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SUPPORTING INFORMATION

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Systemic gene signature of inhaled corticosteroid treatment in allergic asthma to flour

To the Editor,

Inhaled corticosteroids (ICS) are aimed to suppress mucosal inflammation and airway hyperresponsiveness in asthma, but they also have undesired systemic effects.^{1,2} The clinical and immunological heterogeneity of asthma,^{3,4} is partly responsible for different responses to ICS therapy. Therefore, a better mechanistic understanding of the systemic effects of ICS on immune cell functions in asthma is needed.

We studied transcriptomics of peripheral blood mononuclear cells (PBMC) from 38 healthy control subjects before and after budesonide 800 μ g/day for 5–6 weeks and 21 patients with allergic adult-onset asthma to flour (Table S1, Figure S1). The deconvoluted cell fractions (Figure S2) and gene expression profiles of ICS-treated versus untreated samples, and asthmatic versus healthy controls, exhibited distinct clustering patterns (Figure S1). 548 genes were differentially expressed (DE) in healthy controls treated with ICS (Figure 1A). The top biological processes represented by ICS-activated genes were; *Neutrophil degranulation*, and *Regulation of defense response*, while those from ICS-suppressed genes were; *Regulation of T-cell activation* and *Cytokine production* (Figure 1B).

These pathways were predominantly triggered by upstream inactivation of several transcription factors and pro-inflammatory cytokines (Figure S3A,B), that were causally predicted to result in sustained systemic suppression of inflammatory response pathways and decreased immune cell migration (mainly neutrophils) (Figure S3C). In contrast, asthma patients showed 256 DE genes relative to control samples (Figure 1C). The most significant biological processes were enriched by 238 downregulated genes including *Cytokine-mediated signaling*, *Inflammatory response*, and *Prostaglandin biosynthesis* (Figure 1D).

To identify the downstream targets of ICS that interact with the pathophysiological effects of asthma, we compared PBMC gene expressions between healthy controls and asthma patients. Only 15% of all DE genes (106/698) were common between ICS use and asthma (Figure 2A), corresponding to 33% of the enriched biological processes (46/138) in asthma (Figure 2B). These pathways were related to neutrophil functions and cytokine production (Figure 2C). Many of the enriched pathways were predicted to be similarly activated or inhibited in both asthma and ICS-naïve healthy persons (Figure 2D). Only *Cell movement of neutrophils* and *Synthesis of reactive oxygen*

