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Genome-wide Association Study of Susceptibility to Particulate Matter–Associated QT Prolongation

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Background: Ambient particulate matter (PM) air pollution exposure has been associated with increases in QT interval duration (QT). However, innate susceptibility to PM-associated QT prolongation has not been characterized.

Objective: To characterize genetic susceptibility to PM-associated QT prolongation in a multi-racial/ethnic, genome-wide association study (GWAS).

Methods: Using repeated electrocardiograms (1986–2004), longitudinal data on PM $<10\,\mu m$ in diameter (PM_{10}), and generalized estimating equations methods adapted for low-prevalence exposure, we estimated approximately 2.5×10^6 SNP $\times$ PM_{10} interactions among nine Women's Health Initiative clinical trials and Atherosclerosis Risk in Communities Study subpopulations ($n = 22{,}158$), then combined subpopulation-specific results in a fixed-effects, inverse variance-weighted meta-analysis.

Results: A common variant (rs1619661; coded allele: *T*) significantly modified the QT-PM_{10} association ($p = 2.11\times10^{-8}$). At PM_{10} concentrations >90th percentile, QT increased 7 ms across the *CC* and *TT* genotypes: 397 (95% confidence interval: 396, 399) to 404 (403, 404) ms. However, QT changed minimally across rs1619661 genotypes at lower PM_{10} concentrations. The rs1619661 variant is on chromosome 10, 132 kilobase (kb) downstream from *CXCL12*, which encodes a chemokine, stromal cell-derived factor 1, that is expressed in cardiomyocytes and decreases calcium influx across the L-type Ca^{2+} channel.

Conclusions: The findings suggest that biologically plausible genetic factors may alter susceptibility to PM_{10}-associated QT prolongation in populations protected by the U.S. Environmental Protection Agency's National Ambient Air Quality Standards. Independent replication and functional characterization are necessary to validate our findings. https://doi.org/10.1289/EHP347

Introduction

Ambient particulate matter (PM) air pollution contributes substantially to cardiovascular disease morbidity and mortality (Dockery et al. 1993; Miller et al. 2007; Samet et al. 2000). Several studies have attributed part of the contribution to prolonged ventricular repolarization, a known risk factor for cardiovascular events (Dekker et al. 2004; Goldberg et al. 1991; Rautaharju et al. 2006a, b; Schouten et al. 1991), as suggested by PM-associated increases in risk of ventricular arrhythmia/sudden cardiac death (Dockery et al. 2005; Ljungman et al. 2008). Indeed, PM has been associated with increases in QT interval duration (QT) (Liao et al. 2010; Mordukhovich et al. 2016; Van Hee et al. 2011), a quantitative electrocardiographic measure of ventricular repolarization. Although QT prolongation is also related to innate variation in myocardial cation channel proteins (Arking et al. 2014) and the rate at which cation gradients across these voltage-gated channels return to their resting potential, genetic susceptibility to (i.e., modification of) PM-associated QT prolongation has not been evaluated.

The Clean Air Act nevertheless requires the U.S. Environmental Protection Agency (EPA) to create National Ambient Air Quality Standards (NAAQS) that protect populations susceptible to the adverse health effects of PM. Mindful of such regulatory obligations and their public health implications, the present study examined genome-wide variation in susceptibility to PM_{10}-associated QT prolongation among nine longitudinally well-characterized and racially/ethnically diverse populations of men and women living in the 48 contiguous states in the United States (U.S. EPA Regions 1–10).

Methods

Study Design

The study included 22,158 participants in the Atherosclerosis Risk in Communities Study (ARIC) (ARIC Investigators 1989) and the Women's Health Initiative (WHI) clinical trials (National Institutes of Health 1998) cohorts who were examined between 1986 and 2004. They consented to the use of DNA for genetic research, had genomic

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data, and had one or more high-quality, non-paced baseline or follow-up electrocardiograms (ECGs). They were not taking antiarrhythmic medication and did not have heart failure, bundle branch block (QRS interval duration >120 ms),or Wolf-Parkinson-White pattern.

ARIC participants included black and white men and women. White WHI participants were drawn from three substudies: the Genome-wide Association Research Network into Effects of Treatment (GARNET) (National Institutes of Health 2013), Modification of PM-Mediated Arrhythmogenesis in Populations (MOPMAP) (National Institutes of Health 2010), and Women's Health Initiative Memory Study (WHIMS) (Shumaker et al. 1998). Black and Hispanic WHI participants were drawn from the single nucleotide polymorphism (SNP) Health Association Resource Project (SHARe) (National Institutes of Health 2011).

Electrocardiography

At triennial participant examinations and examination sites, trained and certified technicians used standardized procedures and identical electrocardiographs (MAC PC^{TM}; GE Marquette Electronics Inc.) to digitally record and telephonically transmit resting, 10-second, standard, simultaneous 12-lead ECGs 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^{TM} program (GE Marquette) (ARIC Investigators 1987; WHI Study Group 1994). Analysis included reliable estimation of median QT (ms) from the orthogonal XYZ leads and median RR interval duration (RR, ms), i.e., the unit-corrected inverse of heart rate (Schroeder et al. 2004; Vaidean et al. 2005).

