Clinical Bioinformatics Analysis of Airways Inflammation in Asthma

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Abstract

Asthma is a prevalent and heterogeneous respiratory disease that imposes a substantial global burden. This thesis investigated three key factors that contribute to airways inflammation in asthma: type 2 (T2) inflammatory phenotypes, bacterial infections, and sex differences, utilising large-scale clinical data and multi-omics analyses in patients with asthma.

In the RASP-UK cohort, transcriptomic profiling of bronchial biopsy and brush samples from 52 severe asthmatics and 20 healthy controls demonstrated that corticosteroid-resistant T2-high asthma showed upregulation of T2-dependent genes, adaptive immune response, impaired ciliary function, and epithelial development, whereas T2-low asthma showed upregulation of Th1- and IL-17-associated genes, neuroimmune and interferon-γ signalling pathways, and enrichment of neutrophils and mast cells. T2-intermediate asthma displayed a mixed molecular profile between T2-high and T2-low endotypes with activation of pathogen-defence pathways and enrichment of mast and NK cells.

A 27-year retrospective analysis of 4,350 sputum cultures from 1,106 patients with asthma revealed that 31.4% of routine samples were culture-positive, with *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Moraxella catarrhalis* being the most frequently isolated pathogens. 45.8% of isolated bacteria were antibiotic-

resistant, with multidrug resistance common in *Staphylococcus aureus* and *Escherichia coli*. The temporal microbial succession was observed within patients, where *H. influenzae* was replaced by *P. aeruginosa*. Factors independently associated with positive sputum cultures included older age, higher neutrophils, and use of fluticasone propionate, but not beclomethasone or budesonide.

Sex-specific analyses of 568 adults in the U-BIOPRED cohort revealed distinct clinical and molecular patterns in asthma. In mild/moderate asthma, clinical characteristics were largely comparable, although women exhibited a higher prevalence of non-type 2 phenotypes. In severe asthma, men exhibited more pronounced airflow limitation, reflected by lower FEV1 and greater residual volume, while women reported higher symptom burden and exacerbations, alongside enhanced airway remodelling, respiratory epithelial mitochondrial dysfunction, and heightened immune activation. Microbiome profiling indicated a higher prevalence of pathogenic bacteria (*Haemophilus*, *Moraxella*, *Pseudomonas*) in women with severe asthma. Circulating androgen levels were inversely associated with asthma severity in both sexes, suggesting a protective role.

In conclusion, this thesis characterised the roles and mechanisms of these three factors in airways inflammation with advanced clinical data analysis approaches, providing insights for informed personalised management in asthma.

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Abbreviations

Abbreviation	Full name
ACQ	Asthma Control Questionnaire
AHR	Airway hyperresponsiveness
ANOVA	Analysis of variance
ASM	Airway smooth muscle
AQLQ	Asthma Quality of Life Questionnaire
BDP eq	Beclometasone dipropionate equivalent
BHR	Bronchial hyperresponsiveness
BHS	*β-haemolytic streptococci*
BMI	Body mass index
BTS	British Thoracic Society
CI	Confidence interval
CT	Computed tomography
COPD	Chronic obstructive pulmonary disease
CPRD	Clinical Practice Research Datalink
CRP	C-reactive protein
DALYs	Disability-adjusted life years
DAMs	Differentially abundant metabolites
DEGs	Differentially expressed genes
DEPs	Differentially expressed proteins
E. coli	*Escherichia coli*
eHRs	Electronic health records
E. cloacae	*Enterobacter cloacae*
EQTLs	Expression quantitative trait alleles
ER	Emergency room
ESS	Epworth Sleepiness Scale
EWAS	Epigenome-wide association studies
FDR	False discovery rate
FeNO	Fractional exhaled nitric oxide
$FEF_{25-75\%}$	Forced expiratory flow at 25% and 75% of the pulmonary volume
FER	Forced expiratory ratio
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity

Abbreviation	Full name
GEO	Gene Expression Omnibus
GINA	Global Initiative for Asthma
GO	Gene Ontology
GOLD	Global Initiative for Chronic Lung Disease
GORD	Gastro-oesophageal reflux disease
GR	Glucocorticoid receptor
GSEA	Gene set enrichment analysis
GSVA	Gene set variation analysis
GWAS	Genome-wide association studies
HADS	Hospital Anxiety and Depression Scale
H. influenza	*Haemophilus influenzae*
HLA	Human leukocyte antigen
HU	Hounsfield units
HVS	Healthy Volunteer Study
ICS	Inhaled corticosteroids
ICU	Intensive care unit
Ig	Immunoglobulin
IL	Interleukin
ILC2	Type 2 innate lymphoid cell
IMD	Index of Multiple Deprivation
IORD	The Infections in Oxfordshire Research Database
IQR	Interquartile range
Klebsiella spp.	*Klebsiella* species
LAA	Low attenuation areas
LABA	Long-acting $Beta_2$ agonist
LAMA	Long-acting muscarinic antagonist
LLMs	Large langauge models
LOD	Limit of detection
LTRA	Leukotriene receptor antagonist
mAB	Monoclonal antibody
MARS	Medication Adherence Rating Scale
MART	Maintenance and relief therapy (with ICS-formoterol)
M. catarrhalis	*Moraxella catarrhalis*
MCID	Minimal clinically important difference

Abbreviation	Full name
MLD	Mean lung density
MLLMs	Multimodal large language models
Mtb	*Mycobacterium tuberculosis*
NA	Not applicable
NCT	National Clinical Trial
NK cell	Natural killer cell
NLP	Natural language processing
NTM	Non-tuberculous *mycobacteria*
NS	Not significant
OCS	Oral corticosteroids
OUH	Oxford University Hospitals
PC_{20}	Provocative concentration causing 20% fall in FEV_1
PEF	Peak Expiratory Flow
PFP	Proportion of false positives
P. mirabilis	*Proteus mirabilis*
P. aeruginosa	*Pseudomonas aeruginosa*
PBMCs	Peripheral blood mononuclear cells
RASP	Refractory Asthma Stratification Programme
RAST	Radioallergosorbent test
RCT	Randomised controlled trial
REC	Research Ethics Committee
RNA-seq	RNA sequencing
RP/Rsum	Rank product / rank sum
SABA	Short-acting $beta_2$ agonist
SARP	Severe Asthma Research Program
S. aureus	*Staphylococcus aureus*
scRNA-seq	Single-cell RNA sequencing
SD	Standard deviation
sGAW	Specific airway conductance
S. marcescens	*Serratia marcescens*
SNOT	Sino-Nasal Outcome Test
S. maltophilia	*Stenotrophomonas maltophilia*
S. pneumoniae	*Streptococcus pneumoniae*
T2	Type 2 cytokines

Abbreviation	Full name
TCR	T-cell receptor
TFs	Transcription factors
Th2	T-helper type 2
THEO	Theophylline
TLC	Total lung capacity
WGCNA	Weighted gene co-expression network analysis
U-BIOPRED	Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes
VST	Variance stabilising transformation

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Chapter 1 General Introduction

1.1 Asthma: an overview

1.1.1 Definition and clinical characteristics

The Global Initiative for Asthma (GINA) defines asthma as ‘a heterogeneous disease, typically characterised by chronic airways inflammation with a medical history of respiratory symptoms and variable expiratory airflow limitation’ (GINA, 2024).

Asthma is a syndrome in which patients experience a specific constellation of symptoms. Symptoms are typically classified into three domains: respiratory symptoms (e.g., dyspnoea, wheezing), chest symptoms (e.g., chest tightness), and cough-related symptoms (e.g., coughing with mucus or phlegm production) (GINA, 2024). These symptoms often present with marked variability in frequency and intensity. They can be triggered by multiple factors, including exercise, allergen exposure, viral respiratory tract infections, environmental pollutants (such as tobacco smoke or particulate matter), and changes in weather or air temperature (Vernon et al., 2012). Circadian variation is also well recognised, with many patients reporting symptom worsening at night or the early morning (Gautier & Charpin, 2017). Physical examination may reveal expiratory wheeze, reduced breath sounds, or prolonged expiration, although these signs are often absent during asymptomatic periods. Such episodic and reversible patterns of symptoms and signs are central clinical features of asthma (Almond & Chung, 2018) (Table 1.1).