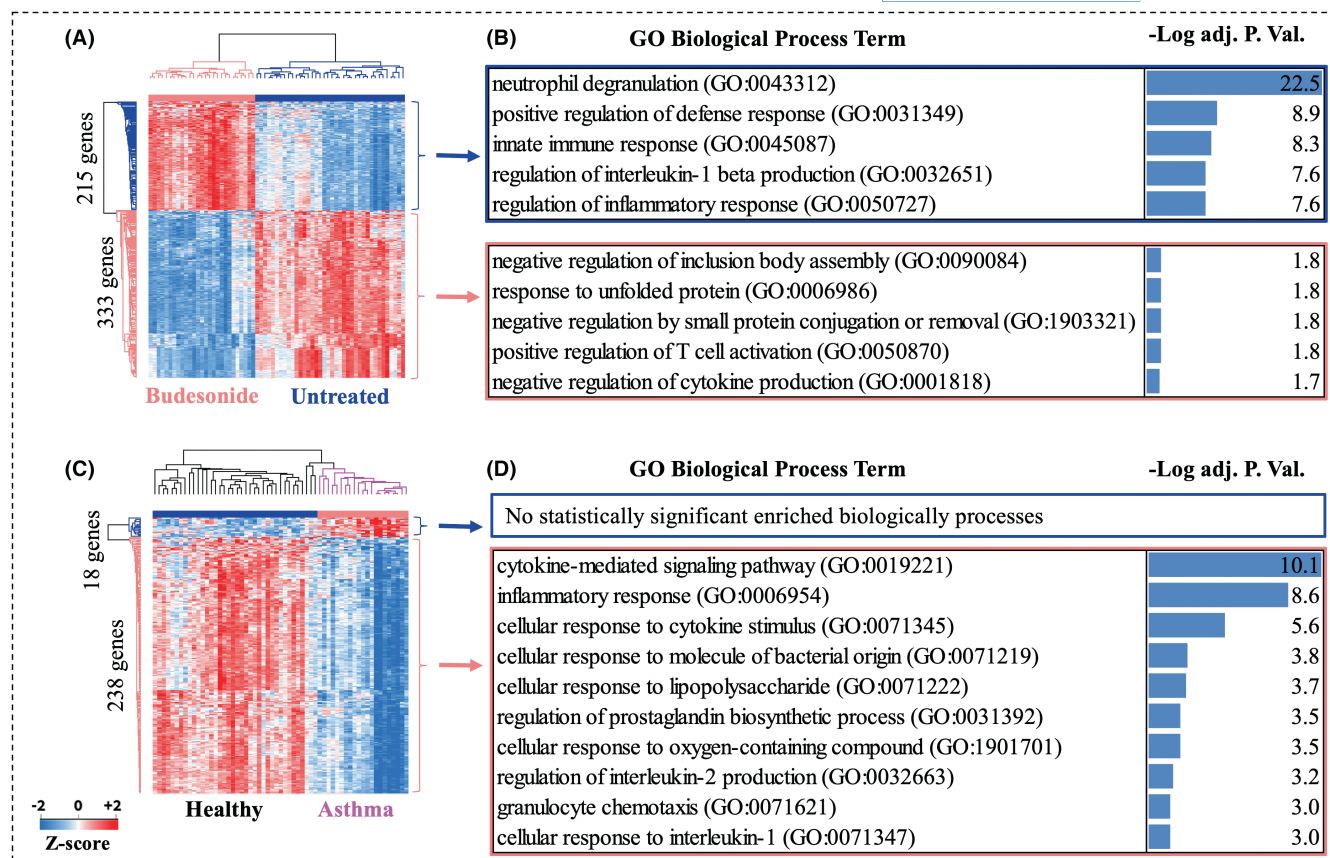


FIGURE 1 Systemic effects of inhaled corticosteroid and allergic asthma. Budesonide triggers significant gene expression change in peripheral blood mononuclear cells (PBMC). These genes separately cluster treated and untreated control samples (A). The top ranked (–log of pathway false discovery rate) gene ontology (GO) terms represented by downregulated or upregulated genes are listed in (B). Gene expression analysis of PBMC from healthy controls and asthma patients revealed 256 differentially expressed (fold-change >1.5, adjusted p-value <0.05) genes, which separated controls from asthma samples (C). GO enrichment analysis (D) show that systemic evidence of disease is dominated by downregulated genes.

species were activated in healthy ICS-treated controls but inhibited in asthma group (Figure 2D). This highlights the importance of neutrophils,⁵ and ICS-dependent mechanisms of action via which ICS counteracts asthma-related disease pathways.

The DE genes from *Cell movement of Neutrophils* (Figure S4A) or *Synthesis of ROS* (Figure S4B) pathways separated PBMC samples of ICS-treated controls from asthma patients, into two hierarchical clusters. Only 9%–18% of the genes in these two pathways were shared between the Asthma/Control and ICS-treated/Naïve control groups (Figure S5A,B). That is, for same molecular pathways, the disease- and drug-specific target genes differed as much as 80%. This suggests that depending on the cellular environment and disease status, ICS treatment may lead to different outcome effects.

In addition to the shared 46 ICS-effective pathways, 92 biological processes were unique to the asthma group, and 102 to immune cells from healthy persons treated with ICS (Figure 2C and Figure S6A). Several asthma-specific biological processes associated with IL-4 and IL-17, while the ICS-specific processes involved IL-1β and TNF (Figure S6B). The asthma-specific processes and associated

DE genes may be considered as robust asthma markers since their expression is not significantly altered by ICS treatment. These pathways might implicate ICS-resistant asthma endotypes.⁶

By comparing immune cell transcriptomics in flour-induced allergic asthma with the effects of ICS identified in healthy controls, we identified systemic ICS-responsive and non-responsive pathways in allergic asthma. These findings emphasize the need to distinguish ICS-related gene expression changes from asthma-related changes in studies with patients using ICS.

AUTHOR CONTRIBUTIONS

HS, PK, JN and HA designed this study. HS, IL and LA undertook patient recruitment and data collection. PK performed analysis of samples in the laboratory. JN performed majority of the bioinformatic data analyses and visualized the results. LW did the cell deconvolution analysis. JN and PK drafted the article. PK and HA did some additional statistical and bioinformatics analyses. All authors contributed to the study design, writing, and editing of the manuscript and have approved the final version for submission.