Genotyping, Quality Control, and Imputation

Genotypes of participants determined on the Affymetrix GeneChip SNP Array 6.0, Illumina Human Omni1-Quad v1-0 B, Affymetrix Genome-wide Human CEU I, or Human OmniExpress Exome-8v1_B Genome-wide Human platforms were subjected to platform-specific quality filters (see Table S1). In GARNET, SNP dosage was imputed using BEAGLE (Browning and Browning 2009) and 1,000 Genomes Project (1000G v3 EUR, 03/2012) reference haplotypes. In the remaining subpopulations, imputation relied on MACH (Li et al. 2010) or Minimac (Howie et al. 2012) and HapMap2 CEU reference haplotypes in ARIC, MOPMAP, and WHIMS whites; a 1:1 mix of HapMap2 CEU/YRI in ARIC and WHI SHARe blacks; and all 1000G ancestry samples in SHARe Hispanics. Coordinate ranges for all HapMap 2 (Build 36) SNPs were converted to Build 37 using liftOver (Kuhn et al. 2012).

PM Exposure Estimation

Participant addresses at the time of ECGs were accurately geocoded (Whitsel et al. 2004; Whitsel et al. 2006), and then national-scale, log-normal ordinary kriging and U.S. EPA Air Quality Systems (AQS) monitor data were used to estimate geocoded address-specific, daily mean concentrations of ambient PM <10 μm in diameter (PM_{10}) (Liao et al. 2006) between 1986 and 2004. Validity of the PM_{10} exposure-estimation method during this period was evaluated using standard cross-validation statistics: the average prediction error (PE = predicted – measured PM_{10} concentration at each monitor site), standardized prediction error (SPE = PE divided by its estimated standard error), root mean square standardized (RMSS = standard deviation of SPE across sites), root mean square prediction error (RMS = empirical standard error based on the mean square of the predictions), and mathematically calculated standard error (SE). Observed values of PE and SPE near 0, RMSS near 1, and RMS near SE have provided evidence of model validity (Liao et al. 2006; Liao et al. 2007) and justification for use of the estimates in published studies of PM_{10}–health outcome associations (Holliday et al. 2014; Liao et al. 2009; Shih et al. 2011; Whitsel et al. 2009; Zhang et al. 2009) in which daily concentrations were averaged over the day of and day before each ECG (lag_{0-1}). Although comparably estimated and accurate, daily mean concentrations of ambient PM <2.5 μm in diameter ($PM_{2.5}$) were not available until 1999, when EPA AQS monitoring data for $PM_{2.5}$ became more widely available, monthly mean concentrations of ambient $PM_{2.5}$ were spatiotemporally estimable at the same geocoded addresses between 1986 and 2004 using generalized additive mixed models, the log-transformed ratio of $PM_{2.5}$ to predicted PM_{10}, and geographic information system (GIS)-based predictors. A 5- to 10-set, 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 the model performed well (Yanosky et al. 2014).

Statistical Analysis

The population was stratified by study, race/ethnicity, and sex. Within these subpopulations, modeling involved a generalized estimating-equations method implemented in R (via the *boss* package) (Voorman and Sitlani 2013) that was designed to detect interactions between SNPs and low-prevalence environmental exposures on a genome-wide scale using repeated measures data (Sitlani et al. 2015), in which i and j denote the i^{th} participant at the j^{th} electrocardiographic examination. Models were given by

$$QT_{ij} = \beta_0 + \beta_1 G_i + \beta_2 E_{ij} + \beta_3 G_i x E_{ij} + \beta_4 C_{ij},$$

where QT_{ij} is QT (ms); β_0 is the intercept; G_i is the HapMap 2 SNP dosage (0–2); E_{ij} is the dichotomized PM_{10} concentration ($\leq$ or $>$ an *a priori* threshold for abnormality, defined as the subpopulation-specific 90th percentile); $G_i x E_{ij}$ is the additive interaction term SNP × PM_{10}, and β_4 is a vector of β coefficients corresponding to C_{ij}, a vector of covariables comprising age (years), geographic region (in WHI) or center (in ARIC), season, calendar year, RR interval (ms), and principal components of ancestry estimated using Eigenstrat (Price et al. 2006). Fit of the fully adjusted model to dichotomized PM_{10} concentrations reinforced its selection over alternatives expressing QT as a linear, quadratic, linear spline, or quadratic spline function of PM_{10} with one to five knots, with and without restriction (Hardin and Hilbe 2012; Pan 2001). To reduce type-1 errors in detecting SNP × PM_{10} interactions at low-prevalence exposure, the t-reference distribution with degrees of freedom based on Satterthwaite's approximation was used (Pan and Wall 2002; Satterthwaite 1946; Sitlani et al. 2015). SNPs with a low minor allele frequency and imputation quality also were excluded from subpopulation-specific analyses (Sitlani et al. 2015) when

$$2 \times MAF \times IQ \times N_{\text{exposed}} \leq 10,$$

where MAF is the minor allele frequency, IQ is the SNP imputation quality, and N_{exposed} is the estimated number of observations with a PM_{10} concentration >90th percentile. For each of the approximately 2.5 million SNPs remaining across subpopulations, subpopulation-specific SNP × PM_{10} interaction estimates were adjusted using genomic control, tested for homogeneity (Cochran's Q), and combined using fixed-effects, inverse

Table 1. Characteristics of subpopulations, by study, race/ethnicity, and sex.

