Published in final edited form as:

Environ Int. 2017 May ; 102: 57–65. doi:10.1016/j.envint.2016.12.024.

Associations between long-term exposure to $PM_{2.5}$ component species and blood DNA methylation age in the elderly: The VA normative aging study

Jamaji C. Nwanaji-Enwerem[a,*], **Lingzhen Dai**[a], **Elena Colicino**[b], **Youssef Oulhote**[a], **Qian Di**[a], **Itai Kloog**[c], **Allan C. Just**[d], **Lifang Hou**[e], **Pantel Vokonas**[f], **Andrea A. Baccarelli**[b], **Marc G. Weisskopf**[a], and **Joel D. Schwartz**[a]

[a]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA

[b]Department of Environmental Health Sciences, Columbia Mailman School of Public Health, New York, NY, USA

[c]Department of Geography and Environmental Development, Ben-Gurion University of the Negev, Beer Sheva, Israel

[d]Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[e]Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

[f]VA Normative Aging Study, Veterans Affairs Boston Healthcare System, The Department of Medicine, Boston University School of Medicine, Boston, MA, USA

Abstract

Background—Long-term $PM_{2.5}$ exposure and aging have been implicated in multiple shared diseases; studying their relationship is a promising strategy to further understand the adverse impact of $PM_{2.5}$ on human health.

[*]Corresponding author at: Department of Environmental Health, Harvard T.H. Chan School of Public Health, Biological Sciences in Public Health, Building 1, Room G7, 665 Huntington Avenue, Boston, MA 02115., United States. jnwanajienwerem@g.harvard.edu (J.C. Nwanaji-Enwerem).

Contributors

JCN and JDS conceived and designed the study. EC, QD, IK, ACJ, LH, and PV gathered data. JCN performed the data analyses and drafted the manuscript. LD, JDS, EC, YO, MGW, and AAB contributed to the analyses. All authors revised and approved the manuscript.

Conflict of interest statement

None declared.

Ethics approval

Boston VA Medical Center, Harvard T.H. Chan School of Public Health (protocol 14027-102).

Data availability

Data are from the Normative Aging Study, from which restricted data are available for researchers who meet the criteria. A subset of the methylation data is deposited at NCBI dbGaP (study accession number: phs000853.v1.p1).

Competing financial interests related to this research

None.

Objective—We assessed the relationship of major $PM_{2.5}$ component species (ammonium, elemental carbon, organic carbon, nitrate, and sulfate) with Horvath and Hannum DNA methylation (DNAm) age, two DNA methylation-based predictors of chronological age.

Methods—This analysis included 552 participants from the Normative Aging Study with multiple visits between 2000 and 2011 (n = 940 visits). We estimated 1-year $PM_{2.5}$ species levels at participants' addresses using the GEOS-chem transport model. Blood DNAm-age was calculated using CpG sites on the Illumina HumanMethylation450 BeadChip. We fit linear mixed-effects models, controlling for $PM_{2.5}$ mass and lifestyle/environmental factors as fixed effects, with the adaptive LASSO penalty to identify $PM_{2.5}$ species associated with DNAm-age.

Results—Sulfate and ammonium were selected by the LASSO in the Horvath DNAm-age models. In a fully-adjusted multiple-species model, interquartile range increases in both 1-year sulfate (95%CI: 0.28, 0.74, $P < 0.0001$) and ammonium (95%CI: 0.02, 0.70, $P = 0.04$) levels were associated with at least a 0.36-year increase in Horvath DNAm-age. No $PM_{2.5}$ species were selected by the LASSO in the Hannum DNAm-age models. Our findings persisted in sensitivity analyses including only visits with 1-year $PM_{2.5}$ levels within US EPA national ambient air quality standards.

Conclusion—Our results demonstrate that sulfate and ammonium were most associated with Horvath DNAm-age and suggest that DNAm-age measures differ in their sensitivity to ambient particle exposures and potentially disease.

Keywords

DNA methylation age; Particulate matter 2.5; Long-term exposure; Epigenetics

1. Introduction

Fine particulate matter ($PM_{2.5}$) remains an inescapable environmental exposure and an enormous global public health concern (World Health, 2014). It is estimated that at least 2.1 million lives could be saved annually if $PM_{2.5}$ guidelines were adhered to worldwide (Apte et al., 2015). For the millions of people exposed to $PM_{2.5}$ daily, understanding the impact of $PM_{2.5}$ on human health is critical for developing interventions aimed at reducing $PM_{2.5}$-related morbidity and mortality globally. Researchers have consistently demonstrated that long-term $PM_{2.5}$ exposure is a major contributor to cardiopulmonary disease (Künzli et al., 2005; Giorgini et al., 2015; Martinelli et al., 2013; Zhong et al., 2015; Kloog et al., 2015; Raaschou-Nielsen et al., 2016), and emerging evidence suggests that $PM_{2.5}$ is a risk factor for previously unconsidered disease outcomes like cognitive decline (Terzano et al., 2010; Schikowski et al., 2015; Power et al., 2011). Nevertheless, much remains to be understood about how $PM_{2.5}$ contributes to even its most well-documented disease outcomes. One promising strategy to better understand the adverse impact of $PM_{2.5}$ on human health, is to study the relationship of $PM_{2.5}$ with aging. Many studies have implicated $PM_{2.5}$ as a contributor to accelerated aging (Chen and Schwartz, 2009; Weuve et al., 2012; Brown et al., 2015; Scheers et al., 2015; Shan et al., 2014; Wilker et al., 2015). Moreover, independent of $PM_{2.5}$ exposures, aging is associated with cardiopulmonary disease, cognitive decline, and many other $PM_{2.5}$-related disease outcomes (Rowe and Kahn, 1987, 2000, 2015; Chung et

al., 2009). Thus, understanding how $PM_{2.5}$ can contribute to aging, may provide additional insight into other adverse $PM_{2.5}$-related health effects.

DNA methylation-based biomarkers of age have proved to be promising tools in understanding the relationship of $PM_{2.5}$ with aging. These biomarkers have surpassed their initial utility of simply predicting chronological age, and have demonstrated remarkable usefulness in assessing individuals' risk of mortality, malignancy, neurocognitive disease, and other biologically-relevant health endpoints (Marioni et al., 2015, 2016; Horvath et al., 2015; Horvath and Ritz, 2015; Levine et al., 2015a, 2015b, 2015c). Evidence also suggests that these biomarkers of age are reflective of individuals' past environmental exposures (Horvath et al., 2014). One such study by our group demonstrated robust associations between $PM_{2.5}$ exposure levels and Horvath DNA methylation (DNAm) age. Horvath DNAm-age is a tissue-independent predictor of chronological age that is calculated from DNA methylation values at 353 chronological age-correlated CpG dinucleotides in Illumina's HumanMethylation450 BeadChip (Horvath, 2013). Specifically, in an elderly cohort and with fully-adjusted models, we observed that a 1 $\mu g/m^3$ increase in 1-year $PM_{2.5}$ exposure was associated with a 0.52-year increase in Horvath DNAm-age (Nwanaji-Enwerem et al., 2016).

Still, $PM_{2.5}$ is a heterogeneous mixture of carbonaceous fractions, inorganics, and metals; and it is widely appreciated that $PM_{2.5}$ component species often differ in their health effects (Zanobetti et al., 2009; Ito et al., 2011; Liu et al., 2016; Laurent et al., 2016). The present study builds upon our previous research and examines the relationships of $PM_{2.5}$ component species with both Horvath and Hannum DNAm-age in elderly men. Hannum DNAm-age is also a DNA methylation-based predictor of chronological age, but it is based on measurements from 71 CpG dinucleotides (Hannum et al., 2013). Only 6 CpG dinucleotides are shared between the Horvath and Hannum metrics. By investigating the relationships of $PM_{2.5}$ component species with these two forms of DNAm-age, we aim to (1) better understand how specific $PM_{2.5}$ species are related to aging, and (2) demonstrate differences in the biological utility of different DNAm-age measures.

