Check for updates

Allergy

WILEY | EAACI

ORIGINAL ARTICLE OPEN ACCESS

Asthma and Lower Airway Disease

Nasal Airway Transcriptome Reflects Selected Asthma-Associated Gene Signatures in the Lower Airways

Hui Wen[1,2] | Tessa Kole[1,2] | Orestes A. Carpaij[1,2] | Tatiana Karp[1,2] | Victor Guryev[1,3] | Alen Faiz[1,4] | Kian Fan Chung[5] | Pankaj Bhavsar[5] | Ian M. Adcock[5] | Salman Siddiqui[5] | Andy Lan[1,4,6] | Katie L. Raby[5] | Nazanin Zounemat-Kermani[7] | Chris Brightling[8] | Dave Singh[9] | Janwillem Kocks[1,2,10,11] | Monica Kraft[12] | Bianca Beghé[13] | Klaus F. Rabe[14] | Alberto Papi[15] | Machteld N. Hylkema[1,6] | Martijn C. Nawijn[1,6] | Maarten van den Berge[1,2]

[1]Groningen Research Institute for Asthma and COPD, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands | [2]Department of Pulmonary Diseases, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands | [3]European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands | [4]Respiratory Bioinformatics and Molecular Biology, University of Technology Sydney, Ultimo, New South Wales, Australia | [5]National Heart and Lung Institute, Imperial College London, London, UK | [6]Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands | [7]Data Science Institute, Imperial College London, London, UK | [8]Institute for Lung Health, National Institute for Health and Care Research, Leicester Biomedical Research Centre, Department of Respiratory Sciences, University of Leicester, Leicester, UK | [9]Centre for Respiratory Medicine and Allergy, Manchester University NHS Foundation Trust, University of Manchester, Manchester, UK | [10]General Practitioners Research Institute, Groningen, the Netherlands | [11]Observational and Pragmatic Research Institute, Singapore, Singapore | [12]Samuel Bronfman Department of Medicine, Icahn School of Medicine, Mount Sinai Medical Center, New York, USA | [13]Department of Respiratory Diseases, University of Modena and Reggio Emilia, Modena, Italy | [14]Department of Medicine, Christian Albrechts University Kiel, Kiel and Lungen Clinic, Grosshansdorf, Germany | [15]Section of Respiratory Medicine, Department of Translational Medicine, University of Ferrara, Ferrara, Italy

Correspondence: Hui Wen (h.wen@umcg.nl)

Received: 12 August 2025 | **Revised:** 8 January 2026 | **Accepted:** 23 January 2026

Keywords: asthma | IL-13 related inflammation | non-invasive | type 2 inflammation | united airways

ABSTRACT

Background: Transcriptomic analysis of bronchial brushes reveals asthma-associated gene signatures but is limited by the invasiveness of bronchoscopy. Based on the "united airways" hypothesis, we evaluated whether and to what extent nasal brushes reflect asthma-associated transcriptomic changes in the lower airways.

Methods: In the ARMS study, we included 26 asthma patients and 28 healthy controls, all fully clinically characterized. Nasal and bronchial brushes were collected for RNA sequencing. We used edgeR and WGCNA to identify asthma-associated genes and modules in bronchial brushes. We assessed their expression patterns in ARMS nasal brushes and validated the findings in the independent ATLANTIS study ($n = 427$).

Results: We identified 51 asthma-associated genes in the lower airways, of which 40 were up-regulated and 11 were down-regulated in asthma compared to healthy controls. Seven of the 40 up-regulated genes were also up-regulated in ARMS nasal brushes and validated in ATLANTIS: CLCA1, FETUB, CST1, NTRK2, CDH26, TPSAB1, and DHX35. WGCNA revealed two modules that were consistently elevated in asthma across bronchial and nasal brushes in ARMS and confirmed in ATLANTIS. One module represents IL-13 related inflammation, whereas the other represents mast cell activity.

Conclusion: The asthma-associated gene signatures of the lower and upper airways show a limited but biologically coherent overlap, with seven genes and two gene modules consistently elevated in asthma. The shared gene signatures reflect two

Machteld N. Hylkema, Martijn C. Nawijn and Maarten van den Berge share last authorship.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2026 The Author(s). *Allergy* published by European Academy of Allergy and Clinical Immunology and John Wiley & Sons Ltd.

separate components of type 2 inflammation: IL-13 related inflammation and mast cell activity. Selected nasal gene signatures offer a non-invasive tool to identify asthma endotypes with distinct molecular mechanisms driving underlying disease biology.

1 | Introduction

Asthma is a chronic respiratory disorder characterized by airway inflammation associated with variable airflow obstruction, leading to symptoms of cough, wheezing, and chest tightness [1, 2].

Over the past decade, the importance of phenotyping has been well established, and targeted treatments have become available for specific subgroups of patients, for example, those with evidence of type 2 (T2) inflammation [2]. Although this has significantly improved the management of severe asthma, variability in treatment response still persists in T2-high patients, and the origins of T2-low disease remain poorly understood. Previous studies have applied transcriptomic analysis to identify underlying molecular mechanisms of the different asthma endotypes associated with varied clinical outcomes and/or treatment responsiveness to inhaled corticosteroids (ICS) [3]. One of the earliest examples of this approach was provided by Woodruff et al., who performed genome-wide expression analysis of bronchial epithelial brushes to identify a distinct gene expression profile associated with T2-asthma and a favorable response to treatment with corticosteroids [4]. Another example comes from the U-BIOPRED study, where transcriptomic analysis from bronchial brushes and biopsies identified several different asthma subgroups, including subsets characterized by T2 inflammation and/or relative corticosteroid insensitivity [5]. Further, Modena et al. used bronchial epithelial gene expression from the Severe Asthma Research Program (SARP), linked to fractional exhaled nitric oxide (FeNO) levels, and clustered asthma patients into five subgroups, uncovering novel molecular mechanisms associated with different asthma endotypes [6].

Since asthma is primarily an inflammatory disease of the lower airways, bronchial brushes or biopsies are considered the gold standard for studying its disease mechanisms. However, the invasive nature of bronchoscopy limits widespread application, and nasal brushes have been suggested as a non-invasive alternative [7]. Poole et al. previously demonstrated that nasal brushes reflect the expression of a subset of asthma-associated T2 inflammation genes in the lower airways [8]. However, this study was conducted in children.

