Beyond the Mean: Quantile Regression to Explore the Association of Air Pollution with Gene-Specific Methylation in the Normative Aging Study

Citation

Published Version
doi:10.1289/ehp.1307824

Permanent link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:21458939

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

Share Your Story
The Harvard community has made this article openly available. Please share how this access benefits you. Submit a story.

Accessibility
Beyond the Mean: Quantile Regression to Explore the Association of Air Pollution with Gene-Specific Methylation in the Normative Aging Study

Marie-Abele C. Bind,1,2 Brent A. Coull,2 Annette Peters,1,3 Andrea A. Baccarelli,1 Letizia Tarantini,4,5 Laura Cantone,4,5 Pantel S. Vokonas,6,7 Petros Koutrakis,1 and Joel D. Schwartz1

1Department of Environmental Health, and 2Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA; 3Institute of Epidemiology, Helmholtz Zentrum München–German Research Center for Environmental Health, Neuherberg, Germany; 4Department of Clinical Sciences and Community Health, Center of Molecular and Genetic Epidemiology, Università degli Studi di Milano, Milan, Italy; 5Fondazione Ca’ Granda, IRCCS Istituto Di Ricovero e Cura a Carattere Scientifico Ospedale Maggiore Policlinico, Milan, Italy; 6VA Normative Aging Study, VA Boston Healthcare System, Boston, Massachusetts, USA; 7Department of Medicine, Boston University School of Medicine, Boston, Massachusetts, USA

BACKGROUND: Air pollution has been related to mean changes in outcomes, including DNA methylation. However, mean regression analyses may not capture associations that occur primarily in the tails of the outcome distribution.

OBJECTIVES: In this study, we examined whether the association between particulate air pollution and DNA methylation differs across quantiles of the methylation distribution. We focused on methylation of candidate genes related to coagulation and inflammation: coagulation factor III (F3), intercellular adhesion molecule 1 (ICAM-1), interferon gamma (IFN-γ), and toll-like receptor 2 (TLR-2).

METHODS: We measured gene-specific blood DNA methylation repeatedly in 777 elderly men participating in the Normative Aging Study (1999–2010). We fit quantile regressions for longitudinal data to investigate whether the associations of particle number, PM2.5 (diameter ≤ 2.5 μm) black carbon, and PM1.5 mass concentrations (4-week moving average) with DNA methylation [expressed as the percentage of methylated cytosines over the sum of methylated and unmethylated cytosines at position 5 (%5mC)] varied across deciles of the methylation distribution. We reported the quantile regression coefficients that corresponded to absolute differences in DNA methylation (expressed in %5mC) associated with an interquantile range increase in air pollution concentration.

RESULTS: Interquantile range increases in particle number, PM2.5 black carbon, and PM1.5 mass concentrations were associated with significantly lower methylation in the lower tails of the IFN-γ and ICAM-1 methylation distributions. For instance, a 3.4-μg/m3 increase in PM2.5 mass concentration was associated with a 0.18%5mC (95% CI: −0.30, −0.06) decrease on the 20th percentile of ICAM-1 methylation, but was not significantly related to the 80th percentile (estimate: 0.07%5mC, 95% CI: −0.09, 0.24).

CONCLUSIONS: In our study population of older men, air pollution exposures were associated with a left shift in the lower tails of the IFN-γ and ICAM-1 methylation distributions.


Introduction

Air pollution affects people every day and especially the elderly, who are a growing population stratum in the United States. Mechanisms by which air pollution causes cardiovascular mortality and morbidity are not fully elucidated (Brook et al. 2010). Recent research has pointed to epigenetics as a potential mechanism for the adverse effects of air pollution (Breton et al. 2012; Jardim 2011; Madrigano et al. 2012a). Epigenetics refers to changes in gene expression that do not modify the genetic code, but influence its expression. The most frequently examined epigenetic mechanism is called DNA methylation because it involves methylation of cytosine in CpG (cytosine–phosphate–guanine) pairs.