2. Materials and methods

2.1. Study population

The participants in this analysis were part of the U.S. Veterans Affairs Normative Aging Study (NAS), a longitudinal investigation of aging men established in Eastern Massachusetts in 1963 (Bell et al., 1966). The men were free of known chronic medical conditions at enrollment, and returned for onsite, follow-up visits every 3–5 years. During these visits, detailed physical examinations were performed, bio-specimens including blood were obtained, and questionnaire data pertaining to diet, smoking status, and additional lifestyle factors that may impact health were collected. All participants provided written informed consent to the VA Institutional Review Board (IRB), and both the Harvard T.H. Chan School of Public Health and VA IRBs granted human subjects approval.

All NAS men with continued study participation as of the year 2000, when $PM_{2.5}$ component levels became available, were eligible for our study sample. After excluding

participants with a diagnosis of leukemia (n = 11), due to its potential influence on the DNA methylation of blood cells (Horvath, 2013), and those incomplete for the covariates of interest (n = 16), we had a total of 552 participants with 940 observations between the years 2000 and 2011. Of the 552 participants, 249 (45%) had one visit, 218 (40%) had two visits, and 85 (15%) had three or more visits.

2.2. DNA methylation and calculation of DNAm-age

Laboratory staff extracted DNA from the buffy coat of whole blood collected from each participant at each NAS follow-up visit (QIAamp DNA Blood Kit, QIAGEN, Valencia, CA, USA). DNA samples were then treated with bisulfite conversion (EZ-96 DNA Methylation Kit, Zymo Research, Orange, CA, USA) and hybridized to the 12 sample Illumina HumanMethylation450 BeadChips (Infinium HD Methylation protocol, Illumina, San Diego, CA, USA). To ensure a similar age distribution and avoid confounding across chips and plates, study staff employed a two-stage age-stratified algorithm to randomize samples. For quality control, study staff removed samples where >5% of probes had a beadcount <3 or >1% of probes had a detection P-value >0.05. The Bioconductor minfi package Illumina-type background correction without normalization was used to preprocess the remaining samples and generate methylation beta values (Aryee et al., 2014). The beta values represent the percentage of methylation for each of the ~480,000 CpG sites in the BeadChip array. The 450 k arrays were run in the Genomics Core Facility at Northwestern University.

To explore potential differences in the relationship of $PM_{2.5}$ and $PM_{2.5}$ species with different forms of DNAm-age, we calculated both Horvath DNAm-age and Hannum DNAm-age using the 450 k beta values and Horvath's publically available online calculator (http://labs.genetics.ucla.edu/horvath/dnamage/). Horvath DNAm-age was derived from an elastic net (penalized regression) using multiple data sets of varying tissue and cell types. 21,369 CpG probes, shared by the Illumina HumanMethylation27 and HumanMethylation450 BeadChip platforms were regressed on a calibrated version of chronological age. The elastic net selected 353 CpGs that correlate with age, and the resulting model coefficients are used by the calculator to predict the age of each DNA sample (DNAm-age) (Horvath, 2013). Hannum DNAm-age was also derived using an elastic net. However, Hannum DNAm-age was based on a single cohort where DNA methylation values were calculated from whole blood. This elastic net selected 71 CpG probes in the Illumina HumanMethylation450 BeadChip that are predictive of chronological age. Hannum DNAm-age was calculated as the sum of the beta values multiplied by the reported effect sizes for the Hannum predictor (Hannum et al., 2013). The Hannum and Horvath DNAm-ages only share 6 CpG probes (cg04474832, cg05442902, cg06493994, cg09809672, cg19722847, and cg22736354).

2.3. Assessment of environmental factors: ambient particles and temperature

We employed the widely used GEOS-chem chemical transport model (http://www.geoschem.org) to generate 1-year exposure estimates for $PM_{2.5}$ and the following major $PM_{2.5}$ component species: organic carbon (OC), elemental carbon (EC), sulfate, nitrate, and ammonium (van Donkelaar et al., 2010). These 5 component species were selected because they make up a large fraction of total $PM_{2.5}$ mass (~ 88.6%) and were best predicted by the model. GEOS-chem incorporates nonlinear chemistry, meteorology, and

detailed emissions inventories to simulate the formation and transportation of atmospheric components to give raw estimates of $PM_{2.5}$ and its major chemical components. Ten-fold cross-validation demonstrated that the model performs well for $PM_{2.5}$ mass and its component species with R2s ranging from 0.70 to 0.88 (Di et al., 2016). We generated daily estimates at the 1 × 1 km area resolution. Each participant's residence was geocoded and linked to an area level grid-point. Time spent away from home (>7 days) and address changes were also accounted for as particle estimates were assigned to each participants' address. Given that >90% of NAS participants are retired, home address exposures are expected to be a good proxy for their individual ambient exposures. We then generated 1-year total $PM_{2.5}$ and $PM_{2.5}$ component species exposure windows by averaging daily exposures for the 365 days prior to the day of each participants' NAS visit. The 1-year $PM_{2.5}$ exposure window was utilized because it has been previously reported to be robustly associated with DNAm-age (Nwanaji-Enwerem et al., 2016).

We used a spatiotemporal prediction multi-step approach to generate temperature (in Celsius) for each participant (Kloog et al., 2014). First, we obtained 1 × 1 km resolution daily physical surface temperature (Ts) data from NASA satellite measurements and daily near surface air (Ta) data from the Environmental Protection Agency, National Climatic Data Center, and Weather Underground Inc. We then used mixed model regression to calibrate Ts to Ta. The model was validated with a mean out of sample R^2 of 0.95. To generate 1-year temperature measurements to complement 1-year particle exposures, we averaged daily temperature measurements over the 365 days prior to participants' NAS visits.

2.4. Statistical analysis

We first used generalized linear mixed effects models to determine the relationship of DNAm-age (Horvath and Hannum independently) with 1-year $PM_{2.5}$ exposure levels and 1-year $PM_{2.5}$ component species exposure levels. All linear mixed effects models included a random participant-specific intercept to account for correlation between repeated measures (*i.e.* multiple visits for a participant).

We adjusted for confounders and covariates that have *a priori* biological/clinical relevance and/or are reported in the existing literature. Specifically, our previous publication was the first study examining associations of ambient particles and DNAm-age (Nwanaji-Enwerem et al., 2016). There, we used a tiered approach of adding confounders and covariates based on known relationships of ambient particles with DNA methylation and known relationships of ambient particles with older markers of aging (Horvath, 2013; Madrigano et al., 2011; Baccarelli et al., 2009; Bind et al., 2015; Peng et al., 2016). Tier one adjusted for chronological age and blood cell types. Tier two made additional adjustments for lifestyle and environmental factors. Tier three expanded on tier two by additionally adjusting for age-related diseases, and tier four expanded on tier two by additionally adjusting for medications of age-related diseases. After considering model fit (assessed *via* AIC) and considering biological factors that are known to be important, the tier two covariates were deemed to be most appropriate. Thus, in line with the previously published tier two framework (Nwanaji-Enwerem et al., 2016), the models for this analysis were adjusted for chronological age (continuous), six blood cell type estimates [*i.e.* plasma cells, CD4+ lymphocytes, CD8+

lymphocytes, natural killer (NK) cells, monocytes, and granulocytes] (continuous) determined *via* Houseman and Horvath methods (Horvath, 2013; Houseman et al., 2012), average 1-year temperature (continuous), cumulative cigarette pack years (continuous), smoking status (current, former, or never), season of visit (spring [March–May], Summer [June–August], Fall [September–November], and Winter [December–February]), body mass index (BMI) (lean [<25], overweight (Horvath and Ritz, 2015; Levine et al., 2015a, 2015b, 2015c; Horvath et al., 2014; Horvath, 2013), obese [>30]), alcohol intake (yes/no ≥2 drinks daily), and maximum years of education (continuous). All $PM_{2.5}$ component species models were additionally adjusted for $PM_{2.5}$ mass (Mostofsky et al., 2012).

