Background

Epigenetic modifications may serve as indicators of past toxic exposures and predict future disease risk. We propose to discover and validate novel methylation biomarkers of air pollution exposure and related phenotypic outcomes of interest. Our understanding about the complex interplay of epigenenvironment interactions remains rudimentary, and it often been based on high-exposure animal models. This array-based methylation study employs the Normative Aging Study (NAS) cohort, followed for over 40+ years, to identify key epigenetic pathways in humans; the Illuma HumanMethylation450 BeadChip was used to query the methylation status of ~480K CpG sites across the human genome. These epigenetic marks may aid in the early diagnosis and prevention of air-pollution-related diseases and the study of basic biological processes in vivo.

Objectives

Collectively, we are looking at changes in DNA methylation with various phenotypes/outcomes: 1) Fasting blood glucose levels 2) Biological age 3) Black carbon (BC) & Lung function decline (FEV1)

Methods

Study design: We analyzed ~46,900 CpG sites with the 10% highest variance in methylation in our cohort. We did a cross sectional analysis, with CpG \( = \alpha \) Phenotype + \( \beta X + \epsilon \),

\[ s \sim N(0, \sigma^2) \]

where the outcome was set as fasting blood glucose, black carbon, or aging, and X specified the covariates to be included in the model. j ranged from 1 to m, where m was the number of CpG sites included (Barfield et al., 2012).

• Confounders for Fasting Blood Glucose: Age, BMI, insulin intake, other diabetes medications, and smoking status.
• Confounders for Aging: BMI, smoking status, physical activity, educational level, and alcohol consumption.
• Confounders for Black Carbon and Lung Function Decline: Age, BMI, smoking status, height, medication intake, education, and disease status

Results

Table 1 Characteristics of participants in NAS-Normative Aging Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.2 (11.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.7 (9.4)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.1 (14.3)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2 (4.0)</td>
</tr>
<tr>
<td>Smoking status</td>
<td>4.2 (1.3)</td>
</tr>
<tr>
<td>Education</td>
<td>16.2 (2.8)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.3 (0.5)</td>
</tr>
</tbody>
</table>

Fig 1 Manhattan Plot for association between methylation and fasting blood glucose level

Among the 46,983 CpG sites queried for an association between fasting blood glucose levels and methylation, 23 sites were significant by the BH method.

Fig 2 Manhattan Plot for association between methylation and biological age

3 sites were found significant by the Holm method
7 sites were found significant by BH method

Discussion

No studies published (to date) have examined associations between short- and long-term exposures to traffic-related air pollution and genome-wide methylation using the 450K. Here we studied 5-methylcytosine from CpGs on the 450K within the top 10% highest ratio of variance above the technical replicates in the study, after removing failed samples and probes (watermelon floater) and background correction.

Fasting blood glucose methylation analysis: One CpG site on chromosome 4 was the most significant hit:

- This CpG site was located at the pericentromeric proliferator-activated receptor pathway gene (TLL1); components of this receptor pathway are molecular targets for the treatment of diabetes (Celli and Shuldiner, 2003).

Age-associated methylation changes: We found that several CpG sites belong to genes previously implicated in aging biology and related processes:

- **ADAMTS1** encodes a member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) protein family, which is a putative tumor suppressor related to nasopharyngeal carcinoma (Li et al., 2010).
- **Glia Cell-Derived Neurotrophic Factor (GDNF)** is a gene significant to epigenetic modifications, contributes to behavioral responses to stress (Usahi et al., 2011).
- **Epigenetic process influence the imprinting of <em>an ono</em>**, a calcium activated chloride channel (Okaw et al., 2012).
- **Adipocyte cyclic 5 (AC5)** is subject to DNA hyper-methylation, which is associated with premature stages of lung adenocarcinoma (Sato et al., 2013).

Conclusions

Understanding the underlying epigenetic basis of human health and disease outcomes is critical to informing prevention efforts, especially as we reconstruct past exposure “signatures” in the epigenome to predict future disease risk. Our current study leverages a rich DNA archive to study the association(s) of air pollution, age, lung function decline, and fasting blood glucose on DNA methylation in vivo, and our preliminary data suggest that we can identify candidate CpGs in relevant genes that function within basic pathophysiological pathways.

References