Several studies have related air pollution exposure to changes in epigenetic outcomes, including DNA methylation, but they have used standard regression methods that report the change in the expected value of an outcome for a given change in exposure (Baccarelli et al. 2009; Bellavia et al. 2013; Soberanes et al. 2012; Tarantini et al. 2009). However, focusing on the mean response may not well describe effects that shift the overall shape, versus the location, of the outcome distribution. Because DNA methylation is a biological mechanism whereby cells control gene expression in a complex manner (stochastic dynamics, phase variation, and bistability) (Riggs and Xiong 2004), we hypothesized that mean regression analyses may not capture associations that occur primarily in the tails of the outcome distribution.

In this study, we examined whether air pollution affects DNA methylation across nine quantiles of the methylation distribution. We focused on methylation of candidate genes related to coagulation and inflammation: coagulation factor III (F3), intercellular adhesion molecule 1 (ICAM-1), toll-like receptor 2 (TLR-2), interferon gamma (IFN-γ), and interleukin 6 (IL-6).

Materials and Methods

Study population. This prospective cohort study included male participants from the Normative Aging Study, an investigation established in Boston, Massachusetts, in 1963 by the U.S. Veterans Administration (Bell et al. 1966). We measured DNA methylation on blood samples collected after an overnight fast and smoking abstinence during the period 1999–2009. Methylation was assessed using blood samples collected at one to five visits completed at 3- to 5-year intervals. About 70% of the participants had more than one medical visit. We excluded individual observations if C-reactive protein levels were > 10 mg/L (74 observations in 71 participants) to reduce the potential influence of current infections (Simón et al. 2004), leaving a total of 1,798 observations in 777 participants.

Address correspondence to M.-A. Bind, Department of Environmental Health, 401 Park Dr., Landmark Center, Suite 415, Boston, MA 02115 USA. Telephone: (617) 384-8740. E-mail: mxbind@mail.harvard.edu

Supplemental Material is available online (http://dx.doi.org/10.1289/ehp.1307824).

This study was supported by U.S. Environmental Protection Agency (EPA) grants RD-827353 and RD-832416; National Institute of Environmental Health Sciences (NIEHS)/National Institutes of Health grants RO1-ES015172, 2RO1-ES015172, ES014663, ES00002, PO1-ES008925; and Harvard–U.S. EPA Clean Air Center (CLARC) grant RD-83479801. The VA Normative Aging Study is supported by the Cooperative Studies Program/Epidemiology Research and Information Center of the U.S. Department of Veterans Affairs and is a component of the Massachusetts Veterans Epidemiology Research and Information Center, Boston, Massachusetts.

The authors declare they have no actual or potential competing financial interests.

Received: 30 October 2013; Accepted: 10 March 2015; Advance Publication: 13 March 2015; Final Publication: 1 August 2015.
DNA methylation. We collected each participant’s blood at every visit and isolated DNA to assess gene-specific DNA methylation using quantitative methods based on bisulfite polymerase chain reaction pyrosequencing (Yang et al. 2004). The degree of methylation was expressed as the percentage of methylated cytosines over the sum of methylated and unmethylated cytosines at position 5 (%5mC).

Nine candidate genes that were expressed in leukocytes and plausibly related to heart or lung disease were chosen a priori for high precision pyrosequencing analysis as part of a previous study. From those nine candidate genes, we focused on five (F3, ICAM-1, IFN-γ, TRL-2, and IL-6) whose associated proteins are related to coagulation and inflammatory pathways. We previously examined the mean association between air pollution exposure and methylation of the same set of genes (Bind et al. 2014).

We measured F3, ICAM-1, IFN-γ, and TRL-2, methylation levels at two to five CpG positions within each gene’s promoter region and calculated the mean values of the position-specific measurements. IL-6 methylation was quantified outside the gene’s promoter region. Exact positions within promoter regions, as well as primers and conditions for the assays, have been previously described (Bind et al. 2012).

Weather variables. Ambient temperatures and relative humidity were measured at the Boston Logan Airport weather station located 8 km from the study center over the 1999–2010 period. Because study participants lived throughout the metropolitan area, we assumed that the monitored temperature and humidity could serve as surrogates of their exposures.