To more rigorously identify the $PM_{2.5}$ component species that may be associated with DNAm-age, we applied the adaptive LASSO (least absolute shrinkage and selection operator) (Schelldorfer et al., 2011). Given that $PM_{2.5}$ component species are correlated, placing them together within the same standard linear regression model can result in unaccounted for stochastic errors. The LASSO is a regression shrinkage and selection approach that helps overcome such limitations. The LASSO applies an l_1 penalty on the component regression coefficients, which minimizes the sum of squared errors subject to the sum of the absolute values of the coefficients being less than a given value (Tibshirani, 1996). The adaptive LASSO improves upon this procedure by utilizing weights for penalizing different coefficients in the l_1 penalty to identify a subset of model predictors to achieve asymptotic normality (Zou, 2006). Furthermore, the adaptive LASSO has been successfully applied in air pollution and health research (Dai et al., 2016a, 2016b).

To identify and select $PM_{2.5}$ component species associated with DNAm-age, we applied a penalty to all $PM_{2.5}$ component species, but not to $PM_{2.5}$ mass and the other covariates in the model. λ, the penalty parameter, determines how strongly the magnitude of the $PM_{2.5}$ species regression coefficients is constrained. When λ is small, the regression coefficients are weakly penalized and mirror those that would be given from a standard linear mixed effects model. When λ is large, the coefficients are strongly penalized, shrinkage is maximized, and all coefficients tend towards zero such that the resulting model includes fixed covariates only. When λ takes a value in between the extremes, the result is a penalized model where some $PM_{2.5}$ component species will have coefficients of zero and others will be non-zero. $PM_{2.5}$ component species with non-zero coefficients are considered as “selected” by the adaptive LASSO. We ran the model across a range of λs, beginning with a λ of 0, and selected the λ resulting in the model with the smallest Bayesian Information Criterion (BIC) (Schwarz et al., 1978). Following LASSO selection, we fit a final multiple-species linear mixed effects model using the selected $PM_{2.5}$ component species and our fixed covariates. From this final model, we were able to estimate component species effect sizes and their corresponding 95% confidence intervals.

Additionally, we considered that the LASSO may not select the $PM_{2.5}$ species that are most correlated with total $PM_{2.5}$ mass. Thus, we conducted a sensitivity analysis where we performed LASSO selection without adjusting for $PM_{2.5}$ mass. From this sensitivity analysis model, we fit a multiple-species linear mixed effects model using the selected $PM_{2.5}$ component species and estimated component species effect sizes and their corresponding 95% confidence intervals.

After finding that Horvath DNAm-age alone was significantly associated with $PM_{2.5}$ component species, we evaluated the relationships of the DNA methylation values of each of the 353 Horvath CpG probes with the particles in the aforementioned LASSO-selected multiple-species linear mixed effects model. In addition to the already described covariates, we included technical covariates (450 k plate, chip, row, and column) to this analysis. To account for multiple hypothesis testing, we also performed FDR correction in this analysis. We then performed gene ontology analysis on the list of significant CpGs (FDR P-value < 0.05) using the publically available GoTermFinder tool (http://go.princeton.edu/cgi-bin/GOTermFinder).

In an additional sensitivity analyses, we re-ran our models excluding participant visits with $PM_{2.5}$ exposures > 12 μg/m^3. This allowed us to assess if our findings persisted even at the $PM_{2.5}$ levels currently deemed acceptable by the U.S. Environmental Protection Agency (EPA) National Ambient Air Quality Standards (NAAQS) (US EPA, O.A.R., n.d.).

All statistical analyses were performed using R Version 3.1.1 (R Core Team, Vienna, Austria) and we considered a P-value <0.05 to be statistically significant.

3. Results

3.1. Descriptive results

Table 1 summarizes the characteristics of the study population. All study participants were Caucasian males with a mean ± SD age of 74.7 ± 6.99 years across all study visits. Average Horvath DNAm-age and Hannum DNAm-age were 74.0 ± 7.92 years and 75.1 ± 8.95 years respectively. Horvath DNAm-age ($r = 0.59$, $p < 0.0001$) and Hannum DNAm-age ($r = 0.77$, $p < 0.0001$) were both strongly correlated with chronological age in the study population. Both measures of DNAm-age were also strongly correlated to each other ($r = 0.69$, $p < 0.0001$).

Table 2 reports 1-year $PM_{2.5}$ and $PM_{2.5}$ component species exposure levels across all study visits. The participants had a mean ± SD 1-year $PM_{2.5}$ exposure level of 10.3 ± 1.60 μg/m^3, with an interquartile range (IQR) of 2.16 μg/m^3. Of the measured $PM_{2.5}$ component species, sulfate accounted for the largest proportion of total $PM_{2.5}$ mass (33%), followed by organic carbon (28.6%), nitrate (11.5%), ammonium (10.1%), and elemental carbon (5.4%). OC was the $PM_{2.5}$ species most correlated with total $PM_{2.5}$ mass ($r = 0.67$). 1-year $PM_{2.5}$ and $PM_{2.5}$ species Pearson correlations across all visits are reported in Table S1. Moreover, 1-year $PM_{2.5}$ and $PM_{2.5}$ species exposure distributions across first visits are reported in Table S2.

3.2. 1-year $PM_{2.5}$ and $PM_{2.5}$ component species as predictors of DNAm-age

Table 3 summarizes the results of three model frameworks where $PM_{2.5}$ and its component species were modeled as predictors of both Horvath and Hannum DNAm-age. Residuals from all models appeared normally distributed. In the model framework 1, $PM_{2.5}$ was modeled as a predictor of Horvath and Hannum DNAm-age independently. In the fully adjusted model, an IQR increase in 1-year $PM_{2.5}$ exposure was significantly associated with a 0.64-year increase in Horvath DNAm-age ($p = 0.005$). However, an IQR increase in 1-year

$PM_{2.5}$ exposure was not significantly associated with Hannum DNAm-age (β = 0.06, p = 0.74).

Under the model framework 2, each $PM_{2.5}$ component species was modeled as an independent predictor of Horvath and Hannum DNAm-age adjusting for all covariates and total $PM_{2.5}$ mass. 1-year IQR increases in OC (β = 0.93, p = 0.001), sulfate (β = 0.59, p < 0.0001), nitrate (β = 0.58, p = 0.01), and ammonium (β = 0.59, p = 0.0004) were all significantly associated with increases in Horvath DNAm-age of at least 0.58 years. No $PM_{2.5}$ component species were significantly associated with Hannum DNAm-age (Table 3).

The model 3 framework reflects the results of the multiple-species fully-adjusted linear mixed effects models with the $PM_{2.5}$ component species selected by the adaptive LASSO. The adaptive LASSO selected sulfate and ammonium as important predictors of Horvath DNAm-age. Fig. 1A depicts the relationship between BIC, the model selection criterion, and λ, the adaptive LASSO penalty parameter. The model with the smallest BIC had λ = 11. Fig. 1B shows the LASSO coefficient paths for the $PM_{2.5}$ component species. Each component species coefficient is expressed as the difference in mean Horvath DNAm-age per an IQR increase in the 1-year component species exposure level. Each curve depicts the rate at which the component species coefficient shrinks towards zero as λ increases. At λ = 0, all components species have a non-zero coefficient.