Study	Race/ethnicity	Sex (mean ± SD)	n (mean ± SD)	Age, y (mean ± SD)	ECGs (mean ± SD)	QT, ms (mean ± SD)	PM_{10}, $\mu g/m^3$[a] (mean ± SD)	P90 (mean ± SD)
ARIC	Black	Men	826	57.6 ± 6.7	3.2 ± 1.0	402 ± 33	34.4 ± 12.7	50.3
ARIC	Black	Women	1,343	57.3 ± 6.4	3.3 ± 0.9	403 ± 33	34.3 ± 12.6	50.9
ARIC	White	Men	3,976	59.0 ± 6.5	3.5 ± 0.9	406 ± 31	33.4 ± 12.9	49.8
ARIC	White	Women	4,462	58.5 ± 6.5	3.6 ± 0.8	405 ± 29	33.3 ± 12.9	49.7
WHI GARNET[b]	White	Women	1,732	68.8 ± 7.1	2.5 ± 0.9	401 ± 30	27.6 ± 10.7	41.5
WHI MOPMAP[b]	White	Women	1,237	67.0 ± 7.0	2.7 ± 0.8	402 ± 30	27.3 ± 10.6	41.2
WHI SHARe	Black	Women	3,538	64.6 ± 7.1	2.4 ± 0.9	400 ± 33	28.1 ± 10.5	41.8
WHI SHARe	Hispanic	Women	1,562	63.5 ± 6.7	2.5 ± 0.8	400 ± 30	29.4 ± 10.6	43.4
WHI WHIMS	White	Women	3,482	73.4 ± 4.5	2.4 ± 0.7	400 ± 30	26.6 ± 10.2	39.7
All	White (67%)	Women (78%)	22,158	64.3	2.9	402	29.9	45.4

Note: ARIC, Atherosclerosis Risk in Communities study; ECG, electrocardiogram; GARNET, Genomics and Randomized Trials Network; MOPMAP, Modification of PM-Mediated Arrhythmogenesis in Populations; P90, 90th percentile; PM_{10}, particulate matter <10 μm in diameter; QT, QT interval duration; SD, standard deviation; SHARe, SNP Health Association Resource; WHI, Women's Health Initiative; WHIMS, Women's Health Initiative Memory Study.
[a]Range, 5.9–124.3 $\mu g/m^3$.
[b]Controls.

variance-weighted meta-analysis implemented in METAL (Willer et al. 2010). Two-stage, split-sample alternatives (in which subpopulations are grouped into discovery and replication populations) were considered but avoided to maximize statistical power (Skol et al. 2006).

Observed genome-wide distributions of meta-analyzed SNP-specific interaction p-values were $-\log_{10}$ transformed and compared with those expected under the χ^2 distribution using a quantile-quantile (QQ) plot and the genomic inflation factor (λ) (Devlin and Roeder 1999). They were also mapped by chromosomal position to produce Manhattan and regional association plots (Pruim et al. 2010).

After accounting for linkage disequilibrium (LD) among the approximately 2.5 million SNPs across racially/ethnically diverse subpopulations, a Bonferroni-corrected threshold of $p < 5.0 \times 10^{-8}$ was used to identify genome-wide significant SNP × PM_{10} interactions (Barsh et al. 2012; Pe'er et al. 2008). Interaction and standard error estimates of SNPs meeting that threshold were forest plotted and used to compute predicted mean QT (ms) and 95% confidence intervals (95% CIs) by genotype and PM_{10} concentration, while adjusting for centered covariables. Sensitivity of results also was examined as follows: to lower PM_{10} thresholds for abnormality (50th–80th percentiles), longer lagged exposure averaging periods (1–4 wk), alternative exposures ($PM_{2.5}$) (Yanosky et al. 2014), use of β-antagonists, additional adjustments [temperature (°C); dew point (°C); barometric pressure (kPa); neighborhood socioeconomic status; smoker status (current, former, never); alcohol drinker status (current, former, never); total caloric intake (kcal); sedentary lifestyle] and separately, application of Bayesian meta-analytic methods allowing for ancestral population heterogeneity implemented in MANTRA (Morris 2011) (genome-wide threshold of importance: $\log_{10}$ Bayes Factor ≥ 6.0, probability of heterogeneity <0.50) (Stephens and Balding 2009).