Statistical methods. We investigated whether air pollution levels averaged over the 4-week period before the jth visit of participant i was associated with the pth percentile of the DNA methylation distribution \( \psi_p (Y_{ij}) \). Because we had repeated methylation measures for 71% of the participants, we fit quantile regressions for longitudinal data and report the associations on the additive scale (Koenker 2004). This approach can be summarized as below:

\[
\psi_p (Y_{ij} | A_{ij}, C_{1ij} = c_1, C_{2ij} = c_2, b_{ip}) = \beta_{1p} + \beta_{2p} c_1 + \beta_{3p} c_2 + \beta_{4p} + \ldots + \beta_{k,p} + \ldots + \beta_{p} + \varepsilon \]

where
- \( A_{ij}, Y_{ij}, C_{1ij}, \) and \( C_{2ij} \) are the air pollution exposure, DNA methylation, set of confounding variables, and the set of risk factors of participant \( i \) at the jth visit, respectively
- \( \psi_p (Y_{ij}) \) is the pth quantile of the \( Y_{ij} \) distribution
- \( b_{ip} \) is the random intercept for participant \( i \) included in the regression model for the pth quantile of the methylation distribution
- \( \beta_{k,p} \) are the coefficients related to the pth quantile regression model (k = 0 to 3)
- Variables in bold represent vectors.

In our regression models, the dependent variable was gene-specific DNA methylation. We reported the quantile regression coefficients, which correspond to differences in DNA methylation (expressed in %5mC) associated with an interquartile range increase in air pollution concentration. The alpha level for statistical significance was 0.05. We adjusted for potential time-varying confounders (\( C_t \)) such as temperature, relative humidity, sine and cosine terms as a function of day of the season, and batch of methylation measurement. We also controlled for time-varying factors likely to influence methylation (\( C_t \)) but not exposure, such as age, diabetes, body mass index, smoking status (former, current, vs. never smoker), statin use, as well as percentages of neutrophils and lymphocytes in differential blood count. We included \( C_t \) in the models for efficiency and blocking any potential backdoor path through unmeasured variables that would be a common cause of air pollution and \( C_t \) (Greenland et al. 1999). We thus assumed no unmeasured confounding between air pollution and methylation, given the random intercept and the \( C_t \) covariates (see Supplemental Material, Figure S1).

We checked for nonlinear dose–response relationships between the methylation mean and air pollutant concentrations, temperature, and relative humidity using generalized additive models and cubic splines. We found no deviation from linear dose–response relationships with respect to methylation: Using cubic splines, we observed no significant improvement in fit relative to a linear model (data not shown). We conducted some sensitivity analyses restricting the study population to never and former smokers (i.e., using individual observations for men whose smoking status changed over follow-up time). Moreover, we assumed the missing mechanisms of the exposures and outcomes to be at random conditional on the covariates, and the measurement error of air pollution to be primarily Berkson (Zeger et al. 2000).

Quantile regression does not specify any distribution for the residuals, and hence is distribution free. Moreover, if one takes as their regression coefficient estimates those values that minimize the sum of the absolute values of the residuals instead of the sum of squared residuals, the result is an estimate of covariate effects on the median, instead of the mean, of the outcome distribution. Quantile regression generalizes this approach by weighting the positive and negative residuals.
differently, which forces the regression line to other percentiles of the distribution. We compared the quantile regression estimates to the ones obtained by a standard mean regression model. Because three methylation distributions (i.e., F3, ICAM-1, and TLR-2) had a point mass at zero and the residuals’ distribution showed important deviation from a Gaussian density, we assumed a Tweedie distribution (with a log-link) for these outcomes and reported associations on the multiplicative scale. For the other two outcome distributions (i.e., IFN-γ and IL-6 methylation), we assumed a Gaussian distribution for the residuals and presented our results on the additive scale. We fit the following linear mixed-effects models:

- Mean model for F3, ICAM-1, and TLR-2 (multiplicative scale)

  \[ \log E[Y_{ij}] = (\gamma_0 + u_i) + \gamma_1 A_{ij} + \Sigma_k \gamma_{ik} C_{kij} + \epsilon_{ij} \]
  \[ \epsilon_{ij} \sim N(0, \sigma^2_u), \]  \[ u_i \sim N(0, \sigma^2_u). \]  \[2\]