In the multiple-species fully-adjusted linear mixed effects model, both sulfate (β = 0.51, p < 0.0001) and ammonium (β = 0.36, p = 0.04) remain significant positive predictors of Horvath DNAm-age. The adaptive LASSO did not select any $PM_{2.5}$ component species as important predictors of Hannum DNAm-age.

In our sensitivity analysis – where LASSO selection was performed without adjusting for total $PM_{2.5}$ mass – sulfate, ammonium, and OC were selected as important predictors of DNAm-age (Fig. S1). Nonetheless, in a multiple-species fully-adjusted linear mixed effects model, both sulfate (β = 0.45, p = 0.0003) and ammonium (β = 0.34, p < 0.05) remained significant positive predictors of Horvath DNAm-age, but OC (β = 0.42, p = 0.16) was not a significant predictor of Horvath DNAm-age (Table S3). Again, the sensitivity analysis adaptive LASSO did not select any $PM_{2.5}$ component species as important predictors of Hannum DNAm-age.

Significant findings from the main analysis multiple-species fully-adjusted linear mixed effects model persisted in the second sensitivity analyses excluding participant visits with $PM_{2.5}$ exposures > 12 $\mu g/m^3$, the annual $PM_{2.5}$ exposure level currently deemed acceptable by the U.S. Environmental Protection Agency (EPA) National Ambient Air Quality Standards (NAAQS) (Table S4).

3.3. Associations between 1-year $PM_{2.5}$ and $PM_{2.5}$ component species levels and methylation values at Horvath DNAm-age CpG sites

After FDR correction, 47 out of 353 Horvath DNAm-age CpG sites had methylation values that were significantly associated with total $PM_{2.5}$ levels in the fully-adjusted multiple-species linear mixed effects model. $PM_{2.5}$ levels were positively or negatively associated

with CpG methylation values depending on the CpG site (Table 4). 46 of the 47 CpG sites mapped to known genes. 9 of these 46 genes (*ENPP2*, *FAM50B*, *LZTFL1*, *SGCE*, *C14orf105*, *ZBTB5*, *TMEM132E*, *CATSPERG*, and *NDUFA13*) were previously reported in a similar, previously published $PM_{2.5}$ Horvath CpG analysis (Nwanaji-Enwerem et al., 2016). Gene ontology of our 46 genes combined with the genes in the previously reported study returned the GO term “regulation of translational initiation” (Table S5).

Only 1 out 353 CpG sites (cg02275294) had methylation values that were significantly associated with ammonium levels in the fully-adjusted multiple-species linear mixed effects model. No individual CpG sites had methylation values that were significantly associated with sulfate levels after FDR correction.

4. Discussion

In this study, we report positive associations of 1-year $PM_{2.5}$ exposure levels with Horvath DNAm-age in a population of community-dwelling, elderly men. Additionally, we utilized the adaptive LASSO to identify 1-year sulfate and ammonium levels as the $PM_{2.5}$ components most robustly associated with Horvath DNAm-age. To our knowledge, this is the first report of associations of multiple $PM_{2.5}$ component species with DNAm-age and the second time that satellite-derived $PM_{2.5}$ exposure levels have been found to be associated with Horvath DNAm-age. In addition to being consistent with the existing literature (Nwanaji-Enwerem et al., 2016), our findings also demonstrate important public health relevance as they persist in sensitivity analyses including only participant visits with 1-year $PM_{2.5}$ levels within current US EPA national ambient air quality standards (US EPA, O.A.R., n.d.). Our study also extends the literature by exploring $PM_{2.5}$ relationships with Hannum DNAm-age although these relationships were found to be null. Furthermore, we identified 47 CpG sites, 9 of which were previously reported, whose methylation values were significantly associated with $PM_{2.5}$ levels in fully-adjusted linear mixed effects models. Only 1 CpG was associated with ammonium levels and 0 were associated with sulfate levels.

Given our prior report of robust associations of $PM_{2.5}$ levels from satellite-based spatiotemporal models with Horvath DNAm-age, we expected to observe a similar positive relationship using $PM_{2.5}$ levels from the GEOS-chem chemical transport model. As expected, we observed that an IQR increase in 1-year $PM_{2.5}$ exposure was associated with a 0.64-year increase in Horvath DNAm-age. Since $PM_{2.5}$ component species are highly related to total $PM_{2.5}$, we also expected that $PM_{2.5}$ component species would be associated with Horvath DNAm-age, even when adjusting for $PM_{2.5}$ mass. Given the existing literature concerning the differential health effects of $PM_{2.5}$ component species, we speculated that some component species may be more robustly associated with Horvath DNAm-age than others. In particular, we expected the carbonaceous fractions to be among the species most robustly associated with DNAm-age due to the extensive literature (including work from our group) on the adverse nature of carbonaceous fraction exposures on health (Nwanaji-Enwerem et al., 2016; Baccarelli et al., 2009; Colicino et al., 2014; Zanobetti et al., 2014; McCracken et al., 2010). In our fully adjusted one-species linear mixed effects models, we observed strong positive associations of 4 out of the 5 component species examined with

Horvath DNAm-age. IQR range increases in organic carbon, sulfate, nitrate, and ammonium were all significantly associated with at least a 0.58-year increase in Horvath DNAm-age.

Despite the results from our fully adjusted one-species linear mixed effects models, we desired a method to more comprehensively identify the component species most associated with DNAm-age. Nevertheless, we were aware that simply modeling highly-correlated $PM_{2.5}$ species together would result in unaccounted for stochastic errors. Thus, we employed the adaptive LASSO as a penalized regression method to help overcome this difficulty. The literature has shown that carbonaceous fractions are robustly associated with age-related health outcomes (Nwanaji-Enwerem et al., 2016; Baccarelli et al., 2009; Colicino et al., 2014; Zanobetti et al., 2014; McCracken et al., 2010); however, neither elemental or organic carbon were selected in our models. Rather, sulfate and ammonium were selected. This difference may be explained by the fact that a majority of the aforementioned studies did not consider other $PM_{2.5}$ component species in addition to the carbonaceous fractions. Even in our single-species linear mixed effects models, we note that organic carbon was among the four species significantly associated with Horvath DNAm-age (Table 3). However, when all five component species are considered together in the adaptive LASSO, only sulfate and ammonium were selected. It is also possible that the LASSO did not select the carbonaceous fractions because the selection was performed under $PM_{2.5}$ adjustment and $PM_{2.5}$ may be capturing most of the variability of organic and elemental carbon. Thus, we performed LASSO selection not adjusting for total $PM_{2.5}$ mass as a sensitivity analysis. This time LASSO did select organic carbon along with sulfate and ammonium. However, when these three component species were modeled with $PM_{2.5}$ in a multiple-species fully-adjusted linear mixed effects model, organic carbon was the only species that was not a significant predictor of DNAm-age. This suggests that organic carbon was selected in the sensitivity analysis because of its strong correlation with $PM_{2.5}$ mass and not because organic carbon itself is a good predictor of DNAm-age. This finding also reiterates the notion that adjustment for $PM_{2.5}$ mass in component species models is very important as $PM_{2.5}$ mass often confounds the relationship between the outcome and species (Mostofsky et al., 2012). Failing to include $PM_{2.5}$ mass may lead to misleading findings about species. In all, our data suggests that of the considered species, sulfate and ammonium have the most important relationships with DNAm-age. Furthermore, existing studies that do consider a range of $PM_{2.5}$ components demonstrate that other non-carbonaceous components are important to age-related outcomes (Dai et al., 2016b; Wu et al., 2013; Wu et al., 2015). These data, together with our findings, also suggests the important need to consider a range of $PM_{2.5}$ components, rather than one or two species, in air pollution and health studies.