Assessment of asthma symptoms is essential for evaluating disease control and guiding management. Validated patient-reported outcome measures (PROMs) are widely used to standardise symptom evaluation (Bonini et al., 2020). The Asthma Control Questionnaire (ACQ) assesses key symptoms and rescue medication use over the previous week, scored 0–6, with lower values indicating better control (≤0.75 well controlled, ≥1.5 poorly controlled), and the minimal clinically important difference (MCID) is 0.5. The Asthma Quality of Life Questionnaire (AQLQ) extends beyond symptom burden to capture the functional and emotional impact, scored 1–7, with higher scores reflecting a better quality of life and an MCID of 0.5 (Bonini et al., 2020). Nevertheless, these tools rely on patient self-report and are subject to recall bias and variability in symptom perception.

A cardinal feature of asthma is marked variability in lung function, which reflects the underlying airway hyperresponsiveness and inflammation (Busse, 2010). This can be measured objectively through dynamic physiological tests. Spirometry commonly demonstrates reduced forced expiratory volume in one second (FEV_1) or FEV_1/forced vital capacity (FVC) ratio, with bronchodilator reversibility (≥12% and ≥200 mL improvement in FEV_1 after administration of a short-acting bronchodilator) (Sánchez-Solís, 2019). Whilst there is a natural diurnal variability in airway calibre in health, in asthma, there is excessive diurnal variability in peak expiratory flow (PEF) ≥20%. Airway hyperresponsiveness can also be confirmed by bronchial provocation testing

(e.g., methacholine or mannitol challenge), which leads to a decrement in FEV_1 with gradually increasing concentrations of inhalant (Brigham & West, 2015). However, most prior studies assessed lung function cross-sectionally rather than longitudinally, which may underestimate its natural variability and dynamic relationship with symptoms.

A dominant immunopathological feature underlying the airway hyperresponsiveness in asthma is type 2 airways inflammation. This is commonly reflected by elevated fractional exhaled nitric oxide (FeNO) levels and peripheral blood eosinophilia, both of which correlate with disease activity and therapeutic responsiveness (Ian D Pavord et al., 2018; Pavord & Corren, 2020). FeNO is a sensitive biomarker closely correlated with IL-13 mediated inflammation of the airways epithelium. Asthma frequently coexists and may be immunopathologically linked with atopic features, such as allergen sensitisation confirmed by skin prick testing or serum-specific IgE assays. Variation in atopic features further contributes to the clinical heterogeneity of asthma (Froidure et al., 2015). Heterogeneity is driven partly by differences in underlying airways inflammatory processes and molecular phenotypes, as well as by differences in symptom perception and comorbidities (Hinks et al., 2015; T. S. C. Hinks et al., 2016).

Table 1.1 Clinical characteristics of asthma.

Clinical characteristics of asthma	
Symptoms	- Wheeze - Shortness of breath - Chest tightness - Cough
Clinical history	- Asthma control assessment (ACQ, AQLQ, etc.) - History of exacerbations (frequency, severity) - Family history of asthma/atopy - Comorbidity screening (rhinitis, GORD, obesity, etc.)
Physical examination	- Wheezing on auscultation - Clinical signs of atopy
Lung function	- Spirometry (FEV_1, FEV_1/FVC, etc.) - Bronchodilator reversibility - Peak Expiratory Flow (PEF) variability - Airway hyperresponsiveness (bronchial provocation test)
Allergy testing	- Skin prick test - Serum specific IgE (RAST, ImmunoCAP, etc.)
Biomarkers	- Fractional exhaled nitric oxide (FeNO) - Peripheral blood eosinophils - Induced sputum eosinophils

Information collated from several current reviews of asthma (Brigham & West, 2015; GINA, 2024; Ioachimescu & Desai, 2019; Papi et al., 2018; Tarasidis & Wilson, 2015).

Abbreviations: ACQ: Asthma Control Questionnaire; ACT: Asthma Control Test; GORD: Gastroesophageal reflux disease; FeNO: Fractional exhaled nitric oxide; FEV_1: Forced Expiratory Volume in 1 second; FVC: Forced Vital Capacity; IgE: Immunoglobulin E; PEF: Peak expiratory flow; RAST: Radioallergosorbent Test.