Among adults, two of our previous studies employing bronchial and nasal epithelial gene-expression datasets demonstrated that nasal gene signatures associated with asthma or COPD reflect specific lower-airway transcriptomic patterns [9, 10]. However, these studies did not include paired bronchial and nasal samples from the same participants. In our previous NORM study, we showed that nasal brush gene-expression profiles can serve as a less invasive proxy for bronchial epithelial gene expression in respiratory-healthy participants [11]. Based on this, the current manuscript analyses paired bronchial and nasal brushes RNA-sequencing data from adult asthma patients and healthy controls in the Asthma Origins and Remission Study (ARMS) to investigate how well asthma-associated genes and gene modules identified in the lower airways are reflected in the upper airways. These shared findings are further validated for their replicability in the independent Assessment of Small Airways Involvement in Asthma (ATLANTIS) cohort [12].

2 | Methods

2.1 | Participants Enrolment

We included both asthma patients and healthy controls from the ARMS study (NCT03141814), a cross-sectional study. In the ARMS study, asthma patients were included if they had an asthma diagnosis confirmed by objective measurements: either an increase in the forced expiratory volume in 1 s (FEV_1) of $\geq 12\%$ from the initial value and ≥ 200 mL after inhaling 400 μg of salbutamol, or airway hyperresponsiveness (AHR) to methacholine (provocative concentration causing a 20% drop in FEV_1 (PC_{20}) ≤ 8 mg/mL). Patients with asthma were required to have clinically stable disease without asthma exacerbation or respiratory infection symptoms for at least 6 weeks before inclusion in the study. Prior to the start of the study, all asthma patients discontinued ICS/long-acting beta-agonists for a period of at least 6 weeks, using only short-acting beta-agonists as needed. Healthy controls were included if they had no respiratory diseases, had normal lung function with an FEV_1% predicted $> 90\%$, FEV_1/forced vital capacity (FVC) ratio above the lower limit of normal, and no AHR to methacholine ($PC_{20} > 16$ mg/mL). All participants underwent a comprehensive clinical assessment, including spirometry, body plethysmography, impulse oscillometry, differential cell counts in blood and induced sputum, nasal brushes, and bronchoscopy with bronchial brushes. Asthma severity was assessed using the Global Initiative for Asthma (GINA) 2012 criteria. In ARMS study, we used RNA-seq data from participants included between 2014 and 2020. The study was approved by the Medical Ethics Committee of the University Medical Center Groningen (ABR nr—NL 53173.042.15) and written informed consent was obtained from all participants prior to the study. To assess the robustness of our identified shared nasal asthma-associated gene signatures in the ARMS study, we validated them in the independent ATLANTIS cohort. ATLANTIS is an international multicenter observational study, with genome-wide nasal gene expression data available from 369 asthma patients and 58 healthy controls [9, 12].

2.2 | RNA-Seq Library Preparation, Alignment, and Quality Control: ARMS Study

Ribosomal RNA depletion and RNA-seq library preparation were conducted by GenomeScan (Leiden, the Netherlands). We used the NEBNext Single Cell/Low Input RNA Library Prep Kit (E6420L) for library preparation, which incorporates

13989995, 2026, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/all.70283 by University Of Technology Sydney, Wiley Online Library on [08/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

unique molecular identifiers (UMIs) to identify PCR duplicates. Sequencing was performed on the Illumina NovaSeq 6000 platform (San Diego, California) with a 2 × 150 bp paired-end configuration aiming for a depth of 30 million read pairs per sample. Quality control (QC) of raw RNA-seq reads was first performed using FastQC, and further QC details were provided in the Supporting Information (Figure S1A,B). We aligned the trimmed reads to the human reference genome (GRCh38) using the STAR aligner (version 2.7.10b). Gene-level quantification was performed using HTSeq (version 0.12.4) with Ensembl GRCh38 release 108 as the gene annotation database.

2.3 | Statistical Analysis

All statistical analyses were conducted using R (version 4.3.2). Descriptive statistics for baseline characteristics were generated using the tableone package (version 0.13.2). For continuous data, we first performed normality tests. A *t*-test or Mann–Whitney *U*-test was used for normally or non-normally distributed data as appropriate. Categorical variables were analyzed using the Chi-square test or Fisher's exact test, as appropriate. In this study, we focused on protein-coding genes identified based on Ensembl protein IDs from the Ensembl database using the biomaRt package (version 2.54.1) by referencing the human reference genome (GRCh38). We conducted differentially expressed gene (DEG) analysis using the edgeR package (version 4.0.16) with trimmed mean of M-values normalization, and lowly expressed genes were filtered using the built-in filterByExpr function. DEGs between asthma patients and healthy controls were identified by fitting the negative binomial generalized linear model using the glmQLFit function, followed by quasi-likelihood *F*-tests with the glmQLFTest function. We adjusted for age and sex in the ARMS DEGs analysis. We additionally adjusted for smoking status in the ATLANTIS DEGs analysis, since the cohort included current smokers. *p* values were adjusted for multiple testing using the Benjamini–Hochberg procedure to control false discovery rate (FDR). An FDR < 0.05 was considered statistically significant for the identification of asthma-associated genes in bronchial brushes. In nasal brushes from the ARMS study, no genes remained significant after FDR correction (FDR < 0.05). As the nasal brush DEGs analysis was primarily used to validate bronchial findings rather than to discover new genes. We therefore evaluated nasal DEGs using nominal *p*-values < 0.05 together with a concordant direction of effect relative to the bronchial DEGs.

2.4 | Weighted Gene Co-Expression Network Analysis (WGCNA)

We applied the WGCNA package (version 1.72-5) to identify modules of co-expressed genes in ARMS bronchial brushes. Module eigengenes (MEs), defined as the first principal component of each gene module, were calculated and correlated with asthma status to pinpoint asthma-associated gene modules. To explore the principal intramodular connectivity within each module, the Molecular Complex Detection (MCODE, version 2.0.2) plugin of Cytoscape (version 3.9.1) was then used to explore hub genes within each gene module. Details of network construction and hub genes' identification are described in the Supporting Information.

2.5 | Gene Set Variation Analysis (GSVA)

GSVA is a non-parametric, unsupervised method shifting focus from individual genes to gene modules by calculating GSVA composite scores for each module in each sample. This allows for the evaluation of gene module activity between asthma patients and healthy controls. Using GSVA package (version 1.50.0), we calculated composite scores of asthma-associated gene modules identified in bronchial brushes. This analysis generated a new matrix of GSVA scores for each of the bronchial and nasal expression datasets. We then performed statistical tests to identify shared gene modules differentially expressed between asthma patients and healthy controls in both ARMS bronchial and nasal brushes. The ARMS findings were subsequently validated in the ATLANTIS study to confirm the shared gene modules in both airways.