Both sulfate and ammonium are classified in the inorganic fraction of $PM_{2.5}$. Sulfates are often produced from oxidation or photochemical reactions involving primary gases derived from sources like coal-burning power plants (Huang et al., 2014). Additionally, ammonia from organic sources including animal feeds and fertilizers can contribute to the existence of sulfates in the form of atmospheric ammonium sulfate (Frank, n.d.). As far as direct ambient sulfate and ammonium toxicity to human health is concerned, existing studies are limited. Yet, there has been extensive evidence describing the ability of acidic sulfates, like ammonium sulfate, to increase the number and toxicity of biologically harmful secondary particles (Mostofsky et al., 2012; Popovicheva et al., 2011; Rubasinghege et al., 2010; Li et

al., 2011; Lepeule et al., 2012; Schwartz and Lepeule, 2012). For instance, ammonium sulfate aerosols have been shown to influence the photo-chemical reactions of nitrogen oxides and toluene hastening the production of secondary organic aerosols (Wu et al., 2007). Moreover, sulfur concentrations have been found to be directly proportional to the ability of soluble particle extracts to generate biologically damaging oxidants (Ghio et al., 1999). Furthermore, a prior study in the NAS has reported a 27% decrease in long interspersed nucleotide element-1 methylation per every IQR increase in 90-day sulfate exposure. This study provides evidence for the influence of sulfates on DNA methylation, which may be a potential pathway for sulfate toxicity (Madrigano et al., 2011). It is still unclear what the molecular relevance of Horvath DNAm-age is, but our findings along with the existing literature will be helpful in providing additional insight for future work.

Following the selection of sulfate and ammonium by the adaptive LASSO, we constructed a final multiple-species linear mixed effects model adjusted for $PM_{2.5}$ mass and all covariates. Even in this model, sulfate and ammonium remained significant positive predictors of Horvath DNAm-age. We then looked to see if there were specific Horvath DNAm-age component CpG sites with methylation values that were associated with $PM_{2.5}$, sulfate, and/or ammonium in our fully-adjusted multiple-species linear model. From this analysis, we identified 47 significant CpG sites after FDR adjustment. These sites mapped to 46 genes, and 9 of them were reported in a previous CpG-level analysis of the same 353 sites in the Horvath algorithm that we conducted using $PM_{2.5}$ levels from a satellite-based spatiotemporal model. To better grasp the impact of $PM_{2.5}$ levels on methylation, we divided the coefficients for each significant CpG site (*i.e.* difference in methylation per IQR increase in particle level) by the standard deviation of the respective particle level. We were pleased to see that 5 of the 9 CpGs that were shared between both $PM_{2.5}$ prediction models were in the top 20% of our gene list. We then combined the gene lists from both $PM_{2.5}$ prediction models (removing any duplicates) and performed a gene ontology (GO) analysis. The GO analysis returned the term “regulation of translational initiation” with the following genes from our list falling into this category: RXRA, EIF3M, EIF31. Though the GO term itself is not highly specific, combining this pathway with what is known about the toxicity of $PM_{2.5}$ will be useful in further understanding how $PM_{2.5}$ may contribute to aging and disease. Only 1 CpG was associated with ammonium levels and it mapped to the gene SOAT1, which is involved in fatty-acyl-CoA binding. SOAT1 has been implicated in a number of diseases including familial hypercholesterolemia (Peters et al., 2011). No CpG sites were specifically associated with sulfate levels. The finding that almost no CpGs sites were associated with ammonium and sulfate further demonstrates that Horvath DNAm-age is simply not a reflection of its 353 component CpGs, and reiterates the need for work focused on defining the molecular relevance of DNAm-age.

Finally, our study demonstrates that all DNAm-age measures are not the same. In the literature there is evidence of both Horvath and Hannum DNAm-age reflecting the same disease outcome and evidence where they differ in their reporting ability. For instance, both Horvath and Hannum DNAm-age appear to be useful in predicting mortality (Chen et al., 2016; Wolf et al., 2016). However, in a study of male and female veterans, Hannum DNAm-age was associated with post-traumatic stress disorder and neural integrity, but Horvath DNAm-age was not (Perna et al., 2016). The differences in these two DNAm-age measures

may stem from the fact that they are derived from almost entirely different CpG sites or from the fact that Horvath DNAm-age was constructed using many datasets of multiple tissue types and the Hannum DNAm-age was based only on blood from one dataset (Horvath, 2013; Hannum et al., 2013). Our results suggest that Hannum DNAm-age is not sensitive to exposure levels of $PM_{2.5}$ and its component species. Additional studies in different populations will be necessary to confirm these findings more broadly. Nonetheless, continued research exploring the specific sensitivity of DNAm-age measures will be a crucial next step in the growth of this field of research. Once more is known about the profiles of these markers, we can begin to use them more effectively in answering questions concerning human health.

Strengths of our study include rigorous statistical methods and access to a large cohort with extensive and repeated information regarding pollutant exposures, potential confounders, and DNA methylation data from multiple study visits. However, our study does have several limitations. First, although we used a validated chemical transport model to estimate the levels of ambient $PM_{2.5}$ and its component species at participants' addresses, we recognize that these estimates may differ from personal exposures. Nonetheless, we know that a majority of NAS participants are retired and spend most of their time at home. Moreover, our approach is expected to result in non-differential misclassification that is likely to underestimate the observed associations rather than bias them away from the null (Kioumourtzoglou et al., 2014). Secondly, it is known that LASSO regression is limited to linear relationships. Given the linear relationship of our particle exposures with DNAm-age and the scope of this paper, the adaptive LASSO was a good tool for identifying $PM_{2.5}$ components that are independently important to DNAm-age. However, for future studies potentially interested in the interactions between $PM_{2.5}$ components, another technique may be necessary as $PM_{2.5}$ species interactions that are important for the prediction of DNAm-age may be more complex (*i.e.* not linear). Third, we note that our findings are based on an elderly cohort of Caucasian males that reside in a lightly-polluted environment. Hence, additional studies involving other demographic groups and in different environments will be necessary to confirm our findings more broadly. Finally, we used the existing literature and *a priori* knowledge of biological/clinical relevance to adjust for potential confounders. Nonetheless, we cannot rule out the possibility of unknown or residual confounding in our analyses.

5. Conclusion

Our study utilizes the GEOS-chem chemical transport model to validate novel positive associations between long-term $PM_{2.5}$ exposure levels and Horvath DNAm-age. For the first time, we demonstrate that sulfate and ammonium are among the $PM_{2.5}$ component species most associated with Horvath DNAm-age in this population of elderly men. In contrast, we observed no relationships of long-term $PM_{2.5}$ and $PM_{2.5}$ component species exposure levels with Hannum DNAm-age. These results suggest that DNA methylation-based biomarkers of age differ in their sensitivity to ambient particle exposures and potentially disease outcomes. Future studies in other populations will be critical for defining the environmental and disease sensitivity profiles of DNAm-age measures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study is supported by grants from the National Institute of Environmental Health Sciences (NIEHS) (R01ES021733 and R01ES025225-01A1). Other support comes from NIEHS grants ES015172, ES014663 and ES020010, and Environmental Protection Agency (EPA) grant RD832416. The US Department of Veterans Affairs (VA) Normative Aging Study (NAS) is supported by the Cooperative Studies Program/ERIC, US Department of Veterans Affairs, and is a research component of the Massachusetts Veterans Epidemiology Research and Information Center (MAVERIC). The views expressed in this paper are those of the authors and do not necessarily represent the views of the US Department of Veterans Affairs. Additional support was provided by the US Department of Agriculture, Agricultural Research Service (contract 53-K06-510).