- Mean models for IFN-γ and IL-6 (additive scale)

  \[ Y_{ij} = (\gamma_0 + u_i) + \gamma_1 A_{ij} + \Sigma_k \gamma_{ik} C_{kij} + \epsilon_{ij} \]
  \[ \epsilon_{ij} \sim N(0, \sigma^2_u) \text{ and } u_i \sim N(0, \sigma^2_u). \]  \[3\]

where \(A_{ij}, Y_{ij},\) and \(C_{kij}\) correspond to the air pollution exposure, DNA methylation, and the set of variables for which we adjusted (i.e., confounders and risk factors) for participant \(i\) at the \(j\)th visit, respectively.

We constructed an alternative way of presenting the decile-specific results by illustrating the actual distributional change of IFN-γ methylation associated with an inter-quartile range increase in particle number concentration. We estimated a predicted curve using the quantile regression coefficients and assuming a constant trend within decile intervals.

**Results**

**Descriptive statistics.** At baseline, the median age of the study population was 72 years. Also, 27% of the participants were obese (defined as body mass index > 30 kg/m²), 14% were diabetics, and only 4% were current smokers. Participants’ characteristics varied according to their total number of visits: Individuals with more visits seemed healthier than participants with fewer visits; that is, at baseline, participants with more medical visits over the study period were less likely to be former smokers, statin users, old, obese, or diabetics (Table 1). Boston has a continental climate with direct influences from the ocean. Although it is mostly cold and dry in winter, it is usually warm and humid in summer. Ambient air pollutants levels in Boston are generally below the U.S. Environmental Protection Agency (EPA) standards. Over the 1999–2009 study period, the 24-hr PM2.5 mass concentrations exceeded the daily standard of 35 \(\mu g/m^3\) for only 13 days: between June and August 2002. Summary statistics of the weather and air pollution as well as Spearman correlations during the study period are presented in Tables 2 and 3, respectively. A substantial number of measurements of particle number concentrations (i.e., 24%) were missing due to a later acquisition of the condensation particle counter or a lack of recording measurements. PM2.5 black carbon was positively correlated with PM2.5 mass (\(p = 0.68\)). We found no statistically significant correlation between other pollutants. Temperature was also negatively correlated with particle number (\(p = -0.69\), but positively correlated with PM2.5 black carbon (\(p = 0.48\)) and PM2.5 mass (\(p = 0.40\)). The gene-specific methylation distributions varied according to genes (Table 4). For instance, at baseline, we observed wider methylation distributions of IFN-γ (5th and 95th percentiles: 75.4, 91.1) and IL-6 (5th and 95th percentiles: 25.4, 62.1), compared with that of F3 (5th and 95th percentiles: 1.0, 4.5), ICAM-1 (5th and 95th percentiles: 2.2, 8.2), and TLR-2 (5th and 95th percentiles: 1.5, 5.3).

**Quantile regression results.** Our results showed that the air pollution association

<table>
<thead>
<tr>
<th>Table 1. Demographic characteristics of the Normative Aging Study participants across visits.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visits</strong> (n = 777)</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Statin user</td>
</tr>
<tr>
<td>Among participants having one visit (n = 221)</td>
</tr>
<tr>
<td>Visit 1</td>
</tr>
<tr>
<td>Visit 2</td>
</tr>
<tr>
<td>Visit 3</td>
</tr>
<tr>
<td>Visit 4</td>
</tr>
<tr>
<td>Visit 5</td>
</tr>
<tr>
<td>Visit 6</td>
</tr>
<tr>
<td>Among participants having four visits (n = 120)</td>
</tr>
<tr>
<td>Visit 1</td>
</tr>
<tr>
<td>Visit 2</td>
</tr>
<tr>
<td>Visit 3</td>
</tr>
<tr>
<td>Visit 4</td>
</tr>
</tbody>
</table>

Three individuals had 5 visits, and their characteristics were fairly healthier than those of the other participants.