1.1.2 Epidemiology and disease burden

Asthma is the most prevalent chronic respiratory disease globally, affecting an estimated 300 million people and causing approximately 1,000 deaths each day (GINA, 2024). Over the course of the 20th century, for as yet unknown reasons, its prevalence rose sharply, transforming asthma from a relatively uncommon condition into a global

epidemic (Upton et al., 2000). This trend has been particularly pronounced in urbanised and industrialising regions. It is thought to result from complex interactions between genetic susceptibility and environmental exposures, including air pollution, allergens, and lifestyle changes (Asher et al., 2021; Lundbäck et al., 2016). Notably, the burden of asthma demonstrates marked heterogeneity across regions and populations. While prevalence is higher in developed countries such as the UK and US (Yuan et al., 2025), mortality is disproportionately concentrated in low- and middle-income countries, which account for about 96% of asthma-related deaths (GINA, 2024). This disparity reflects inequalities in healthcare access and treatment availability (Mattiuzzi & Lippi, 2020). Currently, the population-based data from low- and middle-income regions are limited, restricting our understanding of global heterogeneity.

According to the GINA report (Oh et al., 2025), asthma was responsible for 21.4 (17.0–26.9) million disability-adjusted life years (DALYs) in 2021, corresponding to an age-standardised DALY rate of 264.6 per 100,000 population, which makes it a major global health challenge. Asthma is frequently a lifelong condition linked to impaired quality of life, disability, and high healthcare utilisation (McDonald et al., 2018). Acute exacerbations, often triggered by allergens, viral infections, or environmental pollutants, further amplify this burden by driving emergency visits, hospital admissions, and accelerated disease progression (Crossman-Barnes et al., 2019).

Asthma can be classified according to symptom control and treatment intensity. Mild to moderate asthma was defined as asthma with controlled or partially controlled symptoms while receiving low to moderate dose ICS (<500 µg fluticasone propionate per day or an equivalent dose) according to GINA criteria (GINA, 2024) (D. E. Shaw et al., 2015). Severe asthma was defined as asthma with uncontrolled symptoms and/or at least two exacerbations per year despite treatment with high-dose ICS (≥1000 µg fluticasone propionate daily or equivalent). It is estimated that approximately 17% of all asthma patients have difficult-to-treat asthma, while 3.7% meet the criteria for severe asthma (Figure 1.1). Although severe and uncontrolled asthma represent only a minority of cases, they contribute disproportionately to morbidity, mortality, and healthcare costs (Nunes et al., 2017).

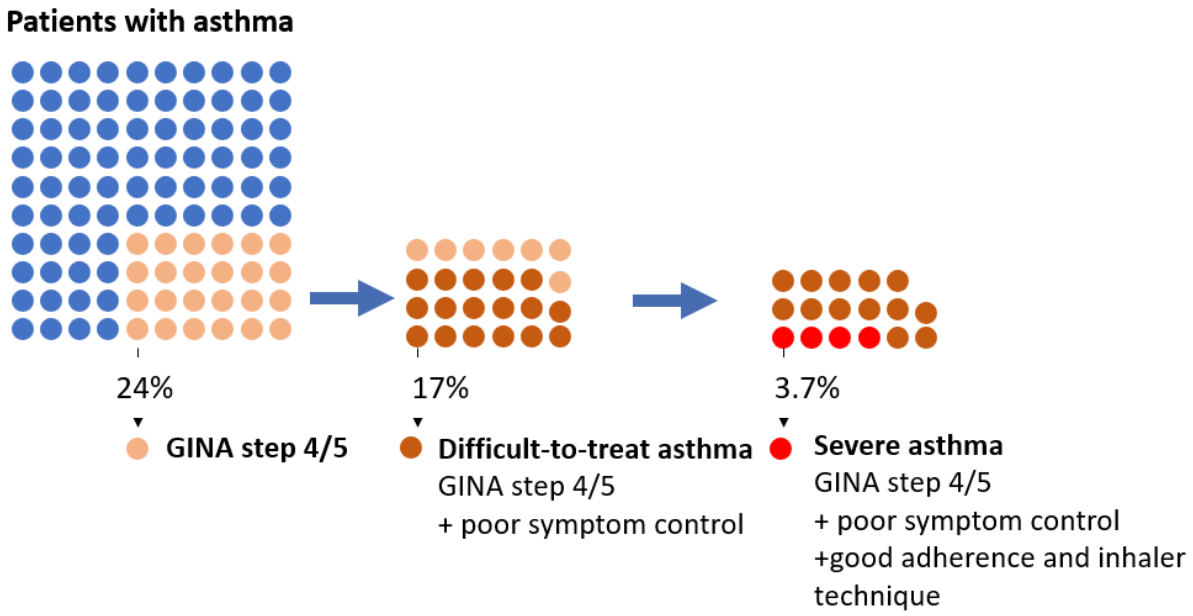


Figure 1.1 Proportion of difficult-to-treat and severe asthma in patients with asthma.

