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LETTER

Association of bronchial steroid inducible methylation quantitative trait loci with asthma and chronic obstructive pulmonary disease treatment response

To the editor,

Large variation in response to inhaled corticosteroids (ICS) has been reported in both asthma and chronic obstructive pulmonary disease (COPD), which may partly be explained by genetic factors. The transcriptome of the airways changes following ICS treatment,[1] which may be directed by single nucleotide polymorphisms (SNPs), that affect deoxyribonucleic acid (DNA) methylation (methylation-Quantitative Trait Loci, meQTL).

A strong and consistent response of the airways to ICS in both asthma and COPD patients[1,2] has been found, and severe childhood asthma has been associated with increased odds of COPD development in later life,[3] showing that overlap between the diseases may exist. We hypothesised that preselection of steroid-inducible meQTL that affect DNA methylation upon ICS treatment may increase power to find SNPs that also clinically affect response to ICS and that these genetic variants might overlap between asthma and COPD. The aim of this study was to identify SNPs that affect change in DNA methylation in the airway wall upon ICS treatment, and to investigate whether these SNPs are associated with asthma exacerbations in children despite treatment with ICS.

For the identification of meQTLs, we investigated 43 Dutch COPD patients from the Groningen and Leiden Universities study of Corticosteroids in Obstructive Lung Disease (GLUCOLD) study (Table S1).[1] Longitudinal airway wall DNA methylation (EPIC 850 K array) and gene expression (ribonucleic acid-sequencing, RNA-seq) was collected from these patients pre- and post-6 months of fluticasone ± salmeterol (500/50 μg twice daily) treatment (Figure S1). We focused on methylation sites that previously were shown to be altered during ICS treatment (1049 CpG sites).[4] This analysis identified 76 inducible meQTL caused by 71 independent SNPs with an false discovery rate (FDR) < 0.05 (Table S2). The most significant association was between cg13086983 and rs10917023, where the G allele (minor allele frequency: 7.7%) induced higher methylation (Beta: 0.849, p value: 4.21×10^{-06}). Of these 76 CpG sites, 24 were associated with 24 gene transcripts (Table S3). The most significant association was found between the Cytosine-phosphate-Guanine (CpG) site cg08570199 and the *CCDC80* gene (Beta coefficient: −1.249, p-value: 2.05×10^{-4}; Figure 1A–D).

Subsequently, we investigated whether the identified meQTL were associated with asthma or COPD exacerbations despite ICS use in children with asthma and adult COPD patients, respectively. The asthma analysis was conducted performing a meta-analysis in eight cohort studies from the Pharmacogenomics in Childhood Asthma (PiCA) consortium[5] stratified by European (n = 1515) or non-European descent (n = 1702) (see online supplementary Table and S4). Two outcomes were defined according to the American Thoracic Society/European Respiratory Society 2009 statement: (1) 'any exacerbation': hospitalisations, asthma-related emergency room visits, or oral corticosteroids (OCS) courses in the past 6–12 months and (2) OCS courses in the past 6–12 months. None of the identified meQTL were associated with the outcomes 'any' exacerbations and OCS courses in the past 6–12 months (Tables S5–S8). The COPD analysis was conducted in the Lung Health Study (LHS)-2, including 1116 COPD patients (ICS, n = 559 or placebo, n = 557) with lung function measurements over 3 years,[6] where a previous pharmacogenomic analysis focusing on genotype-by-ICS treatment effect on 3 years of FEV1 changes (estimated as slope) was investigated for the 71 SNPs None of the identified meQTL SNPs were associated with FEV1 decline after multiple testing correction (Table S9).

In conclusion, corticosteroid inducible meQTL analysis enabled us to identify a set of functional SNPs that may be useful for future (functional) studies. Although our results indicate that overlap in genetic response to steroids may exist, comparing the epigenetic responses in adult COPD patients to clinical effects in children with asthma, we do acknowledge that additional, disease and age-specific effects may be present. However, these SNPs were not significantly associated with exacerbations and OCS courses in children nor with the slope of FEV1 in adults with COPD.

KEYWORDS
children, exacerbations, inhaled corticosteroids, methylation quantitative trait loci (meQTL), pharmacogenetics