\(\text{Body mass index} > 30 \text{ kg/m}^2\).
with DNA methylation was not generally homogeneous across quantiles (Figure 1). We observed that the negative association between particle number and F3 methylation was stronger in the upper deciles of the F3 methylation distribution. Although concentrations of particle number and PM$_{2.5}$ were not associated with ICAM-1 methylation in the upper deciles of the methylation distribution, they were negatively related to the lowest deciles. For instance, a 3.4-μg/m$^3$ increase in PM$_{2.5}$ was associated with a 0.18%5mC (95% confidence interval (CI): –0.30, –0.06) decrease on the 20th quantile of ICAM-1 methylation, and was not significantly related to the 80th quantile (estimate: 0.07%5mC; 95% CI: –0.09, 0.24). PM$_{2.5}$ black carbon concentrations were negatively associated with the 10th to 60th percentiles of the ICAM-1 methylation distribution and positively related to the 90th percentile. Moreover, we observed that the negative association between particle number and IFN-γ methylation was strongest in the lower deciles of the IFN-γ methylation distribution. Particle number concentrations were not related to the 10th and 20th percentiles but were associated with the higher deciles of the IL-6 methylation distribution. We did not find any associations between air pollution and any of the deciles of the TLR-2 methylation distribution. In the analysis restricted to never and former smokers (consisting of

Table 4. Gene-specific methylation (%) across visits (5th, 50th, and 95th percentiles).

<table>
<thead>
<tr>
<th>Visits</th>
<th>F2 mean</th>
<th>ICAM-1 mean</th>
<th>IFN-γ mean</th>
<th>TLR-2 mean</th>
<th>IL-6 mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observations</td>
<td>1,533</td>
<td>1,424</td>
<td>1,726</td>
<td>1,424</td>
<td>1,749</td>
</tr>
<tr>
<td>n CM</td>
<td>265</td>
<td>374</td>
<td>62</td>
<td>374</td>
<td>49</td>
</tr>
<tr>
<td>Baseline (n = 777)</td>
<td>1.0, 2.0, 4.5</td>
<td>2.2, 4.1, 8.2</td>
<td>75.4, 85.2, 91.1</td>
<td>1.5, 2.8, 5.3</td>
<td>25.4, 43.7, 62.1</td>
</tr>
<tr>
<td>Among participants having one visit (n$_1$ = 221)</td>
<td>1.1, 1.9, 3.5</td>
<td>2.6, 4.3, 7.7</td>
<td>72.4, 85.2, 91.8</td>
<td>1.4, 2.8, 5.0</td>
<td>23.7, 43.8, 61.6</td>
</tr>
<tr>
<td>Visit 1</td>
<td>1.0, 2.0, 4.2</td>
<td>2.2, 4.1, 8.4</td>
<td>75.4, 85.5, 90.9</td>
<td>1.5, 2.6, 5.1</td>
<td>23.7, 43.1, 65.3</td>
</tr>
<tr>
<td>Among participants having two visits (n$_2$ = 217)</td>
<td>0.8, 2.3, 4.4</td>
<td>2.2, 3.9, 8.2</td>
<td>75.9, 86.2, 91.4</td>
<td>1.0, 2.6, 5.7</td>
<td>24.7, 42.8, 59.9</td>
</tr>
<tr>
<td>Visit 2</td>
<td>1.0, 2.0, 4.5</td>
<td>2.1, 3.8, 7.6</td>
<td>75.8, 84.7, 91.1</td>
<td>1.3, 2.8, 5.2</td>
<td>28.9, 43.7, 59.8</td>
</tr>
<tr>
<td>Visit 3</td>
<td>0.9, 2.5, 4.5</td>
<td>2.1, 3.6, 7.8</td>
<td>76.4, 86.8, 90.7</td>
<td>1.5, 2.6, 5.3</td>
<td>28.4, 43.0, 57.5</td>
</tr>
<tr>
<td>Among participants having three visits (n$_3$ = 216)</td>
<td>0.9, 1.8, 4.3</td>
<td>2.9, 4.2, 6.7</td>
<td>76.3, 86.2, 91.1</td>
<td>0.9, 2.1, 4.9</td>
<td>24.9, 42.9, 59.7</td>
</tr>
<tr>
<td>Visit 4</td>
<td>0.4, 2.3, 5.2</td>
<td>2.1, 4.0, 9.8</td>
<td>76.9, 84.4, 90.7</td>
<td>1.9, 3.3, 5.9</td>
<td>28.9, 43.8, 61.8</td>
</tr>
<tr>
<td>Among participants having four visits (n$_4$ = 120)</td>
<td>1.0, 2.4, 4.8</td>
<td>2.0, 3.3, 9.9</td>
<td>76.9, 85.6, 91.4</td>
<td>1.7, 3.1, 6.0</td>
<td>25.3, 43.4, 58.4</td>
</tr>
<tr>
<td>Visit 5</td>
<td>1.8, 2.9, 4.5</td>
<td>2.5, 4.4, 6.1</td>
<td>75.0, 89.4, 89.3</td>
<td>1.5, 3.0, 6.3</td>
<td>26.7, 44.6, 62.9</td>
</tr>
<tr>
<td>Visit 6</td>
<td>0.7, 1.5, 3.1</td>
<td>2.8, 4.0, 8.3</td>
<td>77.5, 86.2, 92.7</td>
<td>0.9, 1.6, 4.0</td>
<td>26.3, 44.5, 60.5</td>
</tr>
</tbody>
</table>