Figure adapted from GINA 2018 Pocket guide to severe and difficult to treat asthma (GINA, 2018) and Hekking *et al* (Hekking et al., 2015).

Beyond health outcomes, asthma imposes significant socioeconomic costs, and its prevalence continues to rise. Asthma interferes with daily life, work, and education, with particularly profound consequences for children and their families (GINA, 2024). For children, asthma results in more frequent school absences compared to their peers, with potential long-term implications for educational attainment and social development (Hsu et al., 2016). Among adults, uncontrolled asthma translates into substantial workplace impairment: an international survey reported that nearly 75% experienced reduced productivity, attributable not only to absenteeism but also to presenteeism, where ongoing symptoms such as dyspnoea, fatigue, and cognitive strain undermine work performance (Gruffydd-Jones et al., 2019). These costs encompass both direct medical expenses, such as hospitalisations and healthcare services, and indirect losses, including reduced productivity, work and school absenteeism, and diminished earning potential. In the UK alone, the direct economic burden of asthma exceeds £1.1-1.5 billion per year (Mukherjee et al., 2016), while the total environmental, economic, and societal impacts have been estimated at around £6 billion per year (Asthma+LungUK, 2023). In the US, it is projected to amount to nearly $963.5 billion over the next two decades, encompassing both direct and indirect costs (Yaghoubi et al., 2019).

1.1.3 Risk factors: focus on bacterial exposure and sex

Asthma is a complex chronic disease resulting from the multifactorial interplay of genetic susceptibility, environmental exposures, and host-related factors, with substantial heterogeneity in its pathogenesis and clinical risk profile (Beasley et al., 2015). A broad spectrum of risk factors contributes to the development and progression of asthma, including demographic characteristics, genetic predisposition, allergen sensitisation, environmental exposures, respiratory infections, psychological influences, lifestyle and dietary habits, and socioeconomic status (Merin E Kuruvilla et al., 2019).

Bacterial colonisation and infection have been associated with more severe symptoms, frequent exacerbations, and reduced responsiveness to corticosteroid therapy in asthma, particularly in neutrophilic phenotypes (Huang et al., 2015). Common respiratory pathogens, including *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*, are frequently detected in the airways of patients with asthma, where they can trigger or amplify inflammatory responses and increase the risk of exacerbations (Brown et al., 2022; Smits et al., 2016). Although antibiotic therapies, including long-term macrolides, can alleviate airways inflammation and enhance clinical outcomes. Their use raises significant concerns about the emergence of antimicrobial resistance, which complicates treatment decisions and limits future therapeutic options (Kwok et al., 2025). Close correlation between the presence of specific airway pathogens and type 1 and type 17 inflammatory processes (Mannion

et al., 2023) as well as marked symptomatic and immunological responses to macrolide therapy (P. G. Gibson et al., 2017; Shukla et al., 2021) strongly imply that bacterial infection is not merely a bystander but an active driver of asthma heterogeneity and severity (G. Lavoie et al., 2025), highlighting the need for integrated approaches to characterise pathogen infections in asthma and provide effective treatment strategies. Nevertheless, most existing studies in asthma are small-scale, cross-sectional, and focused on a limited range of pathogens. To date, no research has examined antibiotic resistance in asthma. Therefore, it leaves substantial gaps in our understanding of longitudinal dynamics and the resistance profile of bacterial pathogens in asthma.

Sex differences represent a very important factor in asthma presentation. In childhood, boys bear a greater asthma burden, whereas after puberty, asthma occurs more frequently and tends to be more severe in women with a higher prevalence and greater symptoms (Somayaji & Chalmers, 2022). In women, changes in sex hormone levels across puberty, the menstrual cycle, and pregnancy are associated with the development and progression of asthma (N. U. Chowdhury et al., 2021). Clinically, women with asthma are more prone to severe and treatment-resistant disease, with a higher risk of exacerbations and comorbidities such as obesity, anxiety, and depression. Moreover, sex may influence treatment responses, including differential sensitivity to inhaled corticosteroids (Oh et al., 2025). At the molecular level, sex-specific differences in airways inflammation, immune cell profiles, and gene

expression patterns have been observed, with obesity-related and neutrophilic asthma appearing disproportionately common in women (Borrelli et al., 2025). Understanding these differences is critical for developing sex-informed strategies in asthma management and personalised medicine. However, sex-related differences remain underexplored in clinical data, and the underlying mechanisms, including immunological pathways, hormonal effects, and treatment response, are not fully understood.

