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

Maaike de Vries,1,2 Diana A van der Plaat,1,2 Judith M Vonk,1,2 H Marike Boezen1,2

ABSTRACT

INTRODUCTION Chronic obstructive pulmonary disease (COPD) is a progressive inflammatory lung disease, characterised by persistent airflow limitation. Patients with COPD 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 risk factor for the development of COPD, not every smoker will develop COPD.

METHODS For the current study, 1561 subjects were non-randomly selected from the LifeLines cohort study. We included 903 never smokers and 658 current smokers with and without COPD, defined as pre-bronchodilator forced expiratory volume in 1 s (FEV/FVC)<70%.

RESULTS None of the CpG sites in both the never and the current smokers were genome-wide significantly associated with COPD. CpG site cg14972228 annotated to SIPAL3 was most significant (p=5.66×10−6) in the never smokers, while CpG site cg08282037 annotated to EPS8L1 was most significant (p=1.45×10−5) in the current smokers.

CONCLUSION In contrast to a previous, smaller study, we did not observe any significant association between DNA methylation levels and the presence of COPD, independent of smoking status. Apparently, DNA methylation studies are highly variable.

Key messages

► We did not identify any genome-wide significant association between COPD and DNA methylation in never and current smokers in a large, non-random sample from the general population.
► The results of our study are in contrast to a previous, smaller study, indicating that studies on DNA methylation might be highly variable.
► The high variability in DNA methylation studies should be taken into account when interpreting these types of studies.
pre-bronchodilator forced expiratory volume in 1 s/forced vital capacity (FEV₁/FVC <70%) and job-related exposures. Pre-bronchodilator spirometry was performed with a Welch Allyn Version 1.6.0.489, PC-based Spiroperfect with CA Workstation software according to the ATS/ERS guidelines. Technical quality and results were evaluated by well-trained assistants and abnormal results were re-evaluated by a lung physician.

Measurements
DNA methylation levels in whole blood were determined using the Illumina Infinium Human Methylation 450K Array. After quality control, DNA methylation data presented as beta values were available for 420 938 CpG sites.

Epigenome-wide association study
To analyse the association between genome-wide DNA methylation and COPD, we performed robust logistic regression analysis with COPD as outcome and DNA methylation as predictor, stratified for smoking status (never vs current). We adjusted for the potential confounders age, gender, pack years, batch effects and white blood cell composition and applied false discovery rate (FDR) correction for multiple testing.

RESULTS
Subject characteristics
A total of 903 never smokers and 658 current smokers were included in this study. An overview of the subject characteristics is shown in table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never smokers</td>
</tr>
<tr>
<td>Number of subjects, N (%)</td>
<td>903 (57.8)</td>
</tr>
<tr>
<td>Male, N (%)</td>
<td>508 (56.3)</td>
</tr>
<tr>
<td>Age (years), median (min–max)</td>
<td>46 (18–80)</td>
</tr>
<tr>
<td>Pack years (years), mean (min–max)</td>
<td>–</td>
</tr>
<tr>
<td>COPD (FEV₁/FVC &lt;70%), N (%)</td>
<td>316 (35.0)</td>
</tr>
<tr>
<td>Lung function, mean (SD)</td>
<td></td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>3.5 (0.9)</td>
</tr>
<tr>
<td>FEV₁%predicted (%)*</td>
<td>100.5 (14.5)</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>84.5 (8.2)</td>
</tr>
<tr>
<td>Moderate COPD, N (%) †</td>
<td>56 (6.2)</td>
</tr>
</tbody>
</table>

*Calculated with GLI-2012 if possible.
† COPD GOLD stage ≥2 (FEV₁/FVC <70% and FEV₁ between 50% and 80% of predicted).
COPD, chronic obstructive pulmonary disease; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

RESULTS
In both never and current smokers, none of the CpG sites were genome-wide significantly associated with COPD (see Manhattan plot in figure 1). In never smokers, CpG site cg14972228 on chromosome 19, annotated to Signal-Induced Proliferation-Associated 1-Like Protein 3 (SIPA1L3) and involved in the GTP-ase activating cascade, was most significant (p=5.66×10⁻⁶). While SIPA1L3 has been associated with epithelial cell morphogenesis, polarity and cytoskeletal organisation in eye abnormalities, a similar role for SIPA1L3 in lung-related tissues has not yet been described. In current smokers, CpG site cg08282037 on chromosome 19, annotated to Epidermal Growth Factor Pathway Substrate 8-Related Protein 1 (EPS8L1), was most significant (p=1.45×10⁻⁵). It has been postulated that EPS8L1 can link growth factor stimulation to actin reorganisation, thereby playing a role in the regulation of actin cytoskeletal remodelling. However, the function of EPS8L1 in the respiratory system is currently unknown.

DISCUSSION
To our knowledge, this is the first genome-wide methylation study with the main focus on COPD in a general population-based sample of never and current 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...
COPD was one of the selection criteria, the percentages non-random selection of the LifeLines Cohort Study. Since have to be emphasised. Our DNA methylation cohort is a to our study, some additional cohort specific characteristics phenotypes of COPD in both studies, this might contribute potential DNA methylation itself. Together with the different cannot rule out the fact that COPD may also cause differ-

results of both analyses to some extent.

Furthermore, COPD was defined as a fixed pre-bronchodilator FEV1/FVC ratio below 70%. While this definition of COPD is commonly used, it is known that this definition can overestimate the number of COPD cases, especially in older subjects.13

In conclusion, we did not observe any genome-wide signific-

ance association between DNA methylation levels and the presence of COPD, independent of smoking status. Results of DNA methylation studies appear to be highly variable, which should be taken into account for the interpretation of future studies.

Contributors MdV and HMB: conception and design of the research. MdV and DAvdP: performed the analyses; MdV and HMB: interpreted the results. MdV: prepared the figure and drafted the manuscript. HMB and JMM: critically reviewed and revised the manuscript. All authors read and approved the final version of the manuscript.

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Competing interests None declared.

Patient consent Not required.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

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