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Bronchial Epithelial Cell Transcriptional Responses to Inhaled Corticosteroids Dictate Severe Asthmatic Outcomes

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Abstract

Background: Inhaled corticosteroids (CS) are a backbone of asthma treatment, improving quality of life, exacerbation rates and mortality. Though effective for most, a subset of asthma patients experience CS resistant disease despite receipt of high medication doses.

Objective: Our goal was to investigate the transcriptomic response of bronchial epithelial cells (BEC) to inhaled corticosteroids.

Methods: Independent component analysis was performed on datasets detailing the transcriptional response of BECs to CS treatment. The expression of these CS response components was examined in two patient cohorts and investigated in relation to clinical parameters. Supervised learning was used to predict BEC CS responses using peripheral blood gene expression.

Results: We identified a signature of CS response that was closely correlated with CS use in asthma patients. Participants could be separated based on CS response genes into groups with high and low signature expression. Patients with low expression of CS-response genes, particularly those with a severe asthma diagnosis, showed worse lung function and quality of life. These individuals demonstrated enrichment for T lymphocyte infiltration in endobronchial brushings. Supervised machine learning identified a 7 gene signature from peripheral blood that reliably identified patients with poor CS response expression in BECs.

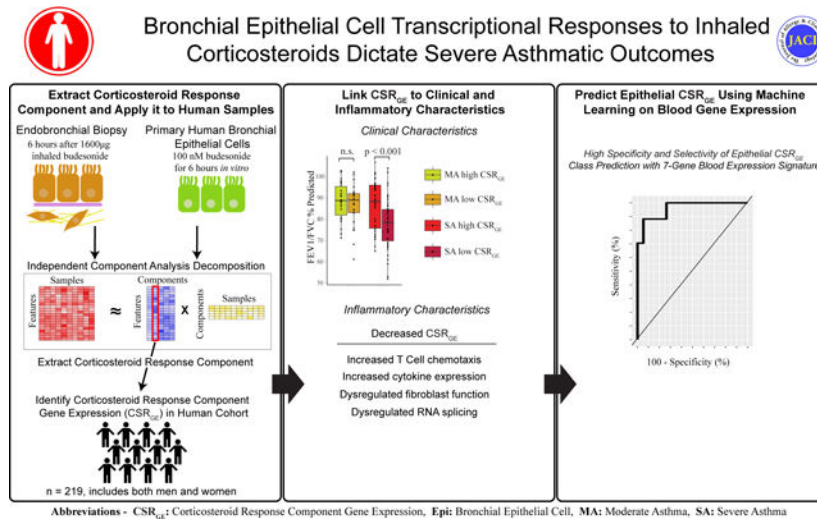
Conclusion: Loss of CS transcriptional responses within bronchial epithelium was related to impaired lung function and poor quality of life, particularly in severe asthma. These individuals were identified using minimally invasive blood sampling, suggesting these findings may enable earlier triage to alternative treatments.

Clinical Implications: The specific transcriptional changes in BECs and blood identified here may guide early use of additional therapies.

Capsule Summary:

Severe asthma patients have reduced corticosteroid-linked transcriptional changes in the bronchial epithelium, show enrichment for T lymphocyte immune processes, and can be identified using a peripheral blood gene signature.

Graphical Abstract



Keywords

Asthma; Corticosteroids; Systems Biology; severe asthma; transcriptomics

Introduction

Asthma is a common disease, affecting more than 300 million people worldwide(1, 2). Inhaled corticosteroids (CS) are the current standard of care, improving quality of life, exacerbation rates and mortality(3). Response is not universal, however, and 5–10% of patients experience poor control despite high dose CS therapy(4). This variation in therapeutic efficacy led to investigations describing heterogeneity amongst asthma patients at the clinical, immunological and molecular levels(2, 5, 6). Though prior work illustrated activation of specific pathways in CS refractory patients, the interplay between CS response in bronchial epithelial cells (BECs) and asthma phenotype remains incompletely understood(5, 7–9).

CS signaling is mediated by the glucocorticoid receptor (GR), which, following ligand binding, translocates to the nucleus to activate target gene expression(10). The GR may also associate with other transcription factors, promoting or suppressing expression of their target genes(11). CS efficacy in the context of asthma has been largely attributed to downregulation of T cell cytokine production and induction of eosinophil apoptosis(12, 13). Though downregulation of immune cell inflammatory cytokines may be crucial for CS response in asthma, growing work illustrates a role for airway epithelium in immune cell activation and propagation of inflammation(14, 15). Prior work clearly demonstrated transcriptional hallmarks of GR activation in human BECs(16). Murine models of asthma also show that epithelial GR expression is required for CS-mediated alleviation of airway hyperresponsiveness and inflammation(17). Together, these data suggest that interplay between BECs and immune cells is crucial for asthma pathogenesis.

Here, we use independent component analysis (ICA) on transcriptomic datasets of CS treatment in healthy volunteers and cell lines to extract the transcriptomic CS response signal in epithelial cells. We then evaluate the expression of this CS response signal in two large, well described asthma patient cohorts, linking poor CS response to clinical traits including impaired lung function and inadequate disease control. We describe a subset of asthma patients with low expression of CS response genes despite self-reported medication adherence, suggesting their disease may be intrinsically recalcitrant to this therapeutic modality. Importantly, patients with low CS response expression in their BECs could be identified using a peripheral blood gene expression signature, offering hope that minimally-invasive monitoring may be one day used to guide therapeutic choices.

Methods

For full methods, see online supplementary methods.

Human Participants

Endobronchial brushings from the Immune Modulation in Severe Asthma (IMSA; GSE158752) and Severe Asthma Research Program (SARP; dbGaP Study Accession: phs001446.v2.p1) cohorts were used as previously described(18). Gene expression counts for all data were normalized using the variance stabilizing transform from the DESeq2 suite(19). Batch effects within and between endobronchial brush data from the two studies were controlled for using the COMBAT algorithm(20), creating a combined epithelial dataset of the two cohorts.

Peripheral blood was also extracted from a subset of the IMSA patients. Transcriptomic data was prepared from the whole peripheral blood using the same methods as the endobronchial brushing and the data are available at GSE207751.

Corticosteroid Signal Source Datasets

Two microarray datasets were downloaded from GEO and used in our analysis. The first dataset, GSE83233, consisted of paired endobronchial biopsies obtained from healthy volunteers from before and after inhaled budesonide(21). The second dataset, GSE161805 consisted of primary human bronchial epithelial cells (pHBECs) from six different donors under control conditions, budesonide, formoterol, or budesonide and formoterol combined(22). For our analysis of GSE161805, only the control and budesonide groups were used.