Significant associations identified lead SNPs and variants in LD with them ($r^2 > 0.8$) for bioinformatic characterization using HaploReg V4 (Ward and Kellis 2012), the UCSC Genome Browser™ (Kuhn et al. 2012), and the WashU Epigenome Browser (Zhou et al. 2011) with data from the Encyclopedia of DNA Elements Project (ENCODE) (Rosenbloom et al. 2010) and Roadmap Epigenomics Project (Bernstein et al. 2010). Their characterization involved searching surrounding regions of the cardiac genome (e.g., in cardiomyocytes, cardiac fibroblasts, and heart tissue) for evidence of active or altered transcription.

Results

The nine ARIC and WHI subpopulations in this study collectively represented 22,158 participants, of whom 26% were black, 7% were Hispanic, and 22% were male. On average, participants were 64.3 years old and contributed 2.9 ECGs with a mean QT of 402 ms. The two-day mean (lag_{0-1}) PM_{10} concentration and its 90th percentile (P90) were 29.9 $\mu g/m^3$ and 45.4 $\mu g/m^3$, i.e., below NAAQS for PM_{10} in place at the time of participant examinations (Table 1) (U.S. EPA 2016).

Manhattan, regional association, and QQ plots (Figures 1 and 2; see Figure S1) of interaction p-values from the trans-ethnic, fixed-effects, inverse variance-weighted meta-analysis identified one genome-wide significant association (rs1619661; $p = 2.11 \times 10^{-8}$) and 22 subthreshold associations ($5 \times 10^{-8} < p < 5 \times 10^{-6}$) across six independent loci (Table 2). The lead SNP, rs1619661 is on chromosome 10, approximately 132 kilobase (kb) downstream of *CXCL12* (Table 2). This variant's coded

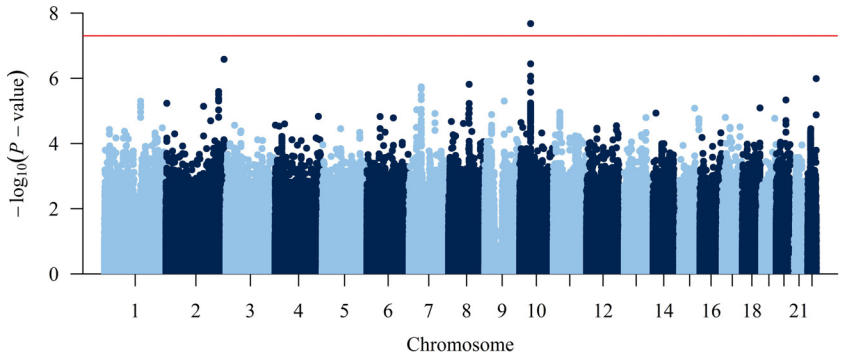

Figure 1. Manhattan plot of $-\log_{10}$ p-value vs. chromosomal position of each SNP from the trans-ethnic, fixed-effects meta-analysis of the SNP × PM_{10} interactions. The red line references the genome-wide significance threshold (p-value $< 5.0 \times 10^{-8}$).