References

Apte JS, et al. Addressing global mortality from ambient PM2.5. Environ Sci Technol. 2015; 49(13): 8057–8066. [PubMed: 26077815]

Aryee MJ, et al. Minfi: a flexible and comprehensive bioconductor package for the analysis of Infinium DNA methylation microarrays. Bioinformatics. 2014; 30(10):1363–1369. [PubMed: 24478339]

Baccarelli A, et al. Rapid DNA methylation changes after exposure to traffic particles. Am J Respir Crit Care Med. 2009; 179(7):572–578. [PubMed: 19136372]

Bell B, Rose CL, Damon A. The veterans administration longitudinal study of healthy aging. The Gerontologist. 1966; 6(4):179–184. [PubMed: 5342911]

Bind MA, et al. 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):759–765. [PubMed: 25769179]

Brown DM, et al. Occupational exposure to PM2.5 and incidence of ischemic heart disease: longitudinal targeted minimum loss-based estimation. Epidemiology. 2015; 26(6):806–814. [PubMed: 26079662]

Chen BH, et al. DNA methylation-based measures of biological age: meta-analysis predicting time to death. Aging (Albany NY). 2016; 8(9):1844–1865. [PubMed: 27690265]

Chen JC, Schwartz J. Neurobehavioral effects of ambient air pollution on cognitive performance in US adults. Neurotoxicology. 2009; 30(2):231–239. [PubMed: 19150462]

Chung HY, et al. Molecular inflammation: underpinnings of aging and age-related diseases. Ageing Res Rev. 2009; 8(1):18–30. [PubMed: 18692159]

Colicino E, et al. Mitochondrial haplogroups modify the effect of black carbon on age-related cognitive impairment. Environ Health. 2014; 13(1):42. [PubMed: 24884505]

Dai L, et al. Fine particles, genetic pathways, and markers of inflammation and endothelial dysfunction: analysis on particulate species and sources. J Expo Sci Environ Epidemiol. 2016a; 26(4):415–421. [PubMed: 26732377]

Dai L, et al. Use of the adaptive LASSO method to identify $PM_{2.5}$ components associated with blood pressure in elderly men: the veterans affairs normative aging study. Environ Health Perspect. 2016b; 124(1):120–125. [PubMed: 26090776]

Di Q, Koutrakis P, Schwartz J. A hybrid prediction model for PM 2.5 mass and components using a chemical transport model and land use regression. Atmos Environ. 2016; 131:390–399.

Frank N. The Chemical Composition of PM2.5 to Support PM Implementation. 2007

Ghio AJ, et al. Sulfate content correlates with iron concentrations in ambient air pollution particles. Inhal Toxicol. 1999; 11(4):293–307. [PubMed: 10380171]

Giorgini P, et al. Air pollution exposure and blood pressure: an updated review of the literature. Curr Pharm Des. 2016; 22(1):28–51. [PubMed: 26548310]

Hannum G, et al. Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell. 2013; 49(2):359–367. [PubMed: 23177740]

Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013; 14(10):R115. [PubMed: 24138928]

Horvath S, Ritz BR. Increased epigenetic age and granulocyte counts in the blood of Parkinson's disease patients. Aging. 2015; 7(12):1130–1142. [PubMed: 26655927]

Horvath S, et al. Obesity accelerates epigenetic aging of human liver. Proc Natl Acad Sci. 2014; 111(43):15538–15543. [PubMed: 25313081]

Horvath S, et al. The cerebellum ages slowly according to the epigenetic clock. Aging. 2015; 7(5): 294–306. [PubMed: 26000617]

Houseman EA, et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinf. 2012; 13:86.

Huang XHH, et al. Characterization of PM2.5 major components and source investigation in suburban Hong Kong: a one year monitoring study. Aerosol Air Qual Res. 2014; 14(1):237–250.

Ito K, et al. Fine particulate matter constituents associated with cardiovascular hospitalizations and mortality in New York City. Environ Health Perspect. 2011; 119(4):467–473. [PubMed: 21463978]

Kioumourtzoglou MA, et al. Exposure measurement error in PM2.5 health effects studies: a pooled analysis of eight personal exposure validation studies. Environ Health. 2014; 13(1):2. [PubMed: 24410940]

Kloog I, et al. Predicting spatiotemporal mean air temperature using MODIS satellite surface temperature measurements across the Northeastern USA. Remote Sens Environ. 2014; 150:132–139.

Kloog I, et al. Effects of airborne fine particles (PM2.5) on deep vein thrombosis admissions in the northeastern United States. J Thromb Haemost. 2015; 13(5):768–774. [PubMed: 25678264]

Künzli N, et al. Ambient air pollution and atherosclerosis in Los Angeles. Environ Health Perspect. 2005; 113(2):201–206. [PubMed: 15687058]

Laurent O, et al. Low birth weight and air pollution in California: which sources and components drive the risk? Environ Int. 2016; 92–93:471–477.

Lepeule J, et al. 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):965–970. [PubMed: 22456598]

Levine AJ, et al. Accelerated epigenetic aging in brain is associated with pre-mortem HIV-associated neurocognitive disorders. J Neurovirol. 2016a; 22(3):366–375. [PubMed: 26689571]

Levine ME, et al. DNA methylation age of blood predicts future onset of lung cancer in the women's health initiative. Aging. 2015b; 7(9):690–700. [PubMed: 26411804]

Levine ME, et al. Epigenetic age of the pre-frontal cortex is associated with neuritic plaques, amyloid load, and Alzheimer's disease related cognitive functioning. Aging. 2015c; 7(12):1198–1211. [PubMed: 26684672]

Li W, et al. Internally mixed sea salt, soot, and sulfates at Macao, a coastal city in South China. J Air Waste Manage Assoc. 2011; 61(11):1166–1173.

Liu S, et al. Fine particulate matter components and emergency department visits among a privately insured population in Greater Houston. Sci Total Environ. 2016; 566–567:521–527.

Madrigano J, et al. Prolonged exposure to particulate pollution, genes associated with glutathione pathways, and DNA methylation in a cohort of older men. Environ Health Perspect. 2011; 119(7): 977–982. [PubMed: 21385671]

Marioni RE, et al. The epigenetic clock is correlated with physical and cognitive fitness in the Lothian Birth Cohort 1936. Int J Epidemiol. 2015; 44(4):1388–1396. [PubMed: 25617346]

Marioni RE, Harris SE, Shah S, McRae AF, von Zglinicki T, Martin-Ruiz C, … Deary IJ. The epigenetic clock and telomere length are independently associated with chronological age and mortality. International journal of epidemiology. 2016; 45(2):424–432.

Martinelli N, Olivieri O, Girelli D. Air particulate matter and cardiovascular disease: a narrative review. Eur J Intern Med. 2013; 24(4):295–302. [PubMed: 23647842]

McCracken J, et al. Annual ambient black carbon associated with shorter telomeres in elderly men: veterans affairs normative aging study. Environ Health Perspect. 2010; 118(11):1564–1570. [PubMed: 21465749]

Mostofsky E, et al. Modeling the association between particle constituents of air pollution and health outcomes. Am J Epidemiol. 2012; 176(4):317–326. [PubMed: 22850792]

Nwanaji-Enwerem JC, et al. Long-term ambient particle exposures and blood DNA methylation age: findings from the VA normative aging study. Environ Epigenet. 2016; 2(2)

Peng C, et al. 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. [PubMed: 27219535]

Perna L, et al. Epigenetic age acceleration predicts cancer, cardiovascular, and all-cause mortality in a German case cohort. Clin Epigenetics. 2016; 8:64. [PubMed: 27274774]

Peters BJ, et al. Genetic variability within the cholesterol lowering pathway and the effectiveness of statins in reducing the risk of MI. Atherosclerosis. 2011; 217(2):458–464. [PubMed: 21741043]

Popovicheva OB, et al. Quantification of the hygroscopic effect of soot aging in the atmosphere: laboratory simulations. J Phys Chem A. 2011; 115(3):298–306. [PubMed: 21186790]

