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To the Editor,

Inhaled corticosteroids (ICS) improve asthma control in most, but not all patients(1,2). Currently, biomarkers that help clinicians to predict individual ICS responsiveness or monitor effects of therapy are limited. Gene expression profiling in bronchial tissue has revealed a signature related to ICS-response(3), but access to this tissue is difficult. We recently showed that nasal gene expression can serve as a proxy to study effects of smoking in the lower airways(4), thereby providing a more accessible sampling alternative. In the current study, we aimed to investigate the effects of ICS-treatment in nasal epithelium and confirmed results both in nasal brushes of patients after ICS-withdrawal and in bronchial biopsies and air-liquid-interface (ALI) cultures after corticosteroid-treatment.

Data were collected from participants of two asthma studies on ICS-treatment: OLiVIA (n=39, 2-week HFA-beclomethasone 200µg b.i.d.(5) and NZRHS (n=28, 12-week budesonide 400µg b.i.d(6)). Detailed information is available in the online supplement. In both studies, nasal brushes were obtained at baseline and after ICS treatment(7). A subpopulation of OLiVIA used ICS prior to the study and therefore had to withdraw their ICS 4-6 weeks. In this subpopulation, an additional nasal brush was taken before ICS withdrawal (Figure E1). In NZRHS, all participants had not used ICS for at least 90 days prior to the study. RNA-sequencing of the samples was performed in OLiVIA and

microarray-based profiling (Affymetrix HuGene 1.0) in NZRHS. We used R-package limma (v3.30.13) to assess ICS-induced gene expression changes in nasal epithelium in OLiVIA and NZRHS and performed a meta-analysis to identify which genes are universally changed by ICS in both studies. P-values were adjusted for multiple testing using Benjamini-Hochberg procedure(8). Next, we compared results with nasal gene expression changes after withdrawal of ICS in OLiVIA using Gene Set Enrichment Analysis (GSEA, v3.0(9)). Additionally, we compared ICS-induced changes in gene expression from nasal samples with two independent asthma datasets of bronchial biopsies using GSEA (dataset 1: n= 12, 8-week budesonide 180µg b.i.d.; dataset 2: n= 20, 1-week fluticasone 500µg b.i.d.). Finally, we compared our findings with results of corticosteroid-induced changes in gene expression in ALI cultures of primary human bronchial epithelial cells (Table E1).

In OLiVIA, mean age was 44 years (± standard deviation [SD] 13), 20 (51%) subjects were current-smokers, 19 (49%) ex-smokers; mean FEV_1 was 84 (±SD 14) %predicted. In NZRHS, mean age was 45 years (±SD 12), 2 (7%) subjects were current-smokers, 10 (36%) ex-smokers and 16 (57%) never-smokers; mean FEV_1 was 88 (±SD 14) %predicted (Table E2). We identified 135 genes that significantly changed in expression with ICS treatment in our meta-analysis, 79 being up- and 56 downregulated (FDR meta-analysis 0.05, nominal $p<0.05$ in both studies; Figure 1; Table E3). Unsupervised clustering of subjects was performed and showed clustering of NZRHS and OLiVIA-subjects, but no clear clustering based on atopy-status, gender or clinical improvement after ICS treatment (responder/non-responder) (Figure E2). Genes downregulated after ICS treatment (n=56) were significantly enriched among genes upregulated after ICS withdrawal (Figure 2A). Genes upregulated after ICS treatment (n=79) were not significantly enriched among genes downregulated after ICS withdrawal (Figure 2B). When comparing our findings in nasal epithelium to those of the two independent studies using bronchial biopsies, GSEA showed that genes upregulated with ICS treatment in nasal epithelium were significantly enriched among genes upregulated with ICS treatment in bronchial biopsies (Figures 2C and 2E). Furthermore, genes downregulated after ICS treatment in nasal epithelium were significantly enriched among genes downregulated after ICS

treatment in bronchial biopsies in both datasets (Figures 2D and 2F). Next, we compared results with corticosteroid-induced gene expression changes in ALI cultures (Table E4). GSEA analysis showed that genes upregulated with ICS treatment in nasal epithelium significantly overlap with upregulated genes in ALI cultures (Figure 2G). For downregulated genes, there was no significant overlap between downregulated genes in nasal epithelium and downregulated genes in ALI cultures (Figure 2H). Gene ontology analysis with g-profiler(10), using the 135 ICS-induced genes as input, revealed pathways involved in death-domain signaling (Table E5).

Together our results show that nasal brushes are suitable to study gene expression changes induced by ICS in asthma patients. We identified 135 genes that significantly change in expression after ICS treatment. Confirming the robustness of our findings, genes downregulated after ICS treatment were commonly upregulated after ICS withdrawal. In addition, ICS-induced gene expression changes in nasal epithelium considerably overlapped with ICS-induced gene expression changes in bronchial biopsies in 2 independent asthma cohorts.