2.6 | Cell-Type Deconvolution

We used CIBERSORTx (https://cibersortx.stanford.edu/) to perform cell-type deconvolution for estimating the proportions of deconvoluted cell types in nasal and bronchial brush samples. We used counts per million (CPM) normalized ARMS RNA-seq data as input mixture file, while single-cell RNA-seq signature reference matrix files were from the Human Lung Cell Atlas (HLCA) [13] based on CPM normalized data. Deconvoluted cell types with a mean estimated abundance below 3% were excluded from further analysis. Subsequently, statistical comparisons of estimated cell proportions between asthma patients and healthy controls were performed to identify cell types with differential abundance. Details about the cell deconvolution process for the ATLANTIS can be found in the previous publication [9].

3 | Results

3.1 | Participants Characteristics

A total of 26 asthma patients and 28 healthy controls in the ARMS study have sufficient-quality RNA-seq data available from bronchial and/or nasal brushes. The results of the QC procedure are provided in the Supporting Information (Figure S1A,B). Their clinical characteristics are presented in Table 1. There were no statistically significant differences in age, sex, or BMI between the groups.

3.2 | Identification of Shared Asthma-Associated Genes in Both Bronchial and Nasal Brushes

In ARMS, we identified 51 asthma-associated genes in bronchial brushes (FDR < 0.05), of which 40 were up-regulated and 11 down-regulated in asthma compared to healthy controls (Figure 1). Among the 40 up-regulated genes in bronchial brushes, we found nine genes to be also up-regulated in nasal brushes from the ARMS study at a nominal *p*-value < 0.05,

13989995, 2026, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/all.70283 by University Of Technology Sydney, Wiley Online Library on [08/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 | Characteristics of ARMS participants.

	Asthma patients	Healthy controls	*p*
Number of participants (*n*)	26	28	—
Age (years)	54 [49, 61]	57 [50, 60]	0.729
BMI (kg/m^2)	26.6 [23.8, 31.4]	24.7 [23.2, 27.8]	0.083
Female/Male (*n*)	9/17	13/15	0.545
GINA treatment stage, *n* (%)			—
1	3 (11.5)	—	
2	7 (26.9)	—	
3	8 (30.8)	—	
4	8 (30.8)	—	
PC_{20} methacholine (mg/mL)	0.69 [0.29, 2.63]	> 16	< 0.001
Never/Ex-smoker (*n*)	3/23	17/11	0.044
Blood leukocytes (10^9/L)	5.94 (1.14)	5.68 (1.63)	0.503
Blood neutrophils (10^9/L)	3.42 (0.95)	3.38 (1.27)	0.906
Blood lymphocytes (10^9/L)	1.61 [1.33, 2.22]	1.62 [1.41, 1.82]	0.539
Blood monocytes (10^9/L)	0.50 (0.15)	0.45 (0.16)	0.239
Blood eosinophils (10^9/L)	0.21 [0.15, 0.40]	0.11 [0.08, 0.16]	< 0.001
Blood IgE (kU/L)	83.5 [45.8, 232.5]	26.5 [14.5, 61.5]	0.002
Sputum neutrophils (%)	60 (19)	57 (16)	0.634
Sputum lymphocytes (%)	2 [2, 4]	3 [2, 4]	0.4
Sputum macrophages (%)	25 [17, 41]	28 [25, 36]	0.474
Sputum eosinophils (%)	2 [1, 8]	1 [0, 1]	0.014
R5 (kPa·s/L)	0.42 [0.33, 0.53]	0.33 [0.27, 0.37]	0.001
R20 (kPa·s/L)	0.34 (0.05)	0.30 (0.06)	0.012
R5-R20 (kPa·s/L)	0.08 [0.03, 0.17]	0.03 [0.00, 0.05]	0.002
X5 (kPa·s/L)	−0.15 [−0.22, −0.10]	−0.09 [−0.12, −0.06]	0.009
AX (kPa/L)	0.88 [0.36, 2.00]	0.23 [0.11, 0.40]	< 0.001
FEV_1% predicted (%)	83 (15)	112 (11)	< 0.001
FEV_1/FVC (%)	63 (9)	76 (6)	< 0.001
RV/TLC (%)	35 [31, 40]	28 [26, 32]	< 0.001
FeNO (ppb)	30 [19, 40]	14 [11, 21]	0.012
Allergic status, *n* (%)			0.065
Allergic	18 (69.2)	11 (39.3)	
Non-allergic	6 (23.1)	15 (53.6)	
Missing	2 (7.7)	2 (7.1)	

Note: Data are presented as number (*n*), percentage (%), median [25th, 75th percentile], Mean (standard deviation, SD). PC20 methacholine, the provocative concentration of methacholine causing a 20% fall in FEV1 from baseline; R5, resistance of the respiratory system at 5 Hz; R20, resistance of the respiratory system at 20 Hz; R5–R20, difference between R5 and R20; X5, reactance of the respiratory system at 5 Hz; AX, area under the reactance curve from 5 Hz to the resonant frequency.
Abbreviations: BMI, body mass index; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; RV/TLC, residual volume/total lung capacity ratio.

a number that is nine times higher than expected by chance alone. Furthermore, seven of these nine up-regulated genes were also higher expressed in asthma in nasal brushes from the independent ATLANTIS study (p-value < 0.05): *CLCA1*, *FETUB*, *CST1*, *NTRK2*, *CDH26*, *TPSAB1*, and *DHX35*. Among the 11 down-regulated genes identified from ARMS bronchial

13969995, 2026, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/all.70283 by University Of Technology Sydney, Wiley Online Library on [08/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