This table does not include three individuals having five visits.

Figure 1. Absolute difference in gene-specific methylation (expressed in %5mC with 95% CI) associated with an IQR increase in exposure [interquartile range (IQR) = 14,599 per cm$^3$ for particle number (PN), 0.26 μg/m$^3$ for PM$_{2.5}$ black carbon, and 3.4 μg/m$^3$ for PM$_{2.5}$ mass], according to the deciles of the methylation distribution.
755 participants and 1,737 individual observations, we found fairly similar results (see Supplemental Material, Figure S2).

The results obtained from mean regression analyses are presented in Table 5. An interquartile range increase in particle number concentration was negatively associated with the means of the F3 and IFN-γ methylation distributions. PM$_{2.5}$ mass concentrations were also negatively related to the mean of the F3 methylation distribution.

We propose another way of presenting the decile-specific results (i.e., finding reported in Figure 1). We focused on the association between particle number and the IFN-γ methylation distribution (presented in third top panel in Figure 1). Particle number was associated with a left shift in the lower tail of the IFN-γ methylation distribution (Figure 2).

Discussion

Our findings suggest a potential impact of air pollution on DNA methylation and heterogeneous associations across quantiles of some gene-specific methylation distributions. In the same cohort, aging has been related to hypomethylation of TLR-2 and hypermethylation of F3 and IFN-γ (Madrigano et al. 2012b); and compared with never and former smokers, current smokers had higher IL-6 methylation and lower TLR-2 and IFN-γ methylation levels (Bind et al. 2014). Moreover, when we used mean regression to conduct mediation analyses in the same cohort (with air pollution as exposure, methylation as mediator, and cardiovascular-related blood markers as outcomes), we estimated a positive indirect effect of PM$_{2.5}$ black carbon on fibrinogen through a decrease in F3 methylation (Bind et al. 2014). Similarly, the positive associations of sulfate and ozone with ICAM-1 seemed to be partly mediated via a decrease in ICAM-1 methylation. This quantile regression study showed that air pollution may be associated with only one extreme of the methylation distribution—which suggests heterogeneity between study participants with respect to potential epigenetic effects resulting from air pollution exposure.

Our results suggest that exposure to fine and ultrafine particles (size between 0.007 and 3 μm in diameter) is associated with decreased methylation in the upper quantiles of F3 methylation and the lower quantiles of IFN-γ methylation. F3, also known as tissue factor, is a major trigger of the coagulation cascade. F3 expression has been observed in vascular smooth muscle cells, endothelial cells, and fibroblasts (which play a role in wound healing) (Holy and Tanner 2010). High F3 levels found in atherosclerotic plaques have been shown to be critical in the pathogenesis of atherothrombosis (Jude et al. 2005). Individuals with acute coronary syndromes, hypertension, dyslipidemia, diabetes, and cancer also have elevated F3 concentrations (measured, for instance, in endothelial cells, monocytes, macrophages, plasma) compared with individuals free of these diseases (Holy and Tanner 2010; Steffel et al. 2006). Furthermore, F3 induces thrombin formation leading to fibrin generation and activation of platelets (Jude et al. 2005). Platelet activation has, in turn, been observed after exposure to ultrafine particles in 57 men with coronary heart disease (Rückerl et al. 2007). An intermediary mechanism could be through inflammatory cytokines and oxidized lipids which have been shown to up-regulate F3 expression (Holy and Tanner 2010; Jude et al. 2005).