Power MC, et al. Traffic-related air pollution and cognitive function in a cohort of older men. Environ Health Perspect. 2011; 119(5):682–687. [PubMed: 21172758]

Raaschou-Nielsen O, et al. Particulate matter air pollution components and risk for lung cancer. Environ Int. 2016; 87:66–73. [PubMed: 26641521]

Rowe JW, Kahn RL. Human aging: usual and successful. Science. 1987; 237(4811):143–149. [PubMed: 3299702]

Rowe JW, Kahn RL. Successful aging and disease prevention. Adv Ren Replace Ther. 2000; 7(1):70–77. [PubMed: 10672919]

Rowe JW, Kahn RL. Successful aging 2.0: conceptual expansions for the 21st century. J Gerontol B Psychol Sci Soc Sci. 2015; 70(4):593–596. [PubMed: 25878054]

Rubasinghege G, et al. Simulated atmospheric processing of iron oxyhydroxide minerals at low pH: roles of particle size and acid anion in iron dissolution. Proc Natl Acad Sci U S A. 2010; 107(15): 6628–6633. [PubMed: 20360560]

Scheers H, et al. Long-term exposure to particulate matter air pollution is a risk factor for stroke: meta-analytical evidence. Stroke. 2015; 46(11):3058–3066. [PubMed: 26463695]

Schelldorfer J, et al. Estimation for high-dimensional linear mixed-effects models using 31-penalization. Scand J Stat. 2011; 38(2):197–214.

Schikowski T, et al. Association of air pollution with cognitive functions and its modification by APOE gene variants in elderly women. Environ Res. 2015; 142:10–16. [PubMed: 26092807]

Schwartz J, Lepeule J. Is ambient PM2.5 sulfate harmful? Schwartz and Lepeule respond. Environ Health Perspect. 2012; 120(12):a454–a455. [PubMed: 23211380]

Schwarz G, et al. Estimating the dimension of a model. Ann Stat. 1978; 6(2):461–464.

Shan M, et al. A feasibility study of the association of exposure to biomass smoke with vascular function, inflammation, and cellular aging. Environ Res. 2014; 135:165–172. [PubMed: 25262090]

Terzano C, et al. Air pollution ultrafine particles: toxicity beyond the lung. Eur Rev Med Pharmacol Sci. 2010; 14(10):809–821. [PubMed: 21222367]

Tibshirani R. Regression shrinkage and selection via the lasso. J R Stat Soc Ser B Methodol. 1996; 58(1):267–288.

US, E.P.A. O.A.R, NAAQS Table. 2015.

van Donkelaar A, et al. Global estimates of ambient fine particulate matter concentrations from satellite-based aerosol optical depth: development and application. Environ Health Perspect. 2010; 118(6):847–855. [PubMed: 20519161]

Weuve J, et al. Exposure to particulate air pollution and cognitive decline in older women. Arch Intern Med. 2012; 172(3):219–227. [PubMed: 22332151]

Wilker EH, et al. Long-term exposure to fine particulate matter, residential proximity to major roads and measures of brain structure. Stroke. 2015; 46(5):1161–1166. [PubMed: 25908455]

Wolf EJ, et al. Accelerated DNA methylation age: associations with PTSD and neural integrity. Psychoneuroendocrinology. 2016; 63:155–162. [PubMed: 26447678]

World Health, O. Burden of Disease from Household Air Pollution for 2012. WHO; Geneva: 2014.

Wu S, et al. Effect of ammonium sulfate aerosol on the photochemical reaction of toluene/NO(x)/air mixture. Huan Jing Ke Xue. 2007; 28(6):1183–1187. [PubMed: 17674719]

Wu S, et al. Blood pressure changes and chemical constituents of particulate air pollution: results from the healthy volunteer natural relocation (HVNR) study. Environ Health Perspect. 2013; 121(1):66–72. [PubMed: 23086577]

Wu S, et al. Association of chemical constituents and pollution sources of ambient fine particulate air pollution and biomarkers of oxidative stress associated with atherosclerosis: a panel study among young adults in Beijing, China. Chemosphere. 2015; 135:347–353. [PubMed: 25981523]

Zanobetti A, et al. Fine particulate air pollution and its components in association with cause-specific emergency admissions. Environ Health. 2009; 8:58. [PubMed: 20025755]

Zanobetti A, et al. Associations between arrhythmia episodes and temporally and spatially resolved black carbon and particulate matter in elderly patients. Occup Environ Med. 2014; 71(3):201–207. [PubMed: 24142987]

Zhong J, et al. Cardiac autonomic dysfunction: particulate air pollution effects are modulated by epigenetic immunoregulation of toll-like receptor 2 and dietary flavonoid intake. J Am Heart Assoc. 2015; 4(1):e001423. [PubMed: 25628407]

Zou H. The adaptive lasso and its oracle properties. J Am Stat Assoc. 2006; 101(476):1418–1429.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2016.12.024.

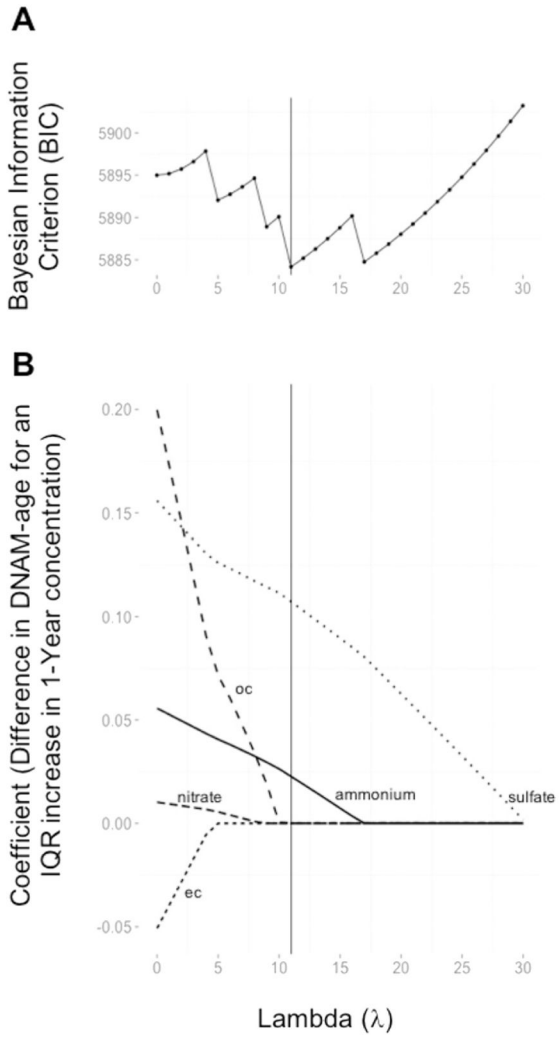

Fig. 1.

A) The relationship between BIC, a criterion for model selection and λ (lambda), the adaptive LASSO penalty parameter, for DNAm-age. The vertical line at λ = 11 denotes the penalty parameter with the lowest BIC. B) LASSO coefficient paths: plot of coefficient profiles for $PM_{2.5}$ components as a function of λ. At λ = 11, sulfate and ammonium are the only $PM_{2.5}$ components with a non-zero coefficient.

Table 1

Characteristics of study subjects (2000–2011).