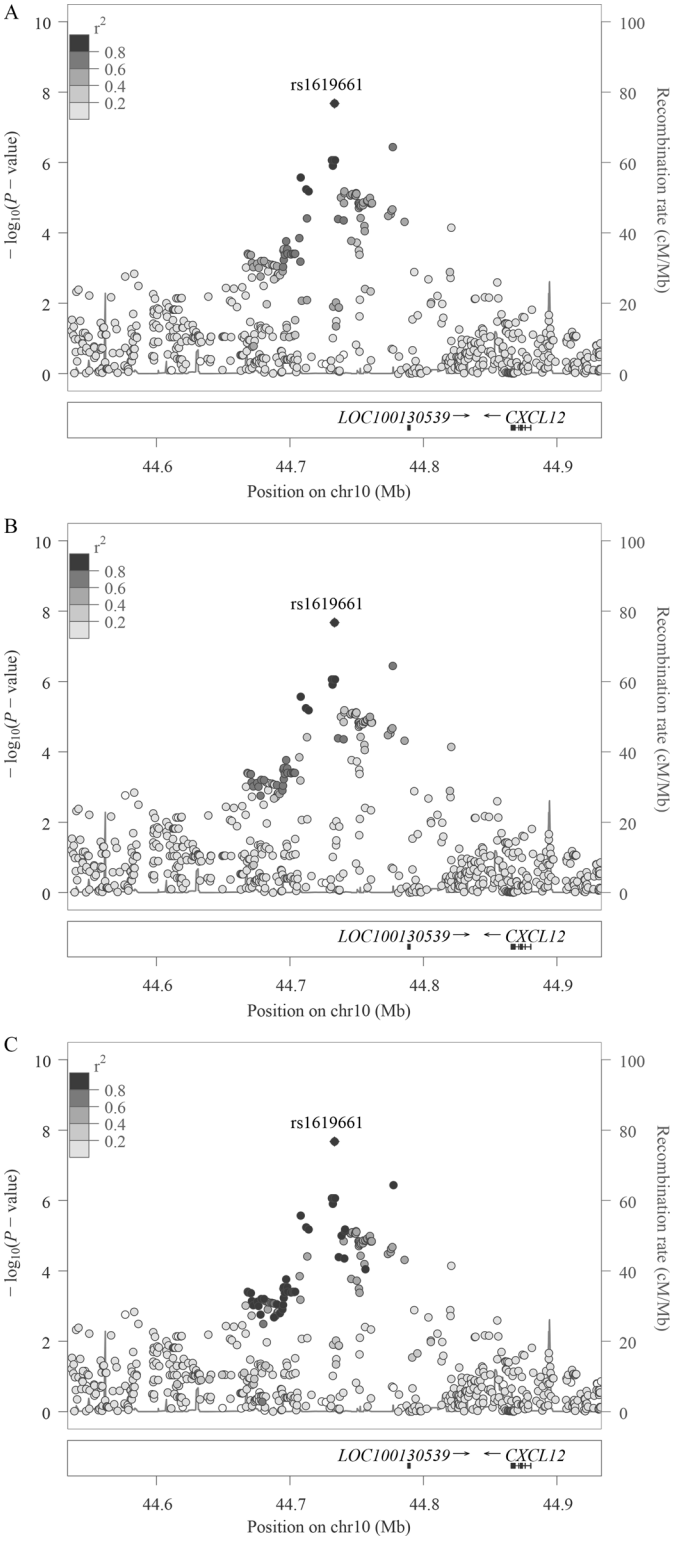

Figure 2. Regional plots of the locus, rs1619661, identified by the trans-ethnic, fixed-effects meta-analysis of the SNP × PM_{10} interactions, on chromosome 10, near *CXCL12*. Each point represents the $-\log_{10} p$-value of a SNP plotted as a function of its genomic position (build 37) and the genome-wide significance threshold (p-value $< 5.0 \times 10^{-8}$). One SNP reached this threshold. The color coding of all other SNPs indicated linkage disequilibrium with this lead SNP, estimated among Africans (*A*), Ad-mixed Americans (*B*), and Europeans (*C*) from 1000G. Recombination rates were estimated from the 1,000 Genomes Project.

allele, *T* (vs. the noncoded allele, *C*), was common among racial/ethnic groups (*T* allele: 81–92%; *CC* genotype: 1.6%, *CT*: 21.8%, *TT*: 76.7%) and associated with QT prolongation in eight (89%) of the nine subpopulations ($P_{Cochran's\,Q} = 0.14$; Figure 3).

At PM_{10} concentrations >90th percentile, QT increased 7 ms across the *CC*, *CT*, and *TT* rs1619661 genotypes: from 397 (95% CI: 396, 399) to 401 (400, 401) to 404 (403, 404) ms, but at PM_{10} concentrations ≤90th percentile, QT only increased from 402 (401, 403) to 403 (402, 403) to 403 (403, 403) ms (Figure 4; Table S2). Associations were insensitive to additional adjustment, Bayesian meta-analysis ($\log_{10}$ Bayes Factor = 6.20; probability of heterogeneity = 0.37), and adoption of a 50-$\mu g/m^3$ PM_{10} threshold, the annual NAAQS for PM_{10}. However, they were attenuated by decreasing PM_{10} thresholds, increasing lagged exposure averaging periods, substituting $PM_{2.5}$, and restricting to β-antagonist users (see Table S3, Figure S2).

In cardiomyocytes, cardiac fibroblasts, and other (including fetal, right atrial, and left/right ventricular) heart tissue, genomic regions surrounding rs1619661 and associated SNPs included deoxyribonuclease (DNAse1) hypersensitivity areas, DNA methylation sites, enhancer/promoter histone marks, and regulatory motifs (see Figure S3 and "TITLE" in Supplemental Material).

Full results from the trans-ethnic, fixed-effects, inverse-variance meta-analysis and rs1619661 characterization using HaploReg Version 4 (Ward and Kellis 2012) and the WashU Epigenome Browser™ (Zhou et al. 2011) are available at https://qtgwaspm.web.unc.edu/EHP/ (Gondalia 2016).

Discussion

This genome-wide association study (GWAS) of gene–environment interactions discovered a genetic variant associated with increased susceptibility of a racially and geographically diverse population of U.S. men and women to prolonged ventricular repolarization during short-duration ambient PM air pollution exposures below annual and daily thresholds established by the U.S. EPA under the Clean Air Act (U.S. EPA 2016).