IFN-γ is a cytokine that plays a central role in the generation and release of reactive oxygen species (ROS). The formation of ROS is associated with lack of important antioxidants, which causes oxidative stress (Schroecksnadel et al. 2006). According to the findings of several studies, oxidative stress appears to be an intermediary process between air pollution and cardiovascular disease (Barregard et al. 2008; Li et al. 2009; Mazzoli-Rocha et al. 2010; Schroecksnadel et al. 2006).

In this study, exposures to particle number, PM$_{2.5}$ black carbon, and PM$_{2.5}$ mass were associated with the lowest quantiles of ICAM-1 methylation. In a previous study, we showed that a decrease in ICAM-1 methylation was also related to a significant increase in the mean of ICAM-1 protein (Bind et al. 2014). ICAM-1 is a glycoprotein that is expressed on endothelial cells and cells of the immune system. Elevated ICAM-1 concentration increases the risk of myocardial infarction or coronary death. Our results suggest that air pollution exposure may decrease ICAM-1 methylation, which may result in ICAM-1 gene de-silencing and ICAM-1 protein overexpression.

![Figure 2. Empirical IFN-γ methylation distribution and its associated predicted distribution assuming an IQR increase in PN number.](image-url)

Table 5. Associations with mean gene-specific methylation for an interquartile range increase in air pollutant concentration.

<table>
<thead>
<tr>
<th></th>
<th>F3</th>
<th>ICAM-1</th>
<th>TLR-2</th>
<th>IFN-γ</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle number</td>
<td>0.78 (0.72, 0.85)*</td>
<td>0.97 (0.92, 1.03)</td>
<td>1.00 (0.94, 1.08)</td>
<td>−0.77 (−1.43, −0.11)*</td>
<td>0.59 (−0.55, 1.74)</td>
</tr>
<tr>
<td>PM$_{2.5}$ black carbon</td>
<td>0.90 (0.85, 0.95)*</td>
<td>0.98 (0.95, 1.02)</td>
<td>1.03 (0.98, 1.08)</td>
<td>−0.41 (−0.87, 0.04)</td>
<td>0.76 (−0.03, 1.54)</td>
</tr>
<tr>
<td>PM$_{2.5}$ mass</td>
<td>0.96 (0.93, 1.00)</td>
<td>0.97 (0.94, 1.00)</td>
<td>1.00 (0.97, 1.03)</td>
<td>−0.18 (−0.49, 0.13)</td>
<td>0.33 (−0.19, 0.84)</td>
</tr>
</tbody>
</table>

*Mean ratio for F3, ICAM-1, and TLR-2 (multiplicative scale): Because the methylation distributions of F3, ICAM-1, and TLR-2 had a point mass at zero and the residuals’ distribution showed important deviation from a Gaussian density, we assumed a Tweedie distribution (with a log-link) for these outcomes and reported associations on the multiplicative scale.

*Mean difference for IFN-γ and IL-6 (absolute scale): For the other outcome distributions (i.e., IFN-γ and IL-6 methylation), we assumed a Gaussian distribution for the residuals and presented our results on the additive scale. *Significant at p < 0.05.
Our findings using quantile regression are fairly consistent with mean regression analyses using distributed-lag models (Bind et al. 2014) or moving averages (Table 5) for exposure in the same cohort. Concentrations of particle number and PM$_{2.5}$ black carbon were associated with F3 hypomethylation in both the mean and quantiles analyses. In the mean regression analysis using moving averages for exposure, concentrations of particle number and PM$_{2.5}$ black carbon were not significantly related to ICAM-1 methylation. This quantile analysis reveals some association between particle number and the low end of the ICAM-1 methylation distribution and no change at the high end of the distribution, demonstrating the added value of the quantile regression approach. In addition, for PM$_{2.5}$ black carbon, we observed significant negative associations with the lower percentiles of the ICAM-1 methylation distribution and a positive association with the 90th percentile, indicating an effect of broadening the distribution at both ends, which resulted in a nonsignificant change on average. Particle number concentration was associated with the lower percentiles and the mean of the IFN-γ methylation distribution. However, the magnitude of the mean estimate was smaller compared with the estimates of the lower percentiles. For example, an interquartile range increase in particle number concentration was associated with a 0.8%5mC (95% CI: 0.1, 1.4; Table 5) and a 1.5%5mC (95% CI: 0.6, 2.4; Figure 1) decrease in mean and the 20th percentile of the IFN-γ methylation distribution, respectively.

