Genes, DNA methylation and exposures underlying COPD in never-smokers
van der Plaat, Dini

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No association between DNA methylation and COPD in never and current smokers

M. de Vries, D.A. van der Plaat, J.M. Vonk, H.M. Boezen

Submitted
To the editor,

Chronic obstructive pulmonary disease (COPD) is a chronic and progressive inflammatory lung disease, characterized by persistent airflow limitation. COPD patients suffer from severe respiratory symptoms, resulting in a worse quality of life. The development of COPD is associated with both genetic and environmental factors and their interactions. Although exposure to cigarette smoke is the main preventable risk factor for the development of COPD, not every smoker will develop COPD. The results of the genome-wide association studies (GWAS) and genome-wide interaction studies (GWIS) that have been performed so far, indicate that only a small part of the so called ‘susceptibility to develop COPD’ can be explained by genetic variation. As a consequence, the epigenome is increasingly recognized as an important link between the inherited genome and environmental exposures. One well-defined epigenetic modification is DNA methylation, which is tissue-specific and involves the binding of a methyl group to a cytosine base adjacent to a guanine base, a so called CpG-site, leading to changes in gene-expression. Therefore, differences in DNA methylation might partly explain the variance in the susceptibility to develop COPD. In the current study, we assessed whether differences in DNA methylation levels are associated with COPD in the general population including never and current smokers.

Table 1: Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Never smokers</th>
<th>Current smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects, N (%)</td>
<td>903 (57.8)</td>
<td>658 (42.2)</td>
</tr>
<tr>
<td>Male, N (%)</td>
<td>508 (56.3)</td>
<td>375 (57.0)</td>
</tr>
<tr>
<td>Age (yrs), median (min-max)</td>
<td>46 (18–80)</td>
<td>46 (22–79)</td>
</tr>
<tr>
<td>Pack years (yrs), mean (min-max)</td>
<td>-</td>
<td>20.6 (5.1–95.0)</td>
</tr>
<tr>
<td>COPD (FEV₁/FVC&lt; 70%), N (%)</td>
<td>316 (35.0)</td>
<td>279 (42.4)</td>
</tr>
<tr>
<td>Lung function, mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>3.5 (0.9)</td>
<td>3.4 (0.9)</td>
</tr>
<tr>
<td>FEV₁%predicted (%) *</td>
<td>100.5 (14.5)</td>
<td>94.4 (14.9)</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>84.5 (8.2)</td>
<td>71.7 (8.8)</td>
</tr>
<tr>
<td>Moderate COPD, N (%) **</td>
<td>56 (6.2)</td>
<td>100 (15.2)</td>
</tr>
</tbody>
</table>

* Calculated with GLI-2012 if possible
** COPD GOLD stage 2 and up (FEV₁/FVC < 70% and FEV₁ between 50% and 80% of predicted)

We selected 903 never smokers and 658 current smokers (> 5 pack years) with and without COPD from the LifeLines cohort study. COPD was defined as pre-bronchodilator...
FEV$_1$/FVC < 70% (see subject characteristics in table 1). We determined whole blood DNA methylation levels of 430,930 CpG-sites (after quality control) using the Illumina Infinium Human Methylation 450K Array. To analyze the association between genome-wide DNA methylation and COPD, we performed robust logistic regression analysis with COPD as outcome and DNA methylation as predictor. We adjusted for the potential confounders age, gender, pack years, batch effects and white blood cell composition and applied Bonferroni correction for multiple testing.

The results show that in never smokers, CpG-site cg14972228 on chromosome 19, annotated to Signal-Induced Proliferation-Associated 1-Like Protein 3 (SIPA1L3), was most significant ($p = 5.66 \times 10^{-6}$). In current smokers, CpG site cg08282037 on chromosome 19, annotated to Epidermal Growth Factor Receptor Pathway Substrate 8-Related Protein 1 (EPS8L1), was most significant ($p = 1.45 \times 10^{-5}$). However, after Bonferroni correction for multiple testing, none of the CpG-sites was genome-wide statistically significant. An overview of all the CpG-sites is presented in the Manhattan plot in figure 1.

To our knowledge, this is the first genome-wide methylation study in a general population-based sample including current and never smokers. The only other study exploring the association between genome-wide DNA methylation and COPD so far, focused on a much more severe COPD phenotype than the common COPD phenotype we have studied.\textsuperscript{6} Our results are in contrast with those from Qui et al, which described a strong association between DNA methylation and COPD. Several possible reasons can be put forward to explain why the outcome of these two, on first sight, comparable studies is so different.

First, Qiu et al used the Illumina Infinium Human Methylation 27K array to determine the methylation levels of the CpG-sites, while we used the Illumina Infinium Human Methylation 450K array. Of the CpG-sites on the 27K array, 24,064 CpG-sites were also present among the 430,930 CpG sites that passed quality control in our analysis. Moreover, Qui et al identified 349 CpG-sites that were significant for their three selected phenotypes COPD, FEV$_1$, and FEV$_1$/FVC and replicated at $p < 10^{-3}$ in their replication cohort. All these 349 CpG-sites were available in our study too. Hence, the different Illumina platforms cannot explain the differences in the results of both studies.
Second, we adjusted for age, gender, pack years, batch effects and white blood cell composition while Qui et al decided to avoid potential over adjustment for age and sex, arguing that these confounders are already included in the definition of COPD. Nevertheless, they do show that in their covariate-adjusted models the association between COPD and DNA methylation remains highly significant. However, the $p$-values are considerably higher than in the unadjusted models and none of the CpG-sites were significantly replicated in their replication cohort. Interestingly, an unadjusted analysis in our cohort revealed several genome-wide significant CpG-sites (data not shown), although we did not replicate any of their top hits. Since Qiu et al. did not adjust for white
blood cell composition, we could not assess if differences in white blood composition might explain the differences between both studies. Overall, adjustments for confounders definitively affects the results of the studies, but it is not clear if the adjustments can be held completely responsible for the observed differences between the studies.

Third, our cohort is a selected sample from the general population including subjects with mild to moderate severe COPD and controls (Table 1). In contrast, Qiu et al included only severe COPD patients based on a strict cut off of $\text{FEV}_1 \%\text{pred}$ of 70% together with a $\text{FEV}_1/\text{FVC} < 70\%$. Furthermore, in our cohort only never smokers and current smokers are included, while ex-smokers are not present. These differences in smoking habits and phenotype between both cohorts might explain the differences between the results in both analyses to some extent.

Finally, we have to keep in mind the cross-sectional design of both studies and that we cannot rule out the fact that COPD may also cause differential DNA methylation itself.

In conclusion, we did not observe any significant association between DNA methylation levels and the presence of COPD. Results of DNA methylation studies appear to be highly variable, which should be taken into account for the interpretation of future studies.
REFERENCES