Although we observed a clinically modest, 7-ms increase in QT among persons in the highest PM_{10} decile with two vs. zero copies of the *T* allele (genotype *TT* vs. *CC*, respectively), the *T* allele of rs1619661 tends to be so common in many U.S. populations that related but seemingly minor population-level shifts in QT may have significant public-health implications. Indeed, upper decile PM_{10}-associated increases in QT exceed the U.S. Food and Drug Administration (FDA) 5-ms threshold used in premarket evaluation of drug safety (U.S. FDA 2015), an increase that may also carry cardiovascular disease morbidity and mortality risk (Zhang et al. 2011).

The attendant cardiovascular risks are plausibly related to *CXCL12* (Table 2)—the locus most proximate to rs1619661—which has been implicated in, for example, GWAS of coronary artery disease (Samani et al. 2007) and early-onset myocardial infarction (Kathiresan et al. 2009). *CXCL12* encodes stromal cell-derived factor 1 (SDF1), an evolutionarily conserved chemokine that is expressed in cardiomyocytes (Pyo et al. 2006) and is induced by pro-inflammatory stimuli, including particulate exposures (Liberda et al. 2010). SDF1 binds to *CXCR4*, a seven-transmembrane, G-protein coupled receptor that is widely distributed on cardiomyocytes and neurons.

In those cell types, the ligand-receptor complex inhibits β-adrenergically activated calcium influx through the L-type Ca^{2+} ion channel (Pyo et al. 2006), recently implicated in the largest GWAS of QT to date (Arking et al. 2014). Resultant shortening of the ventricular action potential (Phase 2) and its electrocardiographic manifestation, QT interval duration, was apparent in the present study among persons in the highest PM_{10} decile with the *C* allele [i.e., those

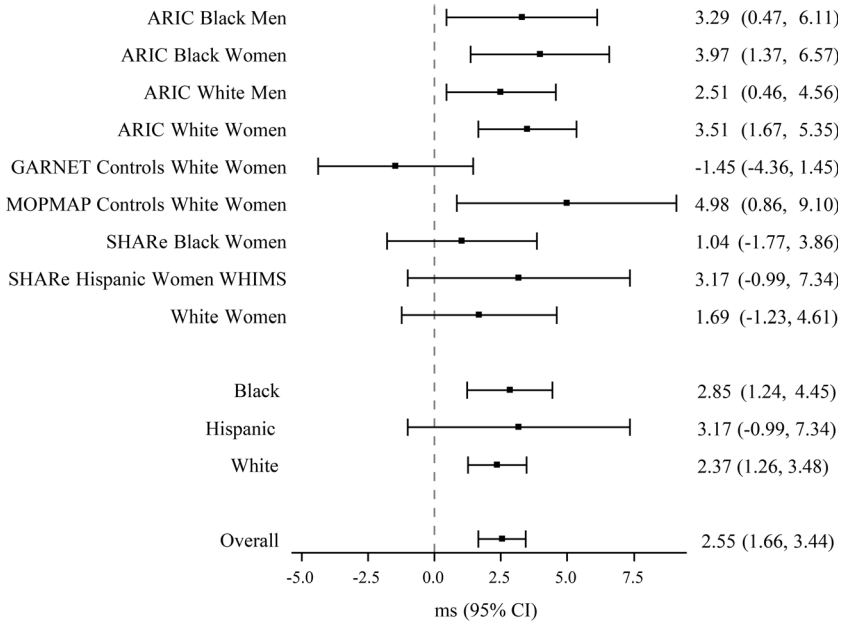

Figure 3. Forest plot of SNP × PM_{10} interaction (95% confidence interval) per *T* allele increase in rs1619661 (genotype *CT*) at PM_{10} concentrations >90th percentile, by study, race/ethnicity, and overall ($P_{Cochran's\ Q} = 0.14$).

individuals with the *CC* or *CT* (vs. *TT*) genotype]. Although contrary to PM-associated increases in QT duration observed in prior studies (Liao et al. 2010; Mordukhovich et al. 2016; Van Hee et al. 2011), this group represents only a minority of the study population. Likewise, its reversibility was reflected, albeit in this observational epidemiologic context, by the attenuation of the observed SNP × PM_{10} interaction among users of β-adrenergic antagonists, the first-line therapy in long-QT syndromes (LaRocca et al. 2010).

The SNP × PM_{10} interaction also was attenuated at longer lagged exposure averaging periods in this context. This form of attenuation highlights the potential role of β-adrenergic receptor-mediated blunting of sympathetic nervous system responses to chronic PM exposure. Indeed, sympathetic responses of the heart to stressors are mediated by the binding of catecholamines to cardiac β-adrenergic receptor s, the density, sensitivity, and activity of which decrease with chronic stress exposure (Konarska et al. 1989; Stone 1983). Chronic stress exposures also lead to adaptive changes of neural and glial cells in the central nervous system (McEwen 2007), which controls the heart via innervation of the sinoatrial node. The attenuated interactions that we observed herein may thereby reflect physiologically desensitizing adaptations to longer-term PM exposures.