Among the top-10 upregulated genes were *FKPB5* and *CD163*. The protein coded by *FKBP5* is known to be upregulated by corticosteroids and functions as an inhibitor of the glucocorticosteroid receptor, thus playing a role in corticosteroid sensitivity(11). Studies investigating nasal and bronchial epithelium confirm our finding that *FKBP5* is upregulated in response to corticosteroids in healthy participants(12), and those with asthma(3,13,14) and COPD(15). CD163 is a hemoglobin scavenger receptor exclusively expressed on monocytes and macrophages, and is a marker of alternatively activated M2 macrophages (16). M2 macrophages are involved in allergic inflammation, wound healing and downregulation of inflammation by releasing the anti-inflammatory cytokine IL-10(17). Among the top-10 downregulated genes was *TRADD*. The protein coded by *TRADD*, Tumor Necrosis Factor (TNF) Receptor Type 1 Associated Death Domain Protein, is an essential member of the TNF-α/NFκB signaling pathway, which contributes to a pro-inflammatory response upon stimulation(18).

Corticosteroids are well known to suppress NFκB-signaling. Therefore, we hypothesize that one of the mechanisms by which corticosteroids inhibit NFκB-activation, is by inhibiting *TRADD* expression.

The overlap between nasal and bronchial gene expression found in the current study is in line with two recent studies from our group, in which we showed strong resemblance between the nasal and bronchial gene expression profiles associated with COPD(7) and smoking(4). This overlap may be explained by multiple factors: 1) It could be speculated that ICS are inhaled through the mouth but exhaled through the nose, which induces direct exposure of the nasal epithelium to corticosteroids, 2) epithelial cells may communicate with each other through excretion of cytokines and other mediating molecules, leading to a similar gene expression profile throughout the airways. Additionally, validation of our findings in ALI cultures showed substantial overlap between ICS-induced upregulated genes in nasal brushes and upregulated genes in corticosteroid-treated ALIs. ALIs solely contain epithelial cells and no other cell types such as fibroblasts or inflammatory cells as can be present in nasal brushes. Therefore, we provide suggestive evidence that, for a subset of genes, expression changes in nasal brushes truly reflect expression changes of epithelial cells and not merely reflect a change in inflammatory cell type composition induced by corticosteroids. A strength of our study is the comparison of changes in nasal gene expression with ICS treatment and ICS withdrawal, as well as the comparison of our findings with corticosteroid-induced bronchial gene expression changes. Limitations of the study are the difference in smoking behavior and ICS-treatment duration between OLiVIA and NZRHS. As a consequence, we might have missed genes that are affected by ICS in non-smokers only, or genes that change after a longer treatment period.

In summary, we show that nasal gene expression is dynamic, changes with ICS treatment in asthma patients and can be used as a proxy for the lower airways to investigate ICS-induced gene expression changes. This opens avenues for future applications of nasal gene expression, such as prediction of therapy response or monitoring treatment.

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Conflicts of interest

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Author's contribution:

The study was designed by MvdB, DSP, MCN, AF, PGW, SAC, JF and RB. IMB and AL performed statistical analyses under supervision of MvdB, DSP, VG and AF. IMB, AF, CAC, SB, SS, SJV,MCN, PGW, SAC, PH, HWF, DC, UB, MW, DSP, JF, RB and MvdB contributed to data acquisition. All authors contributed to interpretation of the data. IMB drafted the manuscript under supervision of MvdB, DSP and VG. All authors critically reviewed and approved the manuscript.

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Figure 1. Delta heatmap showing the change in expression of 135 differential expressed genes in nasal brushes after ICS treatment. Patients are shown in columns and genes are shown in rows. A blue rectangle reflects downregulation of a gene after ICS treatment in a subject while a red rectangle reflects upregulation of a gene after ICS treatment in a subject.

Figure 2. Gene Set Enrichment Analyses. The colored bar represents ranked t values of the association of each gene with change in gene expression after ICS treatment: red represents positive associations while blue represents negative associations. Black lines each represent a differential expressed gene after ICS treatment identified in the meta-analysis. The height of the black lines reflects the running enrichment scores of the GSEA.
A) Genes downregulated in nasal brushes after ICS treatment are significantly enriched among genes upregulated after withdrawal of ICS (FDR=0.03)
B) Genes upregulated in nasal brushes after ICS treatment are not significantly enriched among genes downregulated after withdrawal of ICS (FDR=0.69)
C) and E) Genes upregulated in nasal brushes after ICS treatment are significantly enriched among genes upregulated in bronchial biopsies of asthma patients after ICS treatment (both FDR<0.01)
D) and F) Genes downregulated in nasal brushes after ICS treatment are significantly enriched among genes downregulated in bronchial biopsies of asthma patients after ICS treatment (both FDR<0.01)
G) Genes upregulated in nasal brushes after ICS treatment are significantly enriched among genes upregulated in corticosteroid-treated ALI cultures (FDR<0.01)
H) Genes downregulated in nasal brushes after ICS treatment are not significantly enriched among genes downregulated in corticosteroid-treated ALI cultures (FDR=0.07)