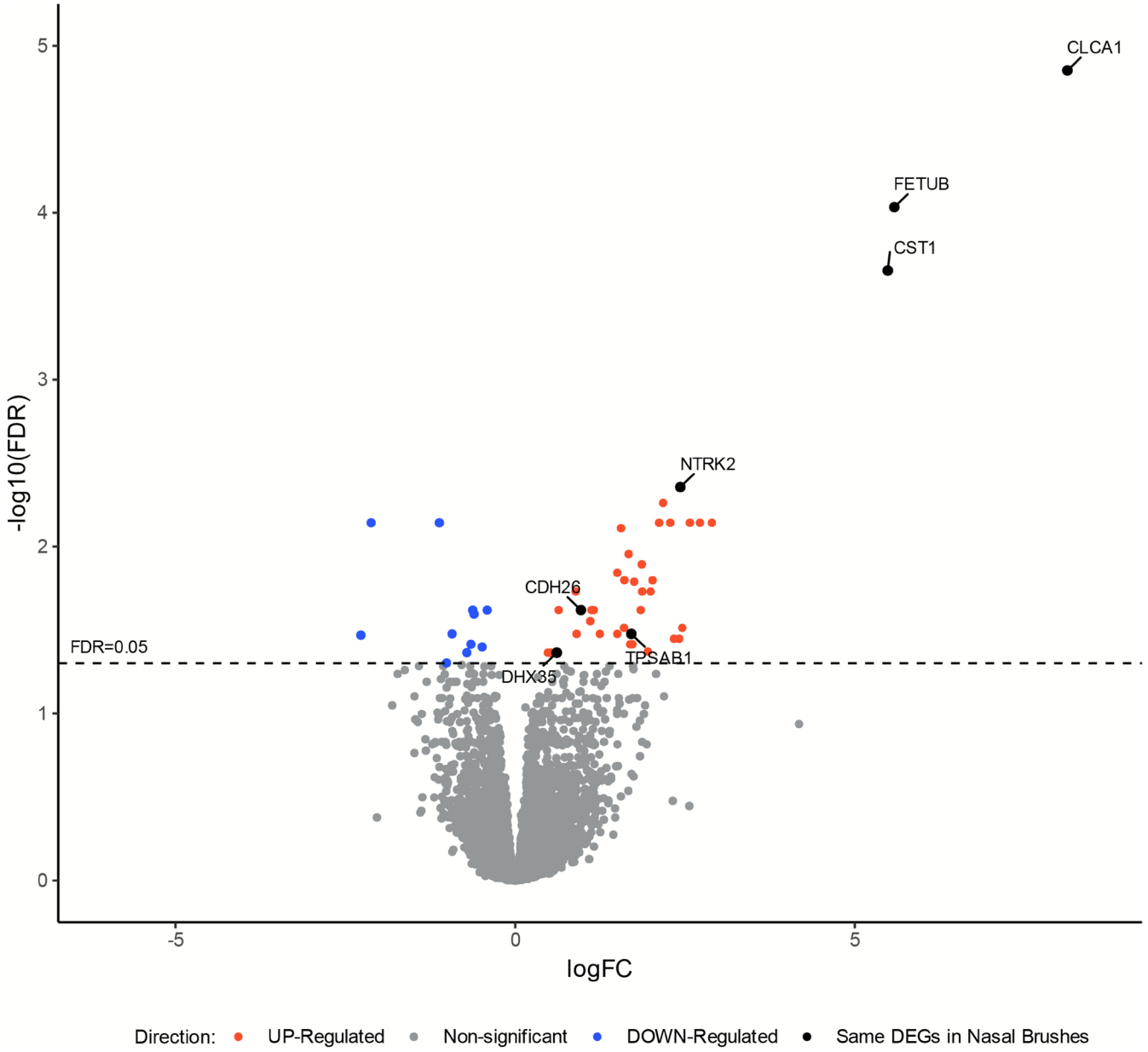


FIGURE 1 | Volcano plot illustrating DEGs in ARMS bronchial brushes comparing asthma patients to healthy controls. The red dots show the genes with higher expression in asthma versus healthy controls, while the blue dots show those with lower expression. The gray dots show genes not significantly different between the groups. The black dots indicate these seven genes with higher expression in asthma versus healthy controls in both ARMS bronchial and nasal brushes as well as in ATLANTIS nasal brushes.

brushes, none were down-regulated in ARMS nasal brushes and one was down-regulated in asthma in ATLANTIS: *EPAS1* (Table S1).

We applied GSVA to calculate GSVA composite scores based on the 40 up- and 11 down-regulated genes from ARMS bronchial brushes. As shown in Figure 2, the GSVA scores of the up-regulated genes were higher in asthma compared to healthy controls in both ARMS bronchial and nasal brushes (p-value <0.0001 and p-value $=0.0073$, respectively) with validation in ATLANTIS nasal brushes (p-value $=0.0019$). For the down-regulated genes, GSVA scores were lower in asthma than in healthy controls from ARMS bronchial brushes (p-value <0.0001), while no significant difference was detected in nasal brushes from either ARMS (p-value $=0.83$) or ATLANTIS (p-value $=0.079$).

3.3 | Identification of WGCNA Modules Consistently Altered in Asthma Across the Lower and Upper Airways

Gene expression patterns in biological systems are often characterized by co-regulated modules of genes that in concert contribute to a certain biological activity. Therefore, we assessed which gene modules were associated with asthma in bronchial brushes. Using WGCNA, we identified 19 asthma-associated modules in ARMS bronchial brushes, with seven modules positively associated with asthma and 12 modules negatively associated with asthma compared to healthy controls (Figure S3).

GSVA was used to quantify the activity of all 19 asthma-associated modules in ARMS bronchial and nasal brushes as

13989995, 2026, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/all.70283 by University Of Technology Sydney, Wiley Online Library on [08/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

well as in ATLANTIS nasal brushes (Figure S4–S6). Among the 19 gene modules, two modules (the IL-13 related inflammation module and the mast cell activity module) consistently exhibited higher GSVA scores in asthma in both ARMS bronchial brushes (p-value = 0.0026 and p-value = 0.00013, respectively) and nasal brushes (p-value = 0.01 and p-value = 0.015, respectively), as well as in the nasal brushes from the independent ATLANTIS study (p-value = 0.0021 and p-value = 0.014, respectively) (Figure 3).

To gain deep insight into the biological relevance of these gene modules, we identified hub genes within each asthma-associated module from WGCNA using the MCODE plugin in Cytoscape (refer to Supporting Information for details). Notably, the hub genes in the IL-13 related inflammation module were *IL13*, *BMP8A*, *CST1*, *CDH26*, *FETUB*, *CEACAM21*, *C1QTNF1*, *CLCA1*, *SERPINB10*, *PTCHD4*, *CCL26*, *NTRK2*, and *GSN*. The hub genes for the mast cell activity module included *TPSAB1*, *TPSB2*, *SIGLEC6*, *IL1RL1*, *CPA3*, *GATA2*, *SLC18A2*, *GCSAML*, *MS4A2*, *CDK15*, and *CTSG*. The lists of hub genes for each of 19 modules are shown in Table S3.

3.4 | Cell-Type Deconvolution

We used CIBERSORTx to test whether the cellular composition was estimated to be different based on bulk RNA-seq data, using single cell references from the HLCA [13]. No significant differences were observed in the estimated proportions of deconvoluted cell types between asthma patients and healthy controls in ARMS bronchial brush samples, including basal, monocytes, and multiciliated cells (Figure S7A). Similarly, ARMS nasal brush samples showed no significant differences in the estimated proportions of deconvoluted cell types, including basal, club, goblet, and multiciliated cells (Figure S7B). Consistently, the previous ATLANTIS study reported no significant differences in deconvoluted cell-type abundance between asthma patients and healthy controls [9].