However, several limitations apply to the study of gene-environment interaction in genome-wide contexts, e.g., low power and overestimation of observed effect sizes in hypothesis-generating discovery efforts (Göring et al. 2001). To increase power, we used all nine subpopulations in the discovery effort. To further increase power, we used generalized estimating equations methods to leverage repeated measures of QT and PM_{10} among 22,158 participants from two well-characterized, multi-ethnic, and environmentally diverse cardiovascular disease cohorts. Furthermore, we established homogeneity and robustness of SNP × PM_{10} interaction estimates among the cohorts, subpopulations, and races/ethnicities in meta-analyses, which were also subjected to additional adjustment for meteorological, neighborhood-socioeconomic, and lifestyle characteristics. Finally, the trans-ethnic, fixed-effects, inverse variance-weighted meta-analysis discovered a genome-wide significant interaction in data that also provided convincing evidence of association in a Bayesian meta-analysis that allowed for racial/ethnic heterogeneity, where the interaction was found to be 1.6 million times more likely under the alternative to the null hypothesis of no association.

The 132-kb separation of rs1619661 and *CXCL12* also limits the biological plausibility of their role in PM-associated QT prolongation. However, causal genes that are megabases away from GWAS-implicated lead SNPs have been identified in other settings (Musunuru et al. 2010; Smemo et al. 2014). For example, obesity-associated SNPs within the well-known *FTO* locus directly interact with promoter regions of *IRX3* that are

Table 2. Findings from the trans-ethnic, fixed-effects, inverse variance-weighted meta-analysis, including sub-threshold associations ($5 \times 10^{-8} < p < 5 \times 10^{-6}$).

Chr	Position	Lead SNP	CA /NCA	Coded allele Frequency			*p*-Value	Interaction Estimate (SE)	*n*	Nearest Gene	SNPs[a]
				Black	Hispanic	White					
10	44,733,383	rs1619661	*T/C*	0.81	0.92	0.91	2.11×10^{-8}	2.55 (0.46)	22,158	*CXCL12*	8
22	51,065,600	rs6151412	G/A	0.90	0.95	0.95	1.02×10^{-6}	3.88 (0.79)	20,921	*ARSA*	1
8	83,252,586	rs10504754	A/G	0.74	0.47	0.43	1.53×10^{-6}	1.54 (0.32)	22,158	*SNX16*	1
7	48,811,506	rs13309098	G/A	0.88	0.88	0.93	1.85×10^{-6}	2.37 (0.50)	22,158	*ABCA13-CDC14C*	4
2	213,065,465	rs6725041	*T/C*	0.78	0.44	0.48	2.55×10^{-6}	1.52 (0.32)	22,158	*ERBB4*	8
20	39,435,700	rs7361259	*T/C*	0.91			4.61×10^{-6}	5.98 (1.39)	2,169	*MAFB*	1

Note: CA, coded allele; CAF, coded allele frequency; Chr, chromosome; NCA, noncoded allele; SE, standard error; SNP, single nucleotide polymorphism.
[a]Total number of significant or sub-threshold SNPs located within the same gene or in LD with the lead SNP ($r^2 \geq 0.80$).