Variable	First visit (N = 552)	All visits (N = 940)
Age (years), mean (SD)	73.3 (6.82)	74.7 (6.99)
Horvath DNAm-age (years), mean (SD)	73.7 (7.77)	74.0 (7.92)
Hannum DNAm-age (years), mean (SD)	73.8 (8.80)	75.1 (8.95)
Temperature (°C), mean (SD)	11.5 (1.12)	11.3 (1.00)
Pack years, mean (SD)	20.7 (24.7)	20.5 (24.4)
Smoking status, N (%)		
Current	25 (4)	40 (4)
Former	355 (64)	614 (65)
Never	172 (32)	286 (31)
Season, N (%)		
Spring	145 (26)	241 (26)
Summer	115 (21)	199 (21)
Fall	177 (32)	313 (33)
Winter	115 (21)	187 (20)
BMI, N (%)		
Healthy/lean	119 (21)	216 (23)
Overweight	302 (55)	493 (52)
Obese	131 (24)	231 (25)
Alcohol consumption, N (%)		
<2 drinks/day	441 (80)	761 (81)
≥2 drinks/day	111 (20)	179 (19)
Education, N (%)		
≤12 years	146 (27)	242 (26)
12–16 years	262 (47)	434 (46)
>16 years	144 (26)	264 (28)

Table 2

Mean 1-year particulate matter 2.5 ($PM_{2.5}$) and component species concentrations across all study visits.

Particle (μg/m^3)	Mean (SD)	IQR	Proportion of $PM_{2.5}$ (%)	Pearson correlation with $PM_{2.5}$	N
$PM_{2.5}$	10.3 (1.60)	2.16	–	–	940
$PM_{2.5}$ Component Species					
EC	0.56 (0.17)	0.23	5.4	0.62	940
OC	2.94 (0.91)	1.28	28.6	0.67	940
Sulfate	3.40 (1.23)	0.82	33.0	0.30	940
Nitrate	1.18 (0.32)	0.42	11.5	0.46	940
Ammonium	1.04 (0.31)	0.3	10.1	0.53	940

Table 3

1-year particulate matter 2.5 ($PM_{2.5}$) and component species as predictors of DNA methylation (DNAm) age.

Particle	Difference in Horvath DNAm-age for IQR (95% CI)	*P*	Difference in Hannum DNAm-age for IQR (95% CI)	*P*	N
Model framework 1					
$PM_{2.5}$	0.64 (0.20, 1.09)	**0.005**	0.06 (−0.28, 0.40)	0.74	940
Model framework 2					
EC	0.27 (−0.25, 0.80)	0.30	−0.09 (−0.48, 0.29)	0.64	940
OC	0.93 (0.37, 1.50)	**0.001**	0.35 (−0.05, 0.77)	0.09	940
Sulfate	0.59 (0.37, 0.81)	<**0.0001**	0.08 (−0.09, 0.25)	0.36	940
Nitrate	0.58 (0.11, 1.04)	**0.01**	0.30 (−0.04, 0.65)	0.08	940
Ammonium	0.59 (0.26, 0.92)	**0.0004**	0.06 (−0.18, 0.30)	0.63	940
Model framework 3					
$PM_{2.5}$	0.18 (−0.30, 0.66)	0.45	–	–	940
Sulfate	0.51 (0.28, 0.74)	<**0.0001**	–	–	940
Ammonium	0.36 (0.02, 0.70)	**0.04**	–	–	940

Model framework 1: adjusted for chronological age, blood cell types, temperature, pack years, smoking status, season, BMI, alcohol consumption, and education. Model frame-work 2: $PM_{2.5}$ component species as independent predictors of DNAm-age adjusted for $PM_{2.5}$ in addition to model 1 covariates. Model framework 3: $PM_{2.5}$, sulfate, and ammonium as joint predictors of DNAm-age (given selection of sulfate and ammonium by the adaptive LASSO) adjusted for model 1 covariates. No species were selected as predictors of Hannum DNAm-age. Bold values indicate significance at $P < 0.05$.

Table 4

1-Year Particle Exposures as Predictors of Horvath CpG Probe.

CpG	Gene	Process/function	Difference in methylation per SD (%)	Direction of association	FDR adjusted *P*
$PM_{2.5}$					
cg15262928	TIMM17A	Mitochondrial protein import	24.18	+	0.001
cg14409958	ENPP2*	Nucleic acid binding	19.85	+	0.001
cg01570885	FAM50B*	Protein binding	19.64	−	0.004
cg08186124	LZTFL1*	Protein binding: cytoplasm	19.59	+	0.004
cg18139769	SGCE*	Calcium binding	19.25	−	0.004
cg15547534	PPP1R35	Phosphatase binding	18.79	+	0.004
cg26456957	PPP1R12C	Protein kinase binding	18.74	+	0.001
cg05847778	BBS5	Transcription initiation	18.01	+	0.006
cg15661409	C14orf105*	Uncharacterized	17.52	−	<0.001
cg02335441	NEK11	DNA replication	17.32	+	0.008
cg17285325	TYMP	Phosphorylase activity	17.23	+	0.007
cg04094160	ZBTB5*	Transcriptional regulation	16.92	+	0.003
cg03682823	SGCE	Calcium binding	16.80	−	0.008
cg07663789	NPR3	Hormone binding/blood volume	16.13	+	0.003
cg15703512	PDZD9	Uncharacterized	15.80	+	0.015
cg22190114	NLRP8	ATP binding	15.60	+	0.015
cg19008809	SFMBT1	Transcription corepressor activity	15.48	+	0.013
cg00374717	ARSG	Sulfatase enzyme activity	15.29	−	0.004
cg12985418	MIB1	Protein binding	15.14	+	0.018
cg03588357	GPR68	G-protein coupled receptor activity	15.09	+	0.020
cg14424579	AGBL5	Metallocarboxypeptidase	15.07	+	0.007
cg14597908	GNAS	G-protein binding	15.04	−	0.015
cg19044674	LEPRE1	Oxidoreductase activity	14.88	+	0.023
cg09441152	PQLC1	Membrane component	14.87	+	0.027
cg07849904	MN1	Transcriptional activator	14.85	+	0.015
cg19273182	PAPOLG	Polynucleotide adenylyltransferase activity	14.81	+	0.025

Environ Int. Author manuscript; available in PMC 2018 May 01.

CpG	Gene	Process/function	Difference in methylation per SD (%)	Direction of association	FDR adjusted *P*
cg17063929	NOX4	Nucleotide binding	14.56	−	0.015
cg24116886	DEFB127	Immunologic response	14.40	−	0.015
cg09191327	PRDM12	Methyltransferase activity	14.30	+	0.027
cg23662675	ZMYND8	Transcription cofactor activity	14.16	+	0.014
cg14992253	EIF3I	Translation initiation	13.12	−	0.018
cg05442902	P2RX6	Channel activity	12.82	−	0.026
cg06557358	TMEM132E *	Integral component of membrane	12.60	+	0.031
cg11932564	TNFRSF13C	Immunologic response	12.51	+	0.037
cg18031008	MRPS21	Mitochondrial ribosome	12.10	+	0.030
cg19945840	SDF4	Calcium binding	12.08	−	0.023
cg19167673	PDGFB	Protein homodimerization activity	12.03	+	0.031
cg25159610	PLK2	Cell division	11.96	+	0.038
cg22006386	CATSPERG *	Ion channel activity	11.89	+	0.026
cg27377450	*unknown*	*unknown*	11.85	−	0.026
cg20100381	NAE1	Protein heterodimerization activity	11.84	+	0.046
cg04268405	CHST3	Sulfotransferase	11.47	−	0.026
cg07595943	ADAD2	RNA binding	11.36	−	0.023
cg25505610	EIF3M	Translation initiation	10.67	+	0.042
cg16744741	PRKG2	Protein kinase activity	10.17	−	0.038
cg21395782	NDUFA13 *	NADH dehydrogenase activity	8.27	+	0.027
cg01459453	SELP	Oligosaccharide binding	8.05	−	0.023
Ammonium					
cg02275294	SOAT1	Fatty-acyl-CoA binding	10.81	+	0.036

All models are fully adjusted.

* CpGs associated with $PM_{2.5}$ levels in a prior publication.