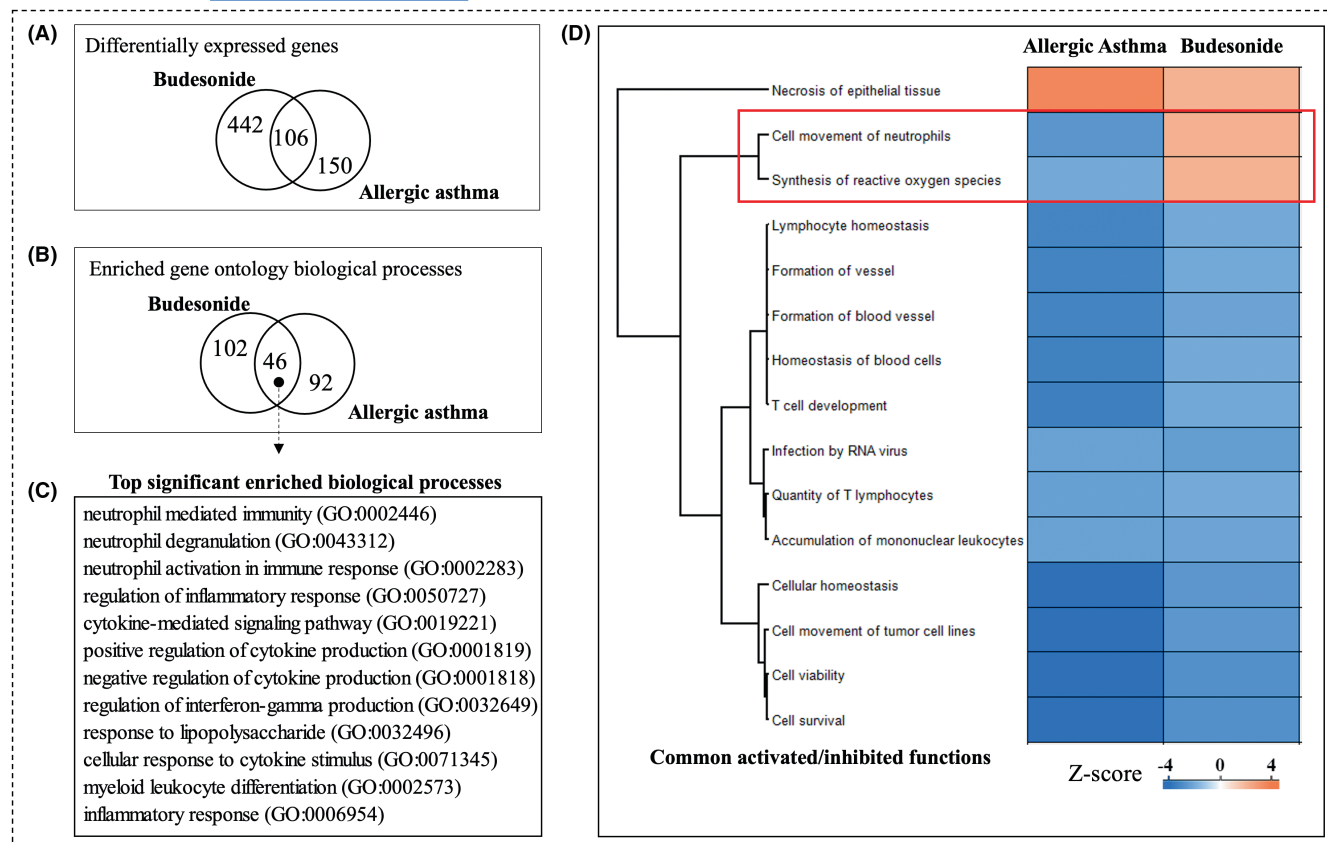


FIGURE 2 Interaction between budesonide target genes and gene signatures of active airway inflammation in allergic asthma. Interaction was deduced from overlapping differentially expressed genes (A) and enriched biological processes (B) from peripheral blood mononuclear cells (PBMC). The top shared biological processes are listed in (C). Causal effect of differentially expressed genes on pathway activity was predicted using a Z-score algorithm; which assesses the degree of overlap between an expected directional relationship (extrapolated from scientific studies curated in IPA knowledgebase) and the observed gene expression in the dataset. Z-scores $\geq +2$ or ≤ -2 correspond to significantly activated or inhibited biological processes, respectively. Overlapping activated/inhibited pathways donor-derived PBMCs are shown in (D).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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