Corticosteroid component extraction with independent component analysis

Joint Approximate Diagonalization of Eigenmatrices (JADE) was used to perform independent component analysis (ICA) on the GEO datasets(23). Each component produced by ICA is a vector containing a score for each gene which denotes the amount that gene contributes to the component.

After extracting the components, paired T-tests were performed to compare the relative expression of each component in the pre-CS samples against the post-CS samples. In

each case, the expression of one of the components was highly significant between the pre- and post-CS samples, and thus deemed as the CS components (Fig 1A). Because the expression of the two CS components in the combined IMSA+SARP epithelial dataset were similar (Fig 2A), we averaged their expression levels to create a set of one CS component expression value per patient.

Computational and Machine Learning Methods

Packages used for computational analysis include ClusterProfiler (24) for gene ontology analysis, GSVA (25) to quantify the expression of the GO term *Response to Steroid Hormone*, ComplexHeatmap (26) for visualization, Deseq2 (19) for differential expression, and WGCNA to identify groups of coexpressed genes (referred to as modules) (27).

Patients in the IMSA cohort that had transcriptomic data for both blood and epithelial cells ($n = 36$) were used for machine learning. For estimation of epithelial CSR_{GE} from blood transcriptomics, elastic net regularized regression analysis was performed with glmnet (28). For supervised modeling, the mixOmics package (29) was used to perform sparse-partial least square discriminant analysis training.

Statistical Analysis

All statistical analysis was performed using the R computing environment (Version 4.1.0) unless otherwise noted(30, 31). Statistical testing and methodology are described within figure legends or above within context specific methodologies. For all figures, significance levels are denoted as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

For information on other computational methods, see the online supplementary material.

Results

Extraction of Corticosteroid Signal

We used Independent Component analysis (ICA) to extract a corticosteroid (CS)-response component from two separate datasets (one CS-response component from each dataset; see methods). The first (GSE83233) consisted of paired endobronchial biopsies before and after inhaled budesonide and the second (GSE161805) of primary human bronchial epithelial cells (pHBEC) cultured with and without budesonide.

We found that many known steroid and inflammation-related genes were among the top genes contributing to the CS response component in both datasets, and that there was a general overall correlation of gene contribution scores in the CS components ($r = 0.186$, $p < 0.00001$, Fig S1, Table S1). To test if the CS-response component from one dataset could be used to examine gene expression patterns in the other, we cross interrogated their expression in control and budesonide treated samples (Fig 1A). Expression of the CS response component derived from endobronchial biopsies was significantly higher in budesonide treatment conditions compared to control in both the original dataset and in pHBECs. A similar expression pattern was observed for the CS response component originating from pHBECs. These results show that a transcriptional signal for CS response can be extracted with ICA and validated across external datasets. Averaging the scores

from the two CS components and performing gene ontology on the top 1% of positively and negatively-contributing genes confirmed that these components are highly enriched for multiple CS-related ontologies (Fig 1B, Table S2).

Expression of CS Signals in Combined IMSA and SARP Dataset

Satisfied that our CS response components could be validated between exposure data sets, we next investigated their utility in assessing asthma patient cohorts. To this end, we examined bronchial epithelial cell (BEC) expression of the extracted CS components in a pooled data set (clinical information in Table S3) from the Immune Modulation in Severe Asthma (IMSA) and Severe Asthma Research Program (SARP) cohorts(18, 32). We calculated an expression value for both of the previously identified CS components in each patient in the IMSA+SARP dataset and found that expression of the two components was highly correlated (Fig 2A; $r = 0.70205$, $p < 0.0001$). For clarity in presentation moving forward, we averaged their expression levels to create a single value of CS-response gene expression (CSR_{GE}) for each cohort participant. Unsurprisingly, CSR_{GE} was higher in patients who had used inhaled CS in the past 12 months than in those who had not (Fig 2B).

We next split the moderate asthma patients into two groups: those who had used inhaled CS in the past 12 months (MA+, $n = 53$) and those who had not (MA-, $n = 21$). We found that there was no significant difference in CSR_{GE} between MA- and HC (Figure 2C). While CSR_{GE} differed between MA+ and MA-, MA+ and SA (who by definition were taking ICS) showed comparable levels. To further support the relationship between CS exposure and CSR_{GE} values, we next created modules of coexpressed genes using Weighted Gene Co-expression Network Analysis (WGCNA)(27). WGCNA identified a module that was strongly correlated with CSR_{GE} (Fig S2A) and included many of the top contributing genes to the combined CS component (Fig S2B). Gene ontology analysis on this module showed enrichment for CS response-related terms (Fig S2C).

To further test the utility of our CSR_{GE} in determining transcriptional response to CS, we compared its performance to gene set variation analysis (GSVA), which scores individuals using known pathways(25). For the purpose of this comparison, we selected the top result from gene ontology analysis of the combined CS component: *GO response to steroid hormone*(33). While there was a strong correlation between the expression of the GSVA *response to steroid hormone* ontology and CSR_{GE} ($r = 0.548$, $p < 0.001$), GSVA was less effective at separating CS+ from CS- patients (Fig. 2D). These data suggest that CSR_{GE} is more effective than currently available GO libraries at determining the transcriptomic response of patients to CS treatment.

CSR_{GE} and Clinical Traits in Asthma

Although CSR_{GE} expression identified asthma patients receiving CSs in aggregate, we noticed high variability in CSR_{GE} amongst MA+ and SA patients, with many having CSR_{GE} values at or below the levels of HCs (Figure 2C). To investigate the nature of this variability in CSR_{GE} , we first correlated CSR_{GE} in MA+ and SA patients with various clinical traits. We found that CSR_{GE} had a strong positive correlation with FEV1% predicted and FEV1/FVC % predicted in SA but not MA+ patients (Fig 3A&B), suggesting that

appropriate CS response may protect lung function in SA. In both MA+ and SA patients there was a correlation between CSR_{GE} and worsening Asthma Control Questionnaire-6 (ACQ6) score, although the negative correlation was stronger in SA patients (Fig 3C). SA patients are older on average than those with MA. As age is known to impact lung gene expression(34), we next determined whether a relationship exists with CSR_{GE} value. We found a positive correlation between CSR_{GE} and age in MA+ patients but not in SA patients (Fig 3D), suggesting that age has minimal relationship to any differences seen in CSR_{GE} value in SA. Overall, these results suggest that age independent, high CSR_{GE} values were linked with improved clinical outcomes in SA but not MA.