4 | Discussion

In this study, we demonstrate that seven genes and two WGCNA modules are consistently elevated in asthma across both the lower and upper airways. Notably, these two gene modules

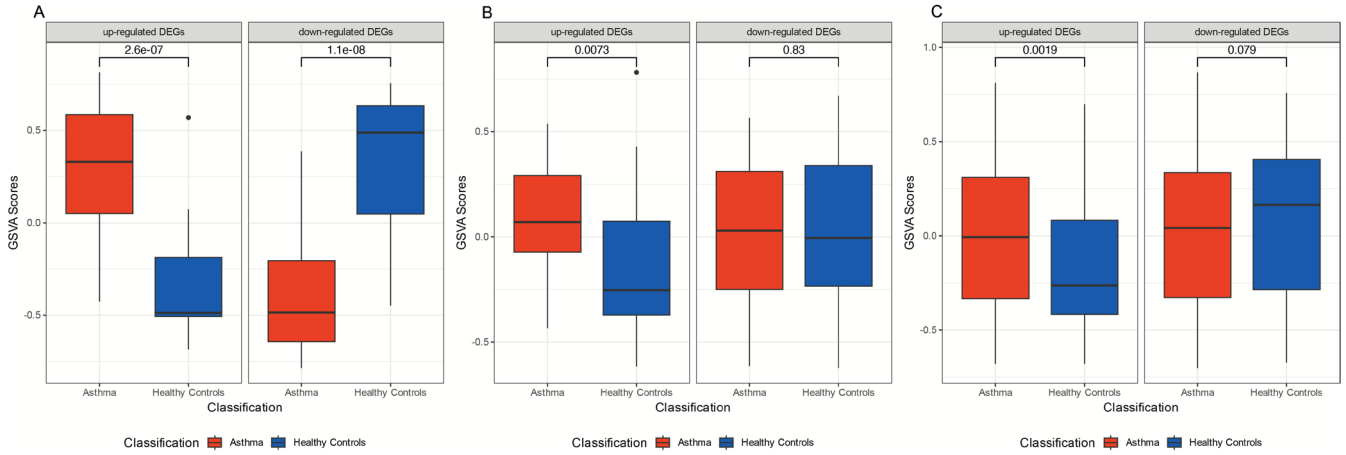


FIGURE 2 | Box plots showing comparisons of GSVA scores for up- and down-regulated genes between asthma patients and healthy controls in (A) ARMS bronchial brushes and (B) ARMS nasal brushes, with validation in (C) ATLANTIS nasal brushes. DEGs, differentially expressed genes.

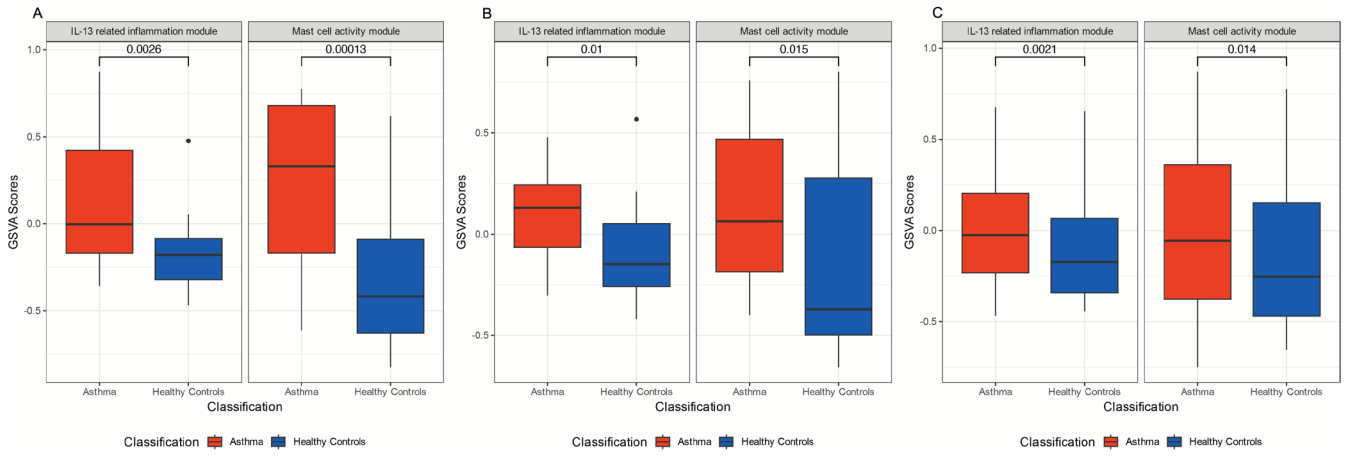


FIGURE 3 | Box plots showing comparisons of GSVA scores for IL-13 related inflammation and mast cell activity modules between asthma patients and healthy controls in (A) ARMS bronchial brushes and (B) ARMS nasal brushes, with validation in (C) ATLANTIS nasal brushes.

13989995, 2026, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/all.70283 by University Of Technology Sydney, Wiley Online Library on [08/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

capture distinct components of T2 inflammation, with one module representing IL-13 related inflammation and the other predominantly consisting of genes highlighting mast cell activity. Together, our findings indicate that selected nasal gene expression profiles reflect specific asthma-associated molecular mechanisms relevant to disease pathogenesis in the lower airways.

Among the seven genes associated with asthma in both bronchial and nasal brushes are *NTRK2* and *DHX35*. Dragunas et al. showed that increased *NTRK2* expression was detected in asthma patients, paralleled by increased cholinergic nerve density possibly contributing to AHR [14]. Furthermore, we found that *DHX35* was up-regulated in bronchial and nasal brushes from asthma patients compared to healthy controls. In this context, the finding of Qiao et al. is of interest. They found that *DHX35* overexpression in human airway epithelial cells increased production of IFN-β, IL-6, and CXCL10, thereby modulating antiviral innate immunity [15]. Together, our findings show that the more easily accessible selected nasal gene expression profiles reflect biologically relevant changes associated with asthma in the lower airways.

Using WGCNA, we identified two distinct gene modules associated with asthma. The first gene module was the IL-13 related inflammation module. The hub genes of this module include *IL13*, *CLCA1*, *FETUB*, *CCL26*, *CDH26*, and *SERPINB10*, all associated with IL-13 driven inflammation [16–21]. IL-13 is a central cytokine in T2 asthma pathophysiology, driving eosinophilic inflammation, mucus secretion, and AHR [16, 22]. Previous studies reported elevated *CLCA1* mRNA expression in airway epithelial cells of asthma patients [4]. Activation of the IL-13 receptor upregulates *CLCA1* expression, whose protein fragments subsequently stimulate MAPK13 and promote *MUC5AC* expression, contributing to mucus production in airway epithelial cells [17]. *FETUB* has also been reported to be induced by IL-13 stimulation in 16HBE cells [18]. Lung-specific knockdown of *FETUB* has been shown to reduce airway inflammation and AHR in asthma [18]. Furthermore, IL-13 upregulates *CST1* and *CDH26* expression in 16HBE cells or bronchial epithelial cells [20, 23]. Du et al. demonstrated that epithelial *CST1* can stimulate airway eosinophilic inflammation in asthma [24]. Additionally, findings from the MESOS randomized controlled trial showed that elevated FeNO levels are associated with high *CST1* expression [25]. *CDH26* has been shown to amplify the IL-4 receptor signaling pathway, enhancing mucin gene expression and eosinophilic chemokines expression in airway epithelial cells [20]. IL-13 additionally promotes the production of *CCL26* and *SERPINB10* in epithelial cells, both associated with airway eosinophilia [19, 21]. Consequently, our finding of the increased expression of the IL-13 related inflammation module genes in asthma may be useful as a non-invasive biomarker to identify IL-13 related T2 high asthma in the lower airways.