1.1.4 Pharmacological treatments and corticosteroid response

Asthma remains an incurable chronic respiratory disease, yet effective management strategies can substantially reduce its burden (Papi et al., 2020; I. D. Pavord et al., 2018). The primary objectives of asthma treatment are to achieve and maintain symptom control, prevent acute exacerbations, preserve lung function, minimise adverse effects of medication, and ultimately improve quality of life (McDonald et al., 2019; Papi et al., 2020).

The introduction of inhaled corticosteroids (ICS) has markedly transformed asthma management over recent decades. As the cornerstone of therapy, ICS effectively suppress airways inflammation, improve long-term lung function, and reduce the risk of exacerbations and death in most patients (Suissa et al., 2000). Their widespread adoption in higher-income nations has contributed to a sustained decline in asthma-related mortality (O'Byrne et al., 2019).

While ICS are highly effective in mild-to-moderate disease, their benefits in severe asthma are often limited, with an estimated one-third to one-half of individuals remaining uncontrolled despite conventional therapy (Buhl et al., 2020), and prolonged use is further associated with systemic side effects (Wadhwa et al., 2019). It is estimated that 10% of patients show resistance to current therapies (Wenzel, 2005), typically presenting with more severe disease, and this subgroup, though a minority, accounts for a disproportionate share of asthma-related healthcare costs, estimated at 50–80% (Barnes & Adcock, 2009). In addition, poor adherence to ICS regimens further limits treatment effectiveness, particularly among patients with non-T2 or neutrophilic asthma, where responses to standard therapy are often suboptimal (Rabe & Schmidt, 2001; Sze et al., 2020).

At the molecular level, corticosteroids exert their effects predominantly through transcriptional regulation. Upon binding, the glucocorticoid receptor (GR) translocates to the nucleus, where it binds glucocorticoid response elements to activate or repress gene transcription. ICS can downregulate airways gene expression of type-2 inflammation, T cell-mediated adaptive immunity, B-cell immunity and innate immunity (Marchi et al., 2024). Beyond direct transcriptional control, corticosteroids also influence chromatin structure and interact with transcription factors such as NF-κB and AP-1, leading to widespread remodelling of airway epithelial and immune cell gene expression programmes (Ingawale et al., 2015). However, the molecular mechanisms

underlying corticosteroid resistance, both in asthma patients and in health, remain poorly understood.

To enhance treatment efficacy, corticosteroids are commonly combined with long-acting β2-agonists (LABAs), which provide sustained bronchodilation and symptom relief (GINA, 2024). For some patients, particularly those with persistent airflow limitation, the addition of long-acting muscarinic antagonists (LAMAs) such as tiotropium can further improve lung function and reduce exacerbations (Cazzola et al., 2020). Leukotriene receptor antagonists (LTRAs), such as montelukast, offer an oral alternative with both anti-inflammatory and bronchodilatory properties, and are particularly effective among patients with concomitant allergic rhinitis or aspirin-exacerbated respiratory disease (Trinh et al., 2019). These step-up approaches represent essential strategies in guideline-based management, but they remain insufficient for a subset of patients with severe or treatment-resistant disease.

Recent advances in understanding asthma heterogeneity, particularly the identification of T2-high endotypes, have paved the way for precision medicine. Monoclonal antibody (mAb) therapies targeting key inflammatory pathways have demonstrated safety and efficacy in reducing asthma attacks, lowering oral corticosteroid (OCS) dependence, and improving quality of life in patients with severe asthma (McGregor et al., 2019). The key biologic classes include: anti-IgE (omalizumab), anti-IL-5 and anti-IL-5R (mepolizumab, reslizumab, benralizumab), anti-IL-4R (dupilumab), and

anti-TSLP (tezepelumab) (Crossingham et al., 2022; I. Howell et al., 2023). However, direct head-to-head trials between these therapies are lacking, and their relative efficacy, safety, and cost-effectiveness across different asthma phenotypes remain uncertain. Robust network meta-analyses are therefore needed to synthesise the available evidence and provide comparative insights to inform personalised treatment selection and guideline development.