Corticosteroid Response Dictates Clinical Outcomes in Severe Asthma

The current ERS/ATS definition of SA includes treatment with high dose inhaled CS, thereby suggesting poor responsiveness to therapy as an intrinsic feature(4). Our data suggest, however, that transcriptional response to CS may in fact vary amongst SA patients and this may have important implications for clinical outcomes. We clustered cohort participants by the top 20 genes in our combined CS component, effectively identifying HCs and MA- patients (Figure 4A). Though the majority of MA+ and SA patients were effectively delineated from those not receiving CSs, there were some individuals whose gene expression profile was similar but not identical to those not on therapy.

To further examine differences in clinical outcomes between high- and low-CSR_{GE} groups, we split both the MA+ and SA patients into high- and low- CSR_{GE} groups by the median CSR_{GE} expression of the patients in the MA+ and SA groups (Fig 4B, Table S4 & S5). As medication adherence may complicate our assessment of CS response, we next investigated prevalence of CS associated comorbidities. We found no difference between MA-, MA+ and SA groups in prevalence of coronary artery disease, osteoporosis or diabetes mellitus. Importantly, we also found no difference in Medication Adherence Report Scale (MARS) score, a validated self-reporting metric for medication compliance, between CSR_{GE}-high or -low patients with either MA+ or SA (Figure 4C)(35). There were, however, large differences in FEV1 % predicted and FEV1/FVC% predicted between high- and low- CSR_{GE} groups in SA, but not MA+ (Figure 4D&E). There was also a significant difference in ACQ6 between high- and low-CSR_{GE} groups in SA, but not MA+ (Figure 4F). Intriguingly, no difference in peripheral blood cell count, including eosinophils, fraction of exhaled nitric oxide (FeNO) or BMI was found between high- and low-CSR_{GE} groups in either SA or MA+ (Figure S3), suggesting currently available biomarkers for evaluation of asthma patients cannot discern BEC CS response.

Transcriptomic Differences in CS Response Between Severe and Moderate Asthma Patients

These differences in clinical traits between CSR_{GE}-high and -low asthma patients suggest that therapeutic response may result in, or be attributed to, broader changes to molecular phenotype. To investigate potential genes and pathways to explain this phenomenon, we performed differential expression between MA+ and SA patients, as well as between high- and low-CSR_{GE} patients. We then plotted the log-fold change from the two differential expression comparisons together and split the plot into quadrants (Fig 5, Table S6). Gene

ontology was then performed on each quadrant (Fig 5 and S4). Unsurprisingly, the high-CSR_{GE}-specific quadrant (top-middle; yellow-green; Fig 5 and S4) was enriched for several CS-response related ontologies. Interestingly, the SA-specific quadrant (middle-left, green, Fig 5 and S4) was enriched for several terms related to bone morphogenic protein (BMP) signaling and prostaglandin pathways. The MA+ high-CSR_{GE} quadrant (upper-right; light blue; Fig 5 and S4), i.e. the gene expression for the patients most responsive to treatment, was enriched for several neurological ontologies. Both the low-CSR_{GE}-specific quadrant (bottom- middle; orange; Fig 5 and S4) and the SA low-CSR_{GE} quadrant (bottom-left; pink; Fig 5 and S4) were enriched for T-cell and lymphocyte related ontologies and cytokines. The bottom SA and low- CSR_{GE} quadrant was also enriched for several ontologies related to peptidase activities.

The SA high- CSR_{GE} quadrant (top-left; dark blue; Fig 5 and S4) expressed many genes associated with activation of fibroblasts and other mesenchymal cells, including *MHY11*, *DES*, *ACTG2*, and surfactant genes(36, 37), suggesting that activation and proliferation of mesenchymal cells could play a role in the pathophysiology of severe asthma. We further note that the contrast between CSR_{GE}-high samples with upregulated mesenchymal gene expression and CSR_{GE}-low samples with upregulated cytokine expression is similar to the contrast between myofibroblastic and inflammatory subpopulations of cancer-associated-fibroblasts(38–40).

Although differential expression can identify genes with different mean expression between groups, there is also the possibility of a gene being correlated with CSR_{GE} in one direction for SA and in the opposite direction for MA+. Such a gene might not be differentially expressed but could still be important in explaining the mechanism behind CS response in SA. Thus, we performed differential correlation analysis between MA+ and SA (Fig 6A, Table S7). Interestingly, among the genes with the lowest ratio of SA vs CSR_{GE} correlation to MA+ vs CSR_{GE} correlation were *DUSP10* (Fig 6B) and *DUSP6*, which both negatively regulate members of the MAP kinase superfamily. Gene ontology on the top differentially correlated genes found that genes differentially correlated with MA+ CSR_{GE} are enriched for multiple ontologies related to RNA splicing. This suggests that dysregulation of RNA splicing in SA may be responsible for blunted response to CS treatment.

Predicting Lung CS Response Using Peripheral Blood

Though bronchial epithelial sampling is an invaluable tool for understanding asthma pathobiology, the resources required and risk imposed by the procedure are prohibitive for regular clinical evaluation of asthma patients. Prior work from our group showed that peripheral blood cell composition may reflect BEC transcriptional phenotype. We next determined whether peripheral blood transcriptional profile can be used to predict BEC CSR_{GE}. Elastic net modeling on IMSA patients that had matched epithelial and peripheral blood (n = 36) showed strong correlation between measured BEC CSR_{GE} values and those predicted by blood gene expression from matched IMSA cohort participants (Figure 7A). As CSR_{GE} response class showed important relationship to both lung function and quality of life, we developed supervised learning models for class prediction. BEC gene expression could be reliably used to predict CSR_{GE} response class using 55 genes (Figure 7B). We

repeated this process using blood gene expression (Figure 7C), with the goal of identifying a minimal set of genes required to meet or exceed the sensitivity of our BEC model. CSR_{GE} class prediction using supervised learning with blood gene expression was highly effective (Figure 7D). A 7-gene model was able to achieve a test sensitivity of 88%, comparable to performance of the original BEC data set. Of these 7 genes, 6 were down-regulated and 1 was up-regulated in CSR_{GE} -high patients (Figure 7E). These data suggest that peripheral blood can be used to predict BEC expression of corticosteroid responsiveness genes.

Discussion

Inhaled CSs have become ubiquitous in the treatment of persistent asthma symptoms. Morbidity and mortality from asthma have been significantly impacted and as a result these can be life changing medicines for many(1, 3). For some, however, disease control is never accomplished, even with dose escalation into ranges that characterize a patient as having severe disease. Prior studies have uncovered heterogeneity in immune cell profile and BEC transcriptional profile that are associated with SA using clustering techniques on patient cohort data(5, 6, 41). Here, we use a different approach to assessing heterogeneity amongst asthma patients, first defining CS response using publicly available data sets that specifically test their effect on BEC transcription. We then identify heterogeneity in CS response that dictates important clinical outcomes such as lung function and asthma control within the SA population.

Correlation between contributors to the CS components from pHBECs and endobronchial biopsies was highly significant but only showed a modest coefficient in statistical comparison of contribution (Figure S1), likely owed to technical and biological considerations. ICA utilizes every gene entry in the transcriptome for component assembly, meaning that many genes included in that calculation had very small component loading values(28). Correspondence between the data sets improved dramatically when considering top contributors to each component. It is important to note that pHBECs represent a more homogenous cell population than endobronchial biopsy samples which contain sub-epithelial stroma and embedded immune cells. It is also important to consider that the experiments in these external datasets were measured 6 hours after exposure to CS, and thus may not contain information regarding long-term repeated exposure to CS.

The lack of significant difference in CSR_{GE} between MA+ and SA patients (Figure 2B) was surprising, as we expected to see a blunted CS response in the SA patients who, by clinical definition, are poorly responsive to CS treatment. Heterogeneity in these individuals instead suggests that CS dose escalation may remain an effective treatment modality for some moderate asthma patients. As CSR_{GE} -low SA patients show decrements in lung function, use of alternative treatment modalities in those that show ineffective CS response may have significant impact on quality of life and objective measures of lung health. The detection of non-responsiveness thereby becomes a critical consideration. Whether CSR_{GE} -low MA+ patients will progress to SA designation, and along the way develop worsening lung function and asthma control, remains to be seen.

Severe asthma patients in CSR_{GE}-low and CSR_{GE}-high groups could not be discerned using peripheral blood cell count or FeNO, two commonly used measures in clinical evaluation of asthma patients. Prior study has associated high levels of Type-2 inflammation, driven by cytokines such as IL-4, IL-5 and IL-13, with CS responsiveness in many but not all patients(42). Here we see that the most commonly used biomarkers of Type-2 inflammation (blood eosinophilia and FeNO) were not significantly different between those with BEC transcriptional response to CS and those without, suggesting that additional biomarkers may be needed to guide expedient patient triage to alternative therapies such as targeted biologics.

As FeNO has been linked to CS-responsiveness (and low adherence(43)) in asthma, a lack of difference between CSR_{GE}-low and CSR_{GE}-high groups also supports the notion that compliance alone does not explain transcriptional changes in BECs(44). Cohort participants identified by CSR_{GE} levels and disease severity were transcriptionally distinct, which would also not be expected if medication adherence was the principal driver. Rather, we would have expected low CSR_{GE} SA patients to resemble MA patients not on ICS. Although we did not have directly observed therapy or pharmacy data available, we did not find difference in self-reported adherence using the MARS scale(35), a scale validated for use across multiple disease states and care contexts, including asthma (35, 45–47).

Both moderate and SA patients with low CSR_{GE} shared upregulation of IFNG, CXCL9 and CXCL10. These cytokines have been previously reported by our group and others in association with CS-resistant inflammation in asthma(6, 8, 9). Interferon- γ is a predominantly T cell derived cytokine; although production from macrophages has been noted, it is unlikely that this signal is being driven by bronchial epithelial cells present in endobronchial brushings. These data suggest that nested T cells may be a critical factor in driving CS resistance. Our recent data confirmed the presence of CD4 and CD8 T cells bearing surface markers of tissue residency within the airways of CS resistant asthma patients(6). Epithelial derived factors that may contribute to tissue resident memory (TRM) cell generation and maintenance have yet to be elucidated, but may offer novel avenues of treatment in asthma and other pathologies.

CS-resistant severe asthma was characterized by expression of CYP1B1, CCL26, IDO1, SLC26A4 and CPA4 in our study. The high expression of these genes could explain the poor prognosis of SA patients with low CSR_{GE}: CYP1B1 and IDO1 are involved in the metabolism of tryptophan, which has been implicated in the pathology of asthma(48, 49). Furthermore, CYP1B1 is involved in the metabolism of steroid hormones and arachidonic acid(50). Curiously, CCL26, CYP1B1, and SLC26A4 are upregulated by IL4, whereas IDO1 expression is suppressed by IL4(51). IDO1 is, however, strongly induced by IFN- γ , suggesting a complex inflammatory microenvironment featuring both T1 and T2 signaling in the airways of these individuals(52, 53). We also found that severe asthma was enriched for terms related to BMP signaling, which has previously been implicated in asthma by a study that found increased BMP-4 and BMP-6 in asthma sputum supernatants with increased neutrophils (54). These data open the possibility for differential signaling within the airway microenvironment and may point to the importance of temporal regulation.

Mesenchymal genes were highly expressed in CSR_{GE}-high patients, whereas cytokine-related genes were highly expressed in CSR_{GE}-low patients. We noticed that this contrast is similar to the contrast between myofibroblastic and inflammatory cancer-associated fibroblasts, suggesting that differentiation of fibroblasts into heterogeneous subpopulations during activation could contribute to variation in treatment response among SA patients. Although further *in vivo* experiments are needed to test this hypothesis, these data suggest that experimental treatments focused on reverting cancer-associated fibroblasts to their quiescent state(55, 56) could also be beneficial in severe asthma.

In addition to their impact on transcription, corticosteroids are also known to exert post-transcriptional and translational effects (57). We identified several genes related to post-translational effects that had differential correlation of CSR_{GE} and gene expression between severe asthma and moderate asthma. Among these was ZFP36 (also known as tristetraprolin), which is known to regulate immune response and inflammation through post-translational degradation of AU-rich element mRNAs, including several cytokines such as TNF α (58–60). Therefore, dysregulation of CS-induced post-transcriptional modification may play a role in resistance to CS in some severe asthma patients.

The ability to identify CS unresponsive patients using peripheral blood assessment may be a valuable tool in evaluation of asthma patients. A 7 gene test is tenable for use in a clinical laboratory setting and may offer value to individuals and health systems for expediting the triage of asthma patients to other treatments such as targeted biologic therapies. Some of the genes identified by predictive modeling may also give insight into biological processes governing systemic immune response of steroid-resistant asthma patients. TAS2R38 codes for a bitter taste receptor expressed by neutrophils and mast cells, as well as ciliated airway epithelium(61). Agonists of the TAS2R family have been shown to curtail allergen-induced inflammatory responses and mitigate airway remodeling in animal models of asthma. Peptidoglycan recognition protein (Pglyrp) 1 is a pattern-recognition protein that mediates antibacterial host defense(62). Mice lacking Pglyrp1 on hematopoietic cells have shown a reduction in HDM-induced eosinophilic and lymphocytic airway inflammation(63).

Using publicly available datasets, we report a transcriptional signal for CS response in BECs that is heterogeneously expressed in asthma patients and linked to lung function and symptom burden in severe disease. Those with poor CS response show hallmarks of T lymphocyte infiltration within their airways and are able to be identified using a blood gene expression signature, suggesting a systemic immune process. Further study may allow us to identify these individuals and restore CS sensitivity or tailor treatment to the specific drivers of their disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declaration of Interests

Sally Wenzel is a consultant for AstraZeneca, Novartis, Knopp, Glaxo Smith-Kline and Sanofi-Genzyme. She is also involved in clinical trials being run by AstraZeneca and an investigator-initiated study with Regeneron. Sally Wenzel and Anuradha Ray have research support from Pieris Pharmaceuticals. Matthew Camiolo is an independent consultant to Pieris Pharmaceuticals. The following companies provided financial support for study activities at the Coordinating and Clinical Centers beyond the third year of patient follow-up: AstraZeneca, Boehringer-Ingelheim, Genentech, GlaxoSmithKline, Sanofi-Genzyme-Regeneron, and TEVA. These companies had no role in study design or data analysis, and the only restriction on the funds was that they be used to support the SARP initiative.

Abbreviations:

ICA	independent component analysis
pHBEC	primary human bronchial epithelial cell
BEC	bronchial epithelial cell
CS	corticosteroid
CSR_{GE}	Corticosteroid response component gene expression
GR	glucocorticoid receptor
FeNO	exhaled nitric oxide
IMSA	Immune Modulation in Severe Asthma
SARP	Severe Asthma Research Program
FDR	false discovery rate
ROC	Receiver operating characteristic
MA	moderate asthma
SA	severe asthma
MA+	moderate asthma with inhaled corticosteroid
MA-	moderate asthma without inhaled corticosteroids

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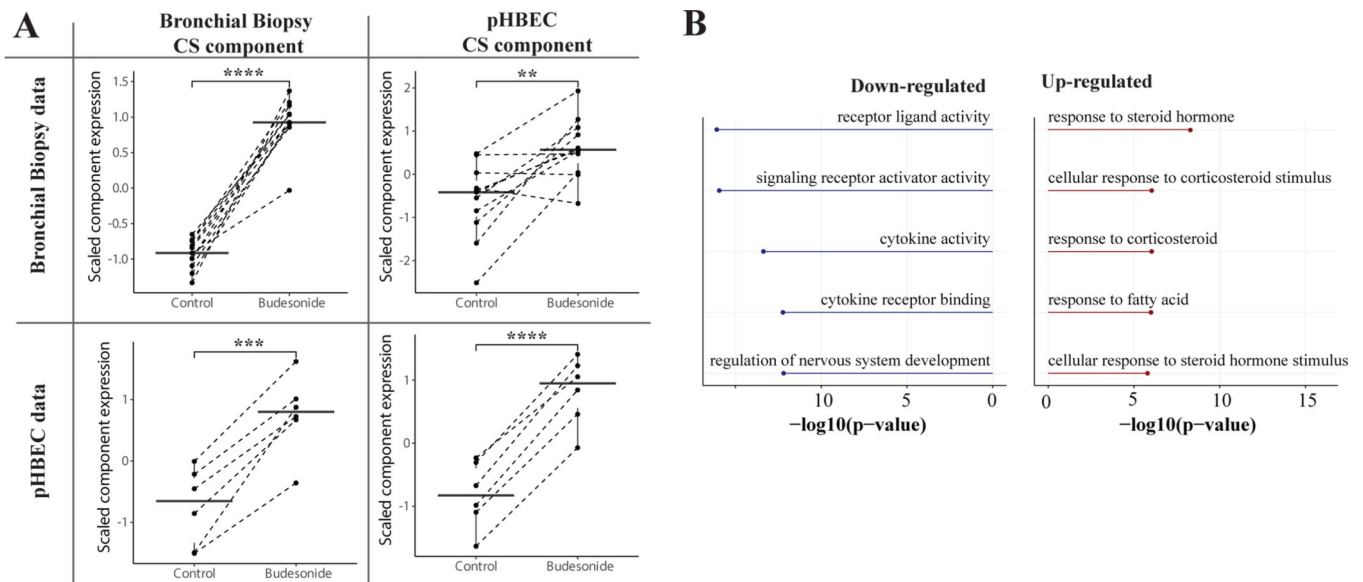


Figure 1: Independent Component Analysis Extracts the Corticosteroid Response Signal.

(A) Corticosteroid components were extracted from both the bronchial biopsy data and the pHBEC data. Each component was then expressed in each of the two datasets.

The corticosteroid components expression levels were significantly different for both components in their native dataset as well as the other dataset. (B) Gene ontology of the top positively and negatively contributing genes for the average of the two corticosteroid components.

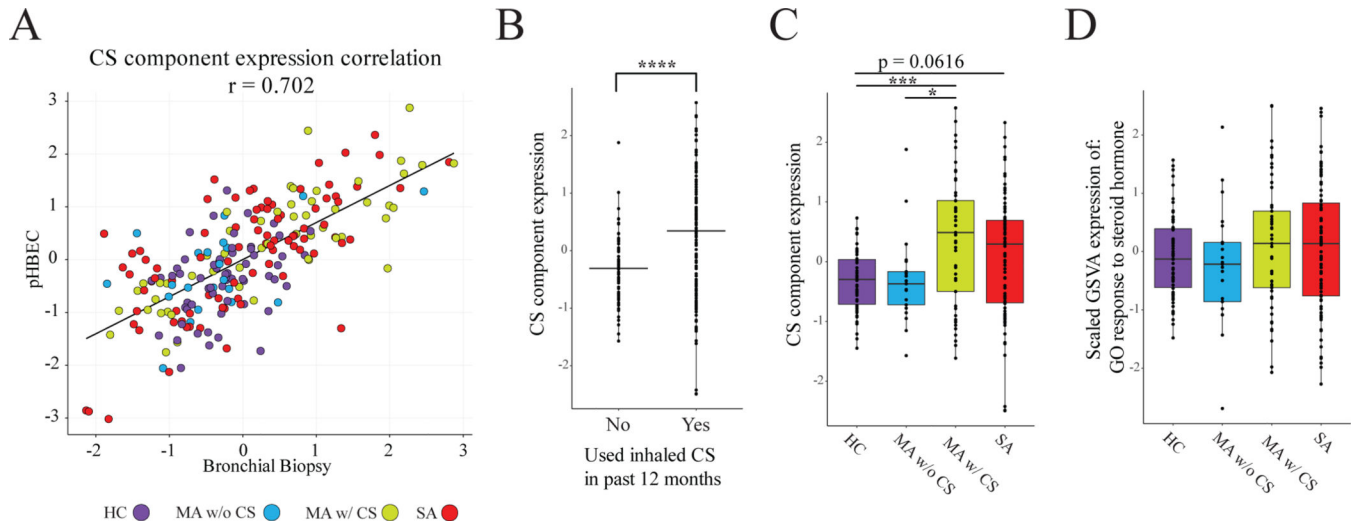


Figure 2: The CS Component is Correlated With CS Usage and Response to Corticosteroid.

(A) The two CS components extracted from GSE161805 (pHBEC) and GSE83233 (Bronchial Biopsy) are highly correlated ($r = 0.702$, $p < 0.00001$) when expressed in the bronchial epithelial cells of the merged IMSA+SARP dataset. (B) There is a significant difference in CS component expression between patients who had used inhaled CS in the past 12 months and those who had not (Student's t-test). (C) Comparison of CS component expression between healthy controls, MA-, MA+ and SA patients. Moderate asthma patients with CS have a significantly higher CS component expression level than moderate asthma patients without CS and healthy controls (ANOVA with Tukey post-hoc test). (D) There is no significant difference between the four groups of GSVA expression of the GO term Response to Steroid Hormone (ANOVA, $p = 0.506$).

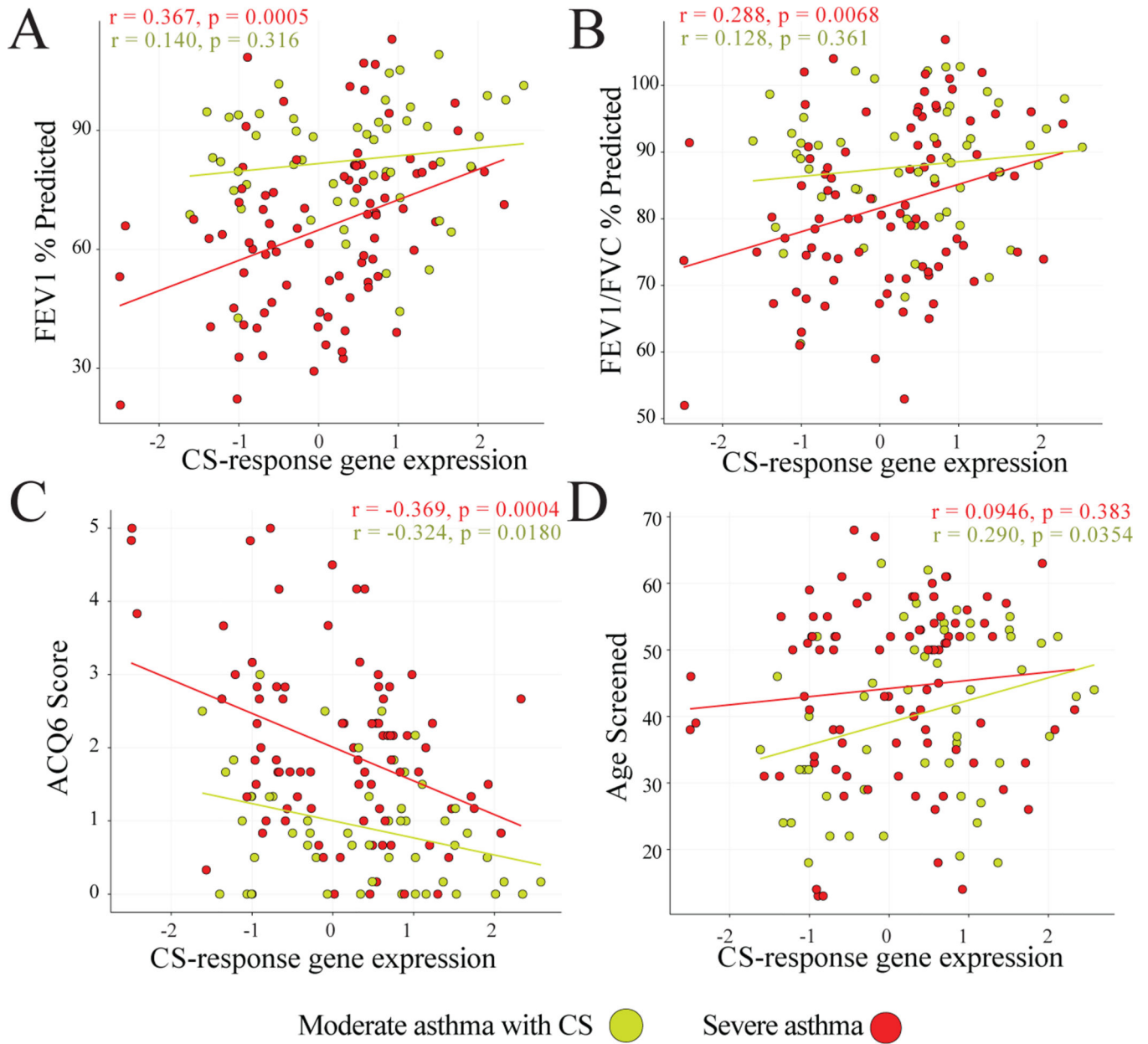


Figure 3: Correlations Between CS-Response Gene Expression and Clinical Traits for Asthma Patients Taking CS.

A-D: Correlation between CSR_{GE} and various clinical traits, split between severe asthma (red) and moderate asthma with CS (yellow).

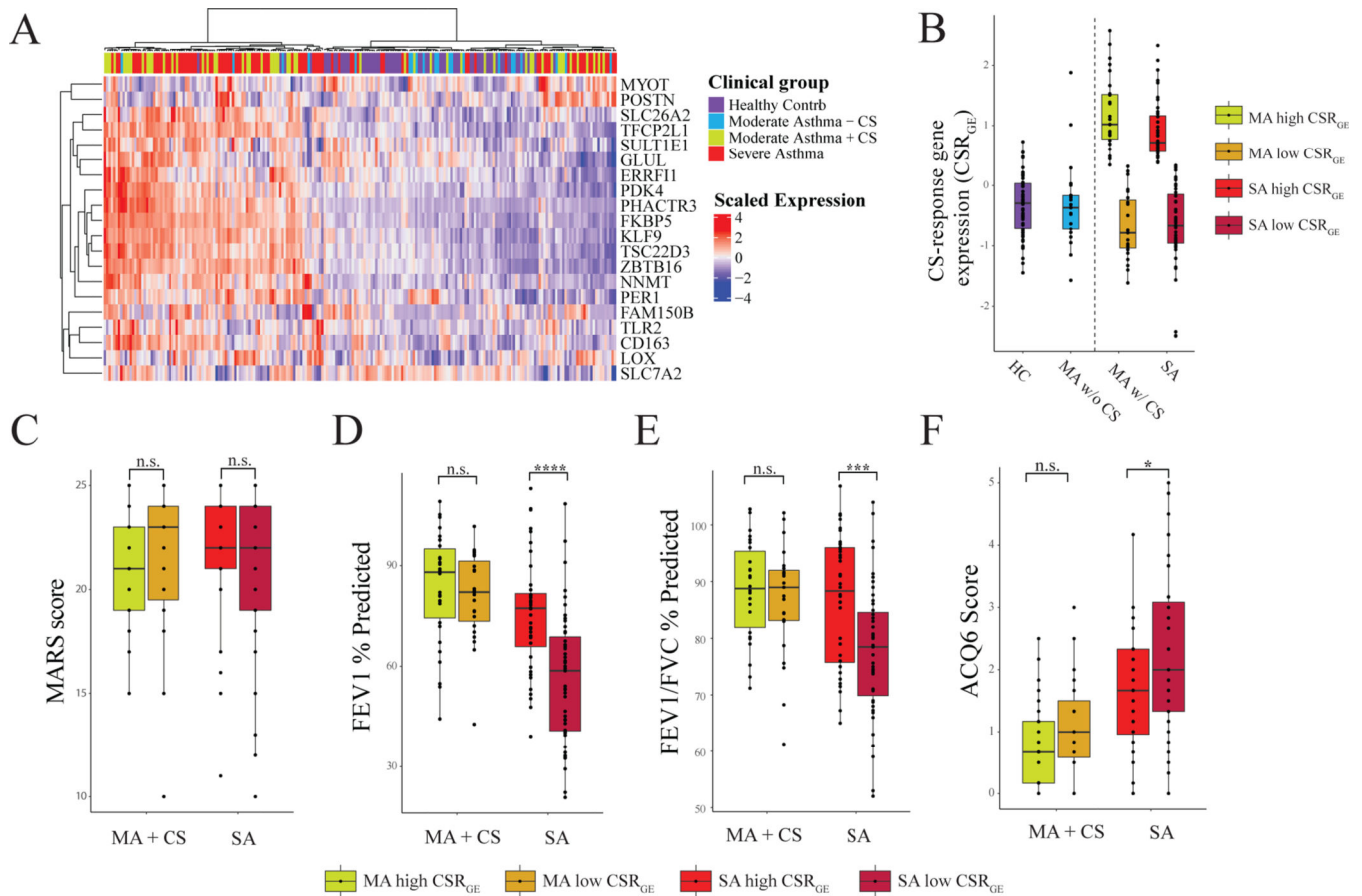
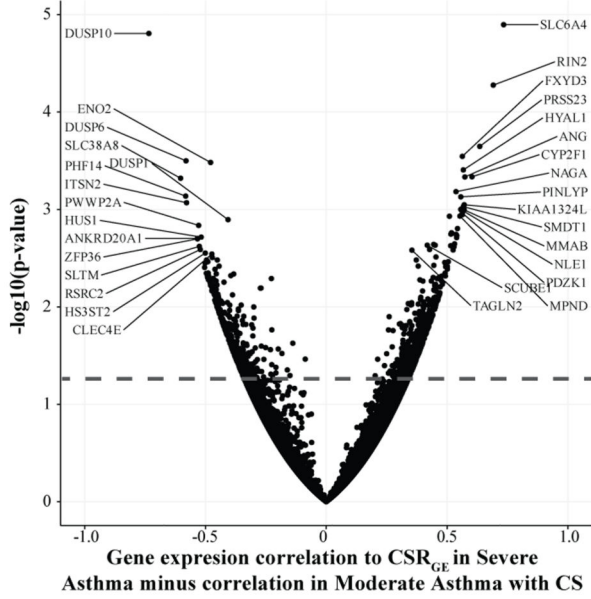


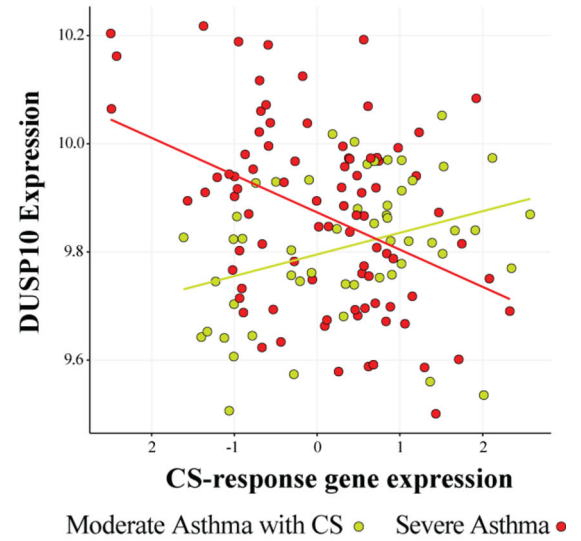
Figure 4: Heterogeneity in CSR_{GE} Dictates Clinical Outcomes in SA.

(A) Heatmap showing clustering of patients for the combined IMSA+SARP dataset by the top 20 contributing genes to the two corticosteroid components (determined by taking the absolute value of the average contribution of each gene to the two components). (B) CSR_{GE} for each diagnosis group, with Severe Asthma and Moderate Asthma with Corticosteroids split into high- and low- CSR_{GE} groups. (C-F): Comparisons of clinical traits between high- and low- CSR_{GE} groups. Statistics are performed with student's t-test comparing high- and low- groups within each diagnosis group.

A



B



C

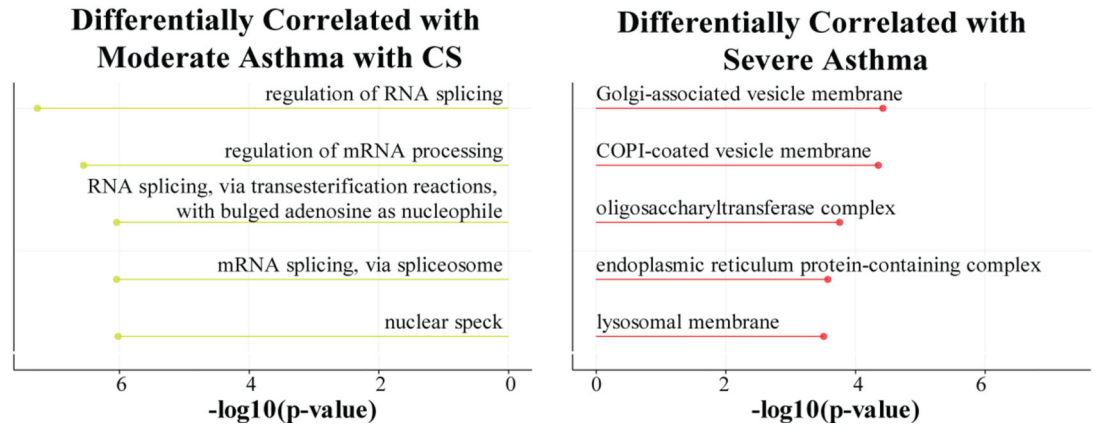


Figure 6: Differential Correlation Analysis by Asthma Severity.

(A) Differential gene correlation between MA-with-CS and SA for CSR_{GE} . (B) Expression levels of DUSP10 compared to CSR_{GE} for Severe Asthma and Moderate-Asthma-with-CS. DUSP10 is negatively correlated with CSR_{GE} in Severe Asthma ($r = -0.404$), but positively correlated with CSR_{GE} in Moderate-Asthma-with-CS ($r = 0.330$). (C) Gene ontology for the top differentially correlated genes for Moderate-Asthma-with-CS and Severe Asthma. Top genes are selected as those with p-value < 0.05 .

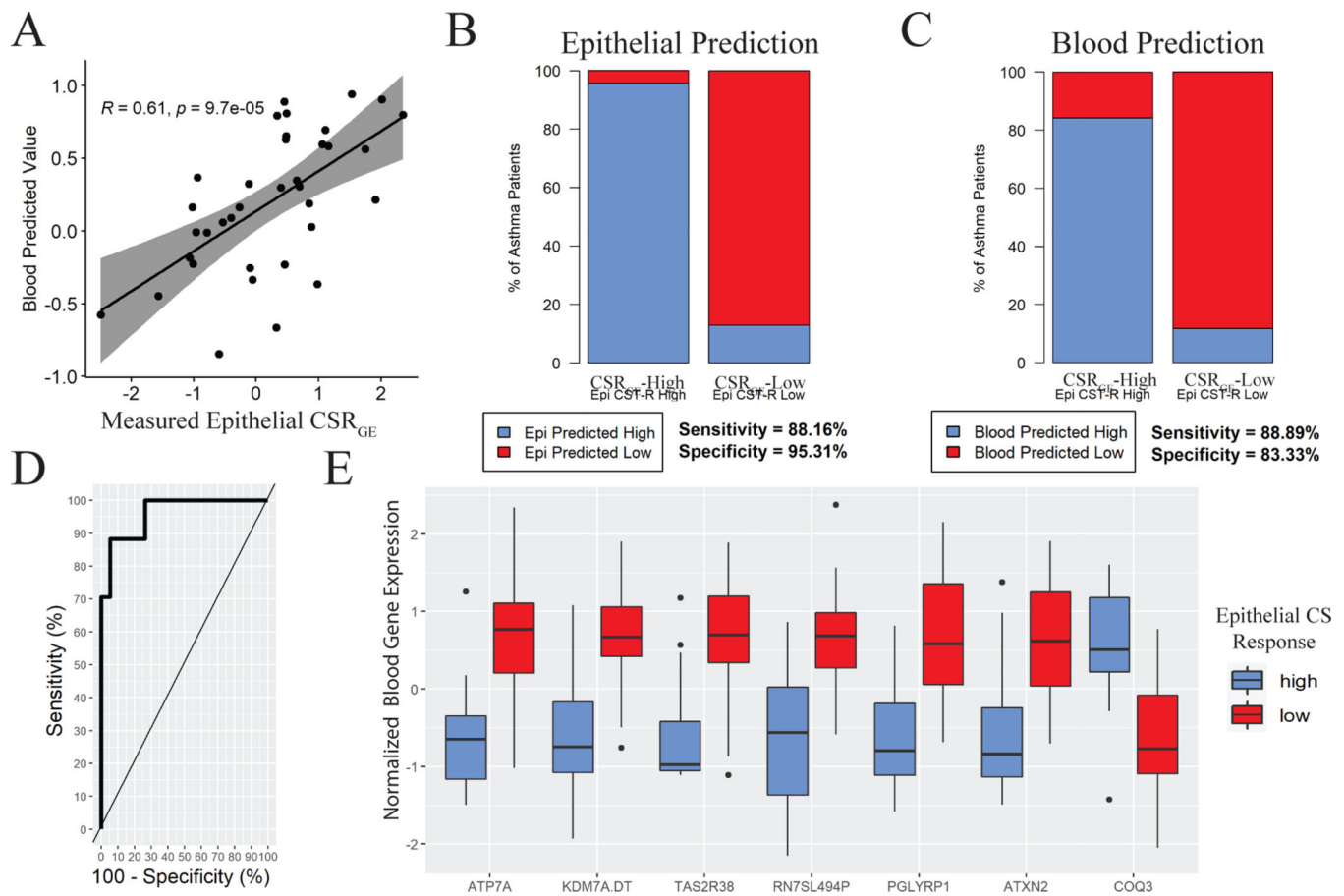


Figure 7: Prediction of BEC CSR_{GE} Using Peripheral Blood.

(A) Elastic Net (EN) predicted BEC CSR_{GE} based on blood gene expression versus measured BEC CSR_{GE} in IMSA. Grayed area indicates the 95% confidence bounds around a linear regression model comparing the two. Spearman's rho and p-value are indicated in plot area. (B) Stacked bar plot of leave-one-out CSR_{GE} response class prediction using BEC transcriptional data. P-value of chi square testing = $4.03e-22$. (C) Stacked bar plot of leave-one-out CSR_{GE} response class prediction using blood transcriptional data. P-value of chi square testing = $6.17e-05$. (D) Receiver operating characteristics (ROC) curve of a sparse-partial least squares discriminant analysis (sPLS-DA) model for CSR_{GE} class prediction. ROC curves were calculated using 5 fold-validation. Area under the curve (AUC) value is 0.9598 based on comparison of predicted scores of one class vs the other. Wilcoxon test of predicted scores < $1e-10$. (E) Boxplot for expression of genes included in the blood prediction model. Upper and lower hinges correspond to the first and third quartiles. The upper whisker extends from the hinge to $1.5 \times$ Inter-quartile range (IQR) and the lower whisker extends from the hinge $1.5 \times$ IQR of the hinge. Data beyond the end of the whiskers are plotted individually.