The second gene module was the mast cell activity module. Hub genes of this module included *TPSAB1*, *TPSB2*, *SIGLEC6*, *IL1RL1*, *GATA2*, *CPA3*, *SLC18A2*, *GCSAML*, *MS4A2*, and *CTSG*, all associated with mast cell activity. Mast cells release a variety of mediators—including cytokines, lipid mediators, and proteases—that contribute to inflammation by promoting immune cell recruitment, disrupting airway structure, and driving airway remodeling [26]. *TPSAB1* encodes either α or β-tryptases, while *TPSB2* encodes β-tryptases including βI-, βII-, or βIII-tryptase isoforms [27, 28]. Meanwhile, β-tryptases stimulate airway smooth muscle proliferation and contraction [27]. In addition, *SIGLEC6* is involved in IgE and non-IgE-mediated mast cell activation [29]. Mast cells express *IL1RL1*. It encodes the receptor for IL-33, a cytokine that modulates type-2 immune responses [30]. *GATA2* is a key transcription factor that regulates *IL1RL1* expression in mast cells [30]. *CPA3* was increased in intraepithelial mast cells of patients with T2-high asthma [31]. In murine models, *CPA3* has been shown to convert angiotensin I into angiotensin II, contributing to airway bronchoconstriction and the enhanced AHR in asthma [31]. The solute transporter, encoded by *SLC18A2*, is involved in the loading of histamine into secretory vesicles, with mast cells being a primary source of histamine [32]. *MS4A2* encodes the β-subunit of FcεRI (FcεRIβ), which facilitates the trafficking of the FcεRI receptor complex to the cell surface and amplifies IgE-mediated mast cell signaling [33]. Both *GCSAML* and *CTSG* are mast- cell associated genes [34]. Our findings support the role of mast cell-related genes in airway inflammation and highlight the potential of the nasal mast cell activity gene module as a non-invasive biomarker for mast cell activity in the lower airways. The identification of two distinct gene modules, reflecting IL-13 related inflammation and mast cell activity is particularly noteworthy because they both represent key, yet separate components of T2 inflammation.

This study has several limitations. First, the relatively small sample size limits our ability to identify specific nasal gene signatures that can reliably reflect bronchial gene expression profiles across diverse asthma subgroups, such as T2 high and T2 low asthma or allergic and non-allergic asthma. Larger, well-characterized cohorts are needed to validate nasal surrogates for bronchial gene signatures, particularly across different asthma subtypes. Additionally, this study utilized bulk RNA sequencing, which lacks the resolution to pinpoint substitute nasal brush genes at the specific cell-type level. Although we observed mast cell-related genes in the upper airways reflecting those of the lower airways, the relationship between mast cell abundance in the two compartments remains unclear. Future studies employing single-cell RNA sequencing of paired nasal and bronchial samples could provide more detailed insights into cell-type specific gene expression.

In conclusion, nasal brush samples can capture selected asthma-associated transcriptomic profiles that reflect those of bronchial brushes. This non-invasive approach could serve as a promising tool to evaluate lower airway inflammation, ultimately contributing to personalized asthma management.

Author Contributions

H.W. performed DEGs and WGCNA analyses; drafted and finalized the manuscript. T. Kole, T. Karp, V.G. performed data management and analysis and edited the manuscript. A.F., O.A.C., K.F.C., P.B., I.M.A., S.S., A.L., K.L.R., N.Z.-K. provided technical guidance and expertise and edited the manuscript. C.B., D.S., J.K., M.K., B.B., K.F.R., A.P. provided guidance and support for the validation analyses. M.N.H., M.C.N., M.B. performed study design. M.B. also drafted sections of the manuscript. All authors approved the final manuscript.

13989995, 2026, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/all.70283 by University Of Technology Sydney, Wiley Online Library on [08/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Funding

This study was supported by Dutch Foundation for Asthma Prevention, GlaxoSmithKline, and the Dutch Ministry of Health via the Public-Private Partnership Program.