Quantile regression allows us to describe effects that shift the overall shape, as opposed to the location, of the outcome distribution. For instance, although we found some evidence that exposure to fine and ultrafine particles (size between 0.007 and 3 μm in diameter) shifts the low quantiles of the IFN-γ methylation distribution toward lower levels, we observed no significant effect on the upper quantiles. Figure 2 shows the distributional change of IFN-γ methylation assuming an interquartile range increase in particle number concentration. Our findings suggest that participants with low IFN-γ methylation may be more susceptible to fine and ultrafine particles. In our study population of older men, air pollution exposures were associated with a left shift in the lower tail of the IFN-γ methylation distribution.

Also, the heterogeneous associations between air pollution and methylation across quantiles of the methylation distribution is seen with mostly particle number for F3 and IFN-γ and is seen with particle number, PM$_{2.5}$ black carbon, and PM$_{1.0}$ mass concentrations for ICAM-1. Different types of pollutants and size of particles may therefore have varying effects on gene-specific methylation.

Method limitations and strengths. Individuals in the top 50% of the F3 methylation distribution (i.e., with methylation levels between 2.0 and 4.5%5mC; Table 4) tend to lose about 1%5mC of methylation per IQR increase in particle number (Figure 1), which corresponds to almost double the loss observed in individuals in the bottom 50% (i.e., with methylation levels between 1.0 and 2.0%5mC). Because a given CpG site in a given homoyzogotic cell is either (fully) methylated or (fully) unmethylated, we acknowledge that the first group of participants has roughly twice as many circulating methylated cells as the second, so the higher impact of exposure in the top 50% group versus the bottom 50% group may not be unexpected. Furthermore, the result is an increase by 1 or 0.5% of the proportion of unmethylated (possibly F3-expressing) cells from a baseline of 95.5–98% and 98–99% (Table 4), respectively, a change whose significance is debatable. On the other hand, for IFN-γ methylation the corresponding picture is that, in people with about 75–85%5mC methylation (i.e., only 15–25% of the circulating cells are unmethylated; Table 4), the frequency of unmethylated cells increases by 1% per IQR in PM$_{2.5}$ mass concentration, a sizable and potentially significant increase. However, these arguments could be reversed if what matters physiologically is a large change on the ratio scale compared with the absolute scale. However, data collected in this study population are limited to address this issue.

Quantile regression is a distribution-free method and allows us to obtain estimates on the additive scale (expressed as a change in %5mC). In contrast, the standard approach using mean regression requires assumptions about the distribution of the residuals or the outcome. This approach using quantile regression can be reused in other disciplines to include the mean effect on an outcome of heterogeneity, reporting the exposure–outcome association along the entire outcome distribution—i.e., among individuals who already had low methylation levels, consistent with a shift on the lower quantiles of the methylation distribution to the left. Although the role of methylation in gene expression is complex, including no role, methylation tends to repress expression (Riggs and Xiong 2004). Hence, it is possible that individuals who already had a higher risk of inflammation may be the ones primarily affected by particles. In summary, quantile regression may capture associations that are only in the tails of the distribution and might be otherwise missed. This approach estimating associations along outcome distribution also allows us to describe distributional outcome changes associated with increasing exposure. This makes it a valuable tool for environmental epidemiology, and for providing results that might allow better risk assessment in future studies.

**References**


Quantile regression: air pollution and methylation

Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA 279(18):1477–1482.