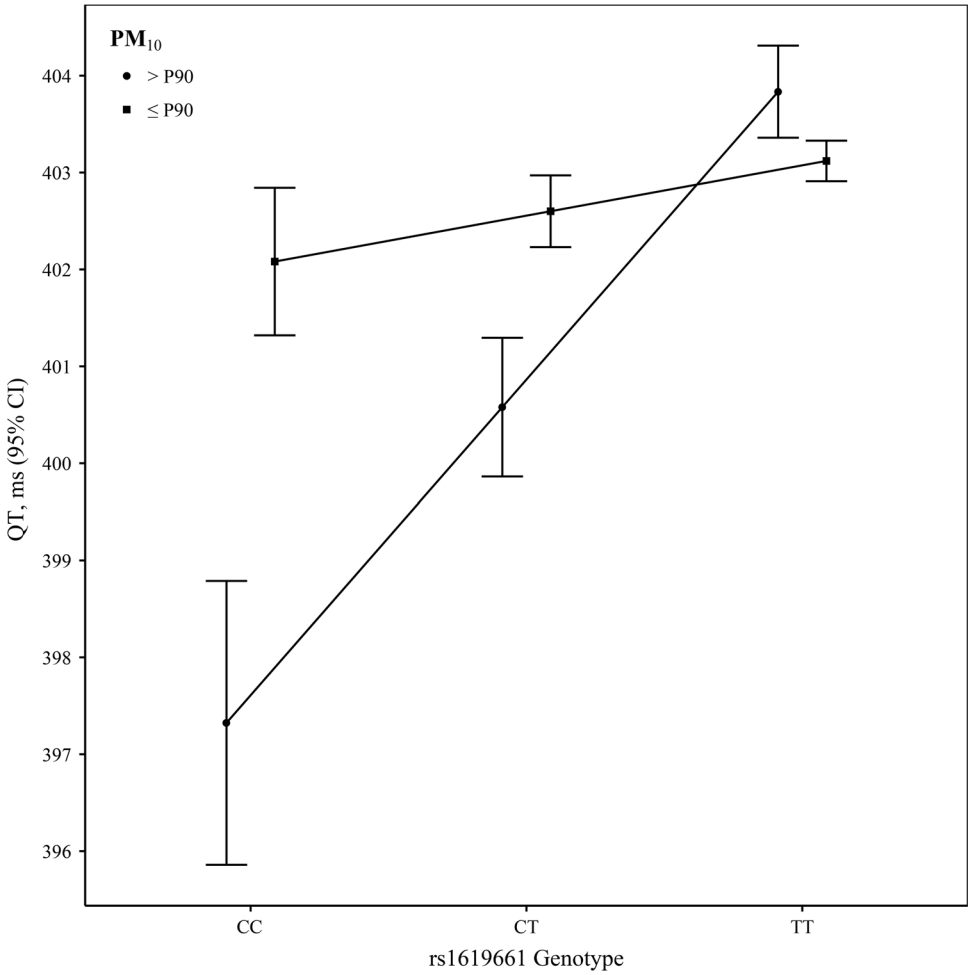

Figure 4. Predicted mean (95% confidence interval) QT (ms) per unit increase in the coded allele (*T*) dosage of rs1619661 at PM_{10} concentrations ≤and >90th percentile (P90), while adjusting for age, geographic region or center, season, calendar year, *RR* interval, and ancestry. *C* allele frequency range: 8–19%.

approximately 500 kb downstream. In fact, *IRX3*, which is causally linked to body mass and composition, participates in long-range interactions across a relatively large, 2-megabase region (Ragvin et al. 2010; Smemo et al. 2014). We also identified potentially active or altered transcription in regions of the cardiac genome surrounding rs1619661 and its associated SNPs with data from ENCODE. Although it is unclear whether these regions are functionally linked to *CXCL12*, it is plausible because of important, long-range (i.e., ∼120 kb) mechanisms of distal gene regulation (Sanyal et al. 2012). Nevertheless, expression assays are needed to confirm the proposed link between the rs1619661 locus and *CXCL12*.

Replication—a suggested gold standard for validating GWAS of main genetic effects—poses a particular challenge for gene-environment interaction studies like the one described here (Aschard et al. 2012; Aslibekyan et al. 2013; Hutter et al. 2013; Thomas et al. 2012). The extent of the challenge is related to the need for similarly powered populations with equally well-harmonized outcomes and exposures, even if they are, e.g., rare, difficult to measure, or peculiar to racial/ethnic minority populations poorly represented in large-scale GWAS to date. In the present study, a well-powered, independent replication was not feasible, given the limited availability of populations with high-quality, 12-lead ECGs; national-scale, kriged daily mean PM_{10} concentrations; and genome-wide SNP data. Moreover, functional validation in model organisms (Gibert et al. 2013; Stevens et al. 2015) was beyond the scope of the original project. We therefore view this discovery effort as hypothesis-generating, and given the importance of replication in protecting against type-1 error (Siontis et al. 2010), we have provided publicly accessible summary statistics (https://qtgwaspm.web.unc.edu/EHP/) to facilitate functional validation and external replication as additional data become available.

Although not reaching genome-wide significance, the sub-threshold loci identified herein (Table 2) may also warrant scrutiny. *ARSA* (rs6151412, synonymous) and *ERBB4* (rs6725041, intronic) are particularly compelling in this setting due to their functional role in Ca^{2+} transport (Brero et al. 2010; Ritzler et al. 1992). *ERBB4* has additionally been associated with cardiac myopathy (García-Rivello et al. 2005), coronary artery calcification (Wojczynski et al. 2013), and cardiomyocyte proliferation (Wadugu and Kühn 2012). *SNX16* (rs10504754, 498 kb upstream) has been associated with heart failure (Smith et al. 2010). *MAFB* (rs7361259, 118 kb upstream) has been implicated in a gene–drug interaction GWAS of rheumatoid arthritis, an inflammatory disorder associated with QT prolongation (Chauhan et al. 2015). *ABCA13-CDC14C* (rs13309098; 124-137 kb downstream) currently has no established link with cardiovascular disease.

Conclusions

We conclude that genetic variation may modify susceptibility to PM_{10}-associated QT prolongation, and pending further follow-

up, cautiously postulate changes in L-type Ca^{2+} ion channel activity triggered by inflammatory responses to PM exposure as a possible mechanism. In lieu of such possibilities, previously hypothesized genetic, inflammatory, and neural mechanisms of PM-mediated arrhythmogenesis would remain largely distinct. The Clean Air Act mandates setting of NAAQS for PM that protect sensitive populations—including persons with innate factors that may increase vulnerability to PM-associated disease. Although we cannot unequivocally link genetic variation to PM-associated QT prolongation, we did discover a biologically plausible variant that may confer susceptibility, a finding that must undergo replication and functional characterization in future studies.

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