1.2 Pathogenesis of asthma

Asthma is a chronic heterogeneous disease characterised by airways inflammation, bronchial hyperresponsiveness (BHR), and airway remodelling. Its pathogenesis reflects a complex interplay of immune dysregulation, epithelial barrier dysfunction, genetic susceptibility, and environmental exposures (Anderson, 2008). These processes converge to generate variable clinical phenotypes and molecular endotypes that determine both disease severity and treatment response (Saglani & Lloyd, 2015).

1.2.1 Airways inflammation and type 2 immunology

Airways inflammation represents the central pathological mechanism of asthma that drives disease progression and variable treatment response. Over the past three decades, advances in immunology and molecular phenotyping have established that asthma can be categorised as T2-high and -low phenotypes, reflecting distinct underlying inflammatory mechanisms and clinical manifestations (Maspero et al., 2022; I. D. Pavord et al., 2018). Understanding these two inflammatory patterns has not only reshaped the classification of asthma but has also led to precision medicine approaches, particularly through the development of biologics.

The T2-high phenotype is prevalent among patients with asthma, especially in children and atopic individuals (Ricciardolo et al., 2021). It is driven by type 2 helper T cells (Th2), group 2 innate lymphoid cells (ILC2s), and the transcription factor GATA3, leading to the production of IL-4, IL-5, and IL-13 as illustrated in Figure 1.2 (Niessen et al., 2022). These cytokines orchestrate IgE synthesis, eosinophil recruitment and

survival, mucus hypersecretion, and airway hyperresponsiveness (B. N. Lambrecht & H. Hammad, 2015). Persistent T2-high inflammation is strongly associated with severe disease, frequent exacerbations, and loss of control, and some patients remain symptomatic despite corticosteroid therapy due to steroid-refractory eosinophilia (Jatakanon et al., 2000). Biomarkers, including sputum and blood eosinophils, FeNO, and periostin, have been extensively studied as indicators of T2 activity and predictors of treatment response (Heaney et al., 2016). Importantly, mechanistic insights have translated into targeted biologics: monoclonal antibodies against IL-5, IL-5Rα, IL-4Rα, and IL-13 have shown substantial benefits in reducing eosinophilic inflammation and exacerbations, while therapies targeting upstream epithelial alarmins, including TSLP, IL-25, and IL-33 are emerging as promising approaches that modulate multiple downstream pathways (McGregor et al., 2019; Peters & Wenzel, 2020). However, existing biomarkers lack optimal specificity and sensitivity, and many patients with T2-high features demonstrate variable treatment response.

The T2-low phenotype, by contrast, is defined by the absence of canonical type 2 pathways and is associated with Th1- and Th17-mediated immune responses, neutrophilic inflammation, and oxidative stress (Chin-See-Chong et al., 2025). It is typically observed in non-atopic, late-onset patients who respond poorly to corticosteroid therapy. The inflammatory milieu involves increased IFN-γ, IL-17A/F, IL-22, and TNF, which promote neutrophil recruitment, epithelial activation, and tissue damage (D. F. Choy et al., 2015). Bacterial colonisation and recurrent airway infections, particularly with *H. influenzae*, contribute to the persistence of neutrophilic inflammation, with IL-17 as a central driver (Essilfie et al., 2012). Notably, corticosteroid therapy may exacerbate bacterial burden in these patients, raising

therapeutic challenges. The heterogeneity of T2-low asthma, including neutrophilic, paucigranulocytic, and infection-driven endotypes, makes it a particularly difficult target for precision medicine. Conventional ICS are largely ineffective in T2-low asthma (Hudey et al., 2020). Clinical studies of macrolides such as azithromycin have demonstrated improvements in exacerbation rates and quality of life in some non-eosinophilic patients with asthma (G. Lavoie et al., 2025). However, it remains unclear whether benefits are due to antimicrobial effects or immune modulation of long-term low-dose macrolides. Targeted biologics have lagged, but current research is exploring therapeutic targets, such as neutrophilic airways inflammation mediators (IL-8, IL-17, IL-1, IL-6, IL-23, TNF-α), agents restoring corticosteroid sensitivity (MAPK, tyrosine kinase, PI3K inhibitors), PDE3 inhibitors and PDE4 inhibitors (Hinks et al., 2021; Kyriakopoulos et al., 2021; Sze et al., 2020).