Conflicts of Interest

H.W., T. Kole, O.A.C., V.G., A.F., A.L., K.L.R., N.Z.-K., J.K., B.B. and M.N.H. declare no conflicts of interest. T. Karp. reports travel support from Chiesi to attend the ERS 2024 Congress in Vienna. K.F.C. reports institutional research grants from the MRC, EPSRC, GSK, and NIEHS; speaking honoraria from GSK, Novartis, and AstraZeneca; participation on advisory boards for GSK, AstraZeneca, Novartis, Roche, Merck, Trevi, Reckitt-Benckiser, Nocion, and Shionogi on asthma, COPD, and chronic cough; and service on the Scientific Advisory Board of the Clean Breathing Institute supported by Haleon. P.B. reports institutional research grants from the MRC, Merck, GSK, and CSA Medical (USA), and an ISS Award from GSK; and payments for lectures from AstraZeneca and GSK. I.M.A. reports support for the present manuscript from the U-BIOPRED programme; institutional research grants from GSK, the MRC, the EPSRC, and Sanofi; consulting fees from GSK; payments for lectures from AstraZeneca and GSK; participation on advisory boards for Kinaset, GSK, and Sanofi; and a leadership role as Head of the Asthma Section at the European Academy of Allergy and Clinical Immunology (EAACI). S.S. reports support for the ATLANTIS study funded by Chiesi; grants from AstraZeneca for research in severe asthma and EGPA; consulting fees from AstraZeneca, GSK, Chiesi, Areteia Therapeutics, and Sanofi; payments for lectures from AstraZeneca, GSK, Chiesi, and Medscape; travel support from AstraZeneca, Chiesi, and GSK; and patents related to breath biomarkers in asthma. C.B. reports support for the ATLANTIS study funded by Chiesi; institutional research grants from Areteia, AstraZeneca, Chiesi, Genentech, GSK, Regeneron Pharmaceuticals, Roche, and Sanofi; and consulting fees from the same companies. D.S. reports consulting fees from Adovate, Aerogen, Almirall, Apogee, Arrowhead, AstraZeneca, Bial, Boehringer Ingelheim, Chiesi, Cipla, CONNECT Biopharm, Covis, CSL Behring, DevPro Biopharma LLC, Elpen, Empirico, EpiEndo, Genentech, Generate Biomedicines, GlaxoSmithKline, Glenmark, Kamada, Kinaset Therapeutics, Kymera, Menarini, MicroA, OM Pharma, Orion, Pieris Pharmaceuticals, Pulmatrix, Revolo, Roivant Sciences, Sanofi, Synairgen, Tetherex, Teva, Theravance Biopharma, Upstream, and Verona Pharma. M.K. reports an institutional research grant from NIH NIAID; consulting fees and lecture payments from Sanofi, Regeneron, Chiesi, AstraZeneca, Kinaset, and Genentech; issued and pending patents related to surfactant protein A peptidomimetics; leadership roles with the Association of Professors of Medicine and the Alliance for the Advancement of Internal Medicine (AAIM); stock in RaeSedo Inc.; and service as a section editor for UpToDate for severe asthma. K.F.R. reports consulting fees from AstraZeneca, Boehringer Ingelheim, Sanofi & Regeneron, and Chiesi, outside the submitted work; payments for lectures from AstraZeneca, Boehringer Ingelheim, Chiesi Pharmaceuticals, CSL Behring, Sanofi & Regeneron, GlaxoSmithKline, Berlin Chemie, and Menarini; participation on advisory boards for AstraZeneca, Boehringer Ingelheim, Sanofi & Regeneron, and CSL Behring; and leadership roles in the German Center for Lung Research (DZL), the German Chest Society (DGP), and the American Thoracic Society (ATS). A.P. reports support for the ATLANTIS study funded by Chiesi; institutional research grants from AstraZeneca, Chiesi, GlaxoSmithKline, and Sanofi; consulting fees from AstraZeneca, Avillion, Chiesi, GlaxoSmithKline, Moderna, Regeneron, Roche, Sanofi, Zambon, and Zentiva; and payments for lectures from AstraZeneca, Avillion, Chiesi, Regeneron, Moderna, GlaxoSmithKline, Roche, Sanofi, and Zambon. M.C.N. reports institutional research grants from AstraZeneca, Roche, Sanofi, and Boehringer Ingelheim; payments for lectures from AstraZeneca; travel support from AstraZeneca and GSK; participation on an advisory board for GSK; and an unpaid leadership role as Coordinator of the Lung Bionetwork within the Human Cell Atlas. M.B. reports institutional grants from GlaxoSmithKline, Chiesi, Genentech, Roche, AstraZeneca.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. B. N. Lambrecht, H. Hammad, and J. V. Fahy, "The Cytokines of Asthma," *Immunity* 50, no. 4 (2019): 975–991, https://doi.org/10.1016/j.immuni.2019.03.018.

2. M. E. Kuruvilla, F. E. Lee, and G. B. Lee, "Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease," *Clinical Reviews in Allergy and Immunology* 56, no. 2 (2019): 219–233, https://doi.org/10.1007/s12016-018-8712-1.

3. A. Ray, J. Das, and S. E. Wenzel, "Determining Asthma Endotypes and Outcomes: Complementing Existing Clinical Practice With Modern Machine Learning," *Cell Reports Medicine* 3, no. 12 (2022): 100857, https://doi.org/10.1016/j.xcrm.2022.100857.

4. P. G. Woodruff, H. A. Boushey, G. M. Dolganov, et al., "Genome-Wide Profiling Identifies Epithelial Cell Genes Associated With Asthma and With Treatment Response to Corticosteroids," *Proceedings of the National Academy of Sciences of the United States of America* 104, no. 40 (2007): 15858–15863, https://doi.org/10.1073/pnas.0707413104.

5. C. S. Kuo, S. Pavlidis, M. Loza, et al., "A Transcriptome-Driven Analysis of Epithelial Brushings and Bronchial Biopsies to Define Asthma Phenotypes in U-BIOPRED," *American Journal of Respiratory and Critical Care Medicine* 195, no. 4 (2017): 443–455, https://doi.org/10.1164/rccm.201512-2452OC.

6. B. D. Modena, J. R. Tedrow, J. Milosevic, et al., "Gene Expression in Relation to Exhaled Nitric Oxide Identifies Novel Asthma Phenotypes With Unique Biomolecular Pathways," *American Journal of Respiratory and Critical Care Medicine* 190, no. 12 (2014): 1363–1372, https://doi.org/10.1164/rccm.201406-1099OC.

7. K. Imkamp, M. Berg, C. J. Vermeulen, et al., "Nasal Epithelium as a Proxy for Bronchial Epithelium for Smoking-Induced Gene Expression and Expression Quantitative Trait Loci," *Journal of Allergy and Clinical Immunology* 142, no. 1 (2018): 314–317.e15, https://doi.org/10.1016/j.jaci.2018.01.047.

8. A. Poole, C. Urbanek, C. Eng, et al., "Dissecting Childhood Asthma With Nasal Transcriptomics Distinguishes Subphenotypes of Disease," *Journal of Allergy and Clinical Immunology* 133, no. 3 (2014): 670–678.e12, https://doi.org/10.1016/j.jaci.2013.11.025.

9. T. Karp, A. Faiz, J. van Nijnatten, et al., "Nasal Epithelial Gene Expression Identifies Relevant Asthma Endotypes in the ATLANTIS Study," *Thorax* 79, no. 10 (2024): 905–914, https://doi.org/10.1136/thorax-2023-221230.

10. I. M. Boudewijn, A. Faiz, K. Steiling, et al., "Nasal Gene Expression Differentiates COPD From Controls and Overlaps Bronchial Gene Expression," *Respiratory Research* 18, no. 1 (2017): 213, https://doi.org/10.1186/s12931-017-0696-5.

11. K. Imkamp, V. Bernal, M. Grzegorzcyk, et al., "Gene Network Approach Reveals Co-Expression Patterns in Nasal and Bronchial Epithelium," *Scientific Reports* 9, no. 1 (2019): 15835, https://doi.org/10.1038/s41598-019-50963-x.

12. D. S. Postma, C. Brightling, S. Baldi, et al., "Exploring the Relevance and Extent of Small Airways Dysfunction in Asthma (ATLANTIS): Baseline Data From a Prospective Cohort Study," *Lancet Respiratory Medicine* 7, no. 5 (2019): 402–416, https://doi.org/10.1016/s2213-2600(19)30049-9.

13. L. Sikkema, C. Ramírez-Suástegui, D. C. Strobl, et al., "An Integrated Cell Atlas of the Lung in Health and Disease," *Nature Medicine* 29, no. 6 (2023): 1563–1577, https://doi.org/10.1038/s41591-023-02327-2.

