Meta-analyzed changes[a] in DNA methylation per 10 μg/m3 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)

Monthly Mean 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 %	95% CI	*P*	$P_{Cochran's\ Q}$
PM_{10}, spatial	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}, spatiotemporal	cg24102420	−0.4	−0.6, −0.2	1.7E-04	0.82	−0.3	−0.5, −0.1	1.0E-03	0.74
$PM_{2.5\text{-}10}$, spatiotemporal	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; HRV, heart rate variability; PM, particulate matter; PM_{10}, PM < 10 μm in diameter; $PM_{2.5\text{-}10}$, 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)

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Table 5.

Meta-analyzed changes[a] 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; 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)

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Table 6.

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]
Monthly Mean Exposure	**CpG**	**ECG[a]**	**Estimate[a]**	**95% CI**	***P***	**Estimate[a]**	**95% CI**	***P***	**%**
PM_{10}, spatial	cg19004594		0.9	−0.2, 0.4	0.58	0.00	−0.02, 0.02	0.79	0
PM_{10}, spatiotemporal	cg24102420	RR[b]	0.6	−0.2, 0.3	0.61	0.00	−0.02, 0.03	0.86	0
$PM_{2.5\text{-}10}$, spatiotemporal	cg12124767		0.2	−0.2, 0.7	0.30	0.01	−0.04, 0.05	0.74	3
PM_{10}, spatial	cg19004594		−0.1	−4.0, 3.9	0.95	0.00	−0.28, 0.29	0.98	--
PM_{10}, spatiotemporal	cg24102420	SDNN[b]	3.9	0.1, 7.8	0.04	−0.10	−0.49, 0.29	0.61	--
$PM_{2.5\text{-}10}$, spatiotemporal	cg12124767		3.6	−2.0, 9.5	0.21	0.08	−0.21, 0.36	0.60	2
PM_{10}, spatial	cg19004594		0.6	−3.4, 4.7	0.78	0.03	−0.27, 0.32	0.86	5
PM_{10}, spatiotemporal	cg24102420	RMSSD[b]	4.7	0.7, 8.8	0.02	−0.09	−0.48, 0.30	0.66	--
$PM_{2.5\text{-}10}$, spatiotemporal	cg12124767		4.3	−1.7, 10.7	0.16	0.12	−0.20, 0.46	0.46	3
PM_{10}, spatial	cg19004594		−0.5	−1.5, 0.5	0.32	0.00	−0.07, 0.08	0.90	--
PM_{10}, spatiotemporal	cg24102420	QT[c]	−0.1	−1.0, 0.9	0.88	−0.01	−0.11, 0.09	0.86	12
$PM_{2.5\text{-}10}$, spatiotemporal	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; HRV, heart rate variability; PM, particulate matter; PM_{10}, PM < 10 μm in diameter; $PM_{2.5\text{-}10}$, 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)

[d]Proportion mediated not estimated when the indirect effect and direct effects were oppositely signed