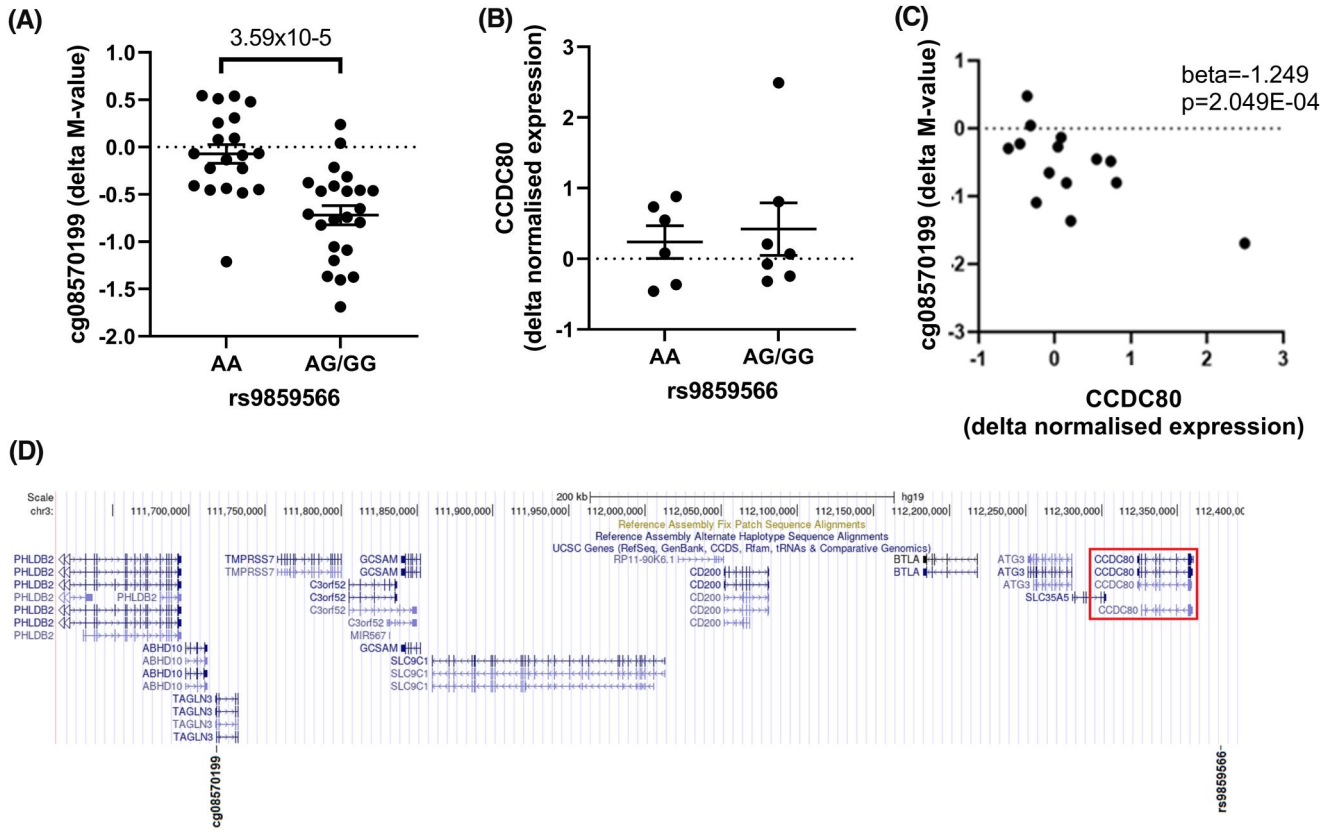

FIGURE 1 The influence of genetics on the changes of methylation during corticosteroid treatment. (A) Inducible meQTL analysis of the change in cg08570199 methylation before and 6 months following ICS treatment in bronchial biopsies and the SNP rs9859566. (B) Change in CCDC80 gene expression before and 6 months following ICS treatment in bronchial biopsies separated by the genotype of rs9859566. (C) EQTM analysis of the change in cg08570199 methylation and CCDC80 gene expression following before and 6 months following ICS treatment in bronchial biopsies. (D) Diagram of the relative positions of rs9859566 and cg08570199 to the gene CCDC80

AUTHOR CONTRIBUTIONS

Elise M. A. Slob contributed to the statistical analysis and interpretation of the data, design of tables and figures, writing of the original draft and review and editing. Alen Faiz contributed to the conception and design, statistical analysis and interpretation of data, design of tables and figures, writing of the original draft and review and editing. Jos van Nijnatten contributed to the statistical analysis and design of the tables and figures and review and editing. Susanne J. H. Vijverberg contributed to the subject recruitment, data collection and review and editing. Maria Pino-Yanes contributed to the subject recruitment, data collection and review and editing. Esteban G. Burchard contributed to the subject recruitment, data collection and review and editing. Uroš Potočnik contributed to the subject recruitment, data collection and review and editing. Colin Palmer contributed to the subject recruitment, data collection and review and editing. Steve Turner contributed to the subject recruitment, data collection and review and editing. Katia Verhamme contributed to the subject recruitment, data collection and review and editing. Somnath Mukhopadhyay contributed to the subject recruitment, data collection and review and editing. Leila Karimi contributed to the subject recruitment, statistical analysis and interpretation of the data, data collection and review and editing. Fook Tim Chew contributed to the subject recruitment, data collection and review and editing. Wim Timens contributed to the subject recruitment, data collection and review and editing. PH contributed to the subject recruitment, data collection and review and editing. Mariëlle W. Pijnenburg contributed to the interpretation of the data and review and editing. Maarten van den Berge contributed to the subject recruitment, conception and design, data collection and review and editing. Hananeh Aliee contributed to the subject recruitment, data collection and review and editing. Vojko Berce contributed to the subject recruitment, data collection and review and editing. Corry A. Brandsma contributed to the subject recruitment, data collection and review and editing. Margaret Neighbors contributed to the subject recruitment, data collection and review and editing. Michele A. Grimbaldeston contributed to the subject recruitment, statistical analysis and data interpretation, data collection and review and editing. Gaik W. Tew contributed to the subject recruitment, data collection and review and editing. Antonio Espuela-Ortiz contributed to the statistical analysis and interpretation of the data and review and editing. Yang Yie Sio contributed to the statistical analysis and interpretation of the data and review and editing. Javier Perez-Garcia contributed to the statistical analysis and interpretation of the data and review and editing. Merve Kutlu contributed to the statistical analysis and interpretation of the data and review and editing. Don D.

Sin contributed to the subject recruitment, data collection and review and editing. Xuan Li contributed to the subject recruitment, data collection and review and editing. Anke H. Maitland contributed to the subject recruitment, data collection and review and editing. Cyrielle Maroteau contributed to the statistical analysis and interpretation of the data and review and editing. Gerard H. Koppelman contributed to the conception and design, statistical analysis and interpretation of data, writing of the original draft, review and editing and is the principle investigator. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

Research data are not shared.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.