13969995, 2026, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/all.70283 by University Of Technology Sydney, Wiley Online Library on [08/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

14. G. Dragunas, M. E. Woest, S. Nijboer, et al., "Cholinergic Neuroplasticity in Asthma Driven by TrkB Signaling," *FASEB Journal* 34, no. 6 (2020): 7703–7717, https://doi.org/10.1096/fj.202000170R.

15. Y. Qiao, S. Zhu, N. Yang, et al., "The RNA Helicase DHX35 Functions as a Co-Sensor for RIG-I-Mediated Innate Immunity," *PLoS Pathogens* 20, no. 7 (2024): e1012379, https://doi.org/10.1371/journal.ppat.1012379.

16. T. A. Wynn, "IL-13 Effector Functions," *Annual Review of Immunology* 21 (2003): 425–456, https://doi.org/10.1146/annurev.immunol.21.120601.141142.

17. Y. G. Alevy, A. C. Patel, A. G. Romero, et al., "IL-13-Induced Airway Mucus Production Is Attenuated by MAPK13 Inhibition," *Journal of Clinical Investigation* 122, no. 12 (2012): 4555–4568, https://doi.org/10.1172/jci64896.

18. P. Sun, Q. Hua, H. Fu, et al., "Epithelial FETUB-Mediated the Inhibition of NEP Activity Aggravates Asthma," *International Immunopharmacology* 152 (2025): 114397, https://doi.org/10.1016/j.intimp.2025.114397.

19. M. C. Larose, J. Chakir, A. S. Archambault, et al., "Correlation Between CCL26 Production by Human Bronchial Epithelial Cells and Airway Eosinophils: Involvement in Patients With Severe Eosinophilic Asthma," *Journal of Allergy and Clinical Immunology* 136, no. 4 (2015): 904–913, https://doi.org/10.1016/j.jaci.2015.02.039.

20. Y. Feng, S. Chen, L. Xiong, et al., "Cadherin-26 Amplifies Airway Epithelial IL-4 Receptor Signaling in Asthma," *American Journal of Respiratory Cell and Molecular Biology* 67, no. 5 (2022): 539–549, https://doi.org/10.1165/rcmb.2021-0109OC.

21. Y. Mo, K. Zhang, Y. Feng, et al., "Epithelial SERPINB10, a Novel Marker of Airway Eosinophilia in Asthma, Contributes to Allergic Airway Inflammation," *American Journal of Physiology. Lung Cellular and Molecular Physiology* 316, no. 1 (2019): L245–l254, https://doi.org/10.1152/ajplung.00362.2017.

22. A. Azim, B. Green, L. Lau, et al., "Peripheral Airways Type 2 Inflammation, Neutrophilia and Microbial Dysbiosis in Severe Asthma," *Allergy* 76, no. 7 (2021): 2070–2078, https://doi.org/10.1111/all.14732.

23. L. Yao, X. Yuan, H. Fu, et al., "Epithelium-Derived Cystatin SN Inhibits House Dust Mite Protease Activity in Allergic Asthma," *Allergy* 78, no. 6 (2023): 1507–1523, https://doi.org/10.1111/all.15739.

24. L. Du, C. Xu, K. Tang, et al., "Epithelial CST1 Promotes Airway Eosinophilic Inflammation in Asthma via the AKT Signaling Pathway," *Allergy, Asthma & Immunology Research* 15, no. 3 (2023): 374–394, https://doi.org/10.4168/aair.2023.15.3.374.

25. S. Diver, S. Sridhar, L. C. Khalfaoui, et al., "Feno Differentiates Epithelial Gene Expression Clusters: Exploratory Analysis From the MESOS Randomized Controlled Trial," *Journal of Allergy and Clinical Immunology* 150, no. 4 (2022): 830–840, https://doi.org/10.1016/j.jaci.2022.04.024.

26. G. H. Banafea, S. Bakhashab, H. F. Alshaibi, P. Natesan Pushparaj, and M. Rasool, "The Role of Human Mast Cells in Allergy and Asthma," *Bioengineered* 13, no. 3 (2022): 7049–7064, https://doi.org/10.1080/21655979.2022.2044278.

27. J. J. Lyons and T. Yi, "Mast Cell Tryptases in Allergic Inflammation and Immediate Hypersensitivity," *Current Opinion in Immunology* 72 (2021): 94–106, https://doi.org/10.1016/j.coi.2021.04.001.

28. J. Chovanec, I. Tunc, J. Hughes, et al., "Genetically Defined Individual Reference Ranges for Tryptase Limit Unnecessary Procedures and Unmask Myeloid Neoplasms," *Blood Advances* 7, no. 9 (2023): 1796–1810, https://doi.org/10.1182/bloodadvances.2022007936.

29. W. Korver, Z. Benet, A. Wong, et al., "Regulation of Mast Cells by Overlapping but Distinct Protein Interactions of Siglec-6 and Siglec-8," *Allergy* 79, no. 3 (2024): 629–642, https://doi.org/10.1111/all.16004.

30. Y. Baba, K. Maeda, T. Yashiro, et al., "GATA2 Is a Critical Transactivator for the Human IL1RL1/ST2 Promoter in Mast Cells/Basophils: Opposing Roles for GATA2 and GATA1 in Human IL1RL1/ST2 Gene Expression," *Journal of Biological Chemistry* 287, no. 39 (2012): 32689–32696, https://doi.org/10.1074/jbc.M112.374876.

31. R. H. Dougherty, S. S. Sidhu, K. Raman, et al., "Accumulation of Intraepithelial Mast Cells With a Unique Protease Phenotype in T(H)2-High Asthma," *Journal of Allergy and Clinical Immunology* 125, no. 5 (2010): 1046–1053.e8, https://doi.org/10.1016/j.jaci.2010.03.003.

32. D. F. Dwyer, N. A. Barrett, and K. F. Austen, "Expression Profiling of Constitutive Mast Cells Reveals a Unique Identity Within the Immune System," *Nature Immunology* 17, no. 7 (2016): 878–887, https://doi.org/10.1038/ni.3445.

33. A. Olivera, M. A. Beaven, and D. D. Metcalfe, "Mast Cells Signal Their Importance in Health and Disease," *Journal of Allergy and Clinical Immunology* 142, no. 2 (2018): 381–393, https://doi.org/10.1016/j.jaci.2018.01.034.

34. E. Motakis, S. Guhl, Y. Ishizu, et al., "Redefinition of the Human Mast Cell Transcriptome by Deep-CAGE Sequencing," *Blood* 123, no. 17 (2014): e58–e67, https://doi.org/10.1182/blood-2013-02-483792.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** all70283-sup-0001-Supinfo.docx. **Table S2:** Overview of the 19 asthma-associated WGCNA modules.

13969995, 2026, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/all.70283 by University Of Technology Sydney, Wiley Online Library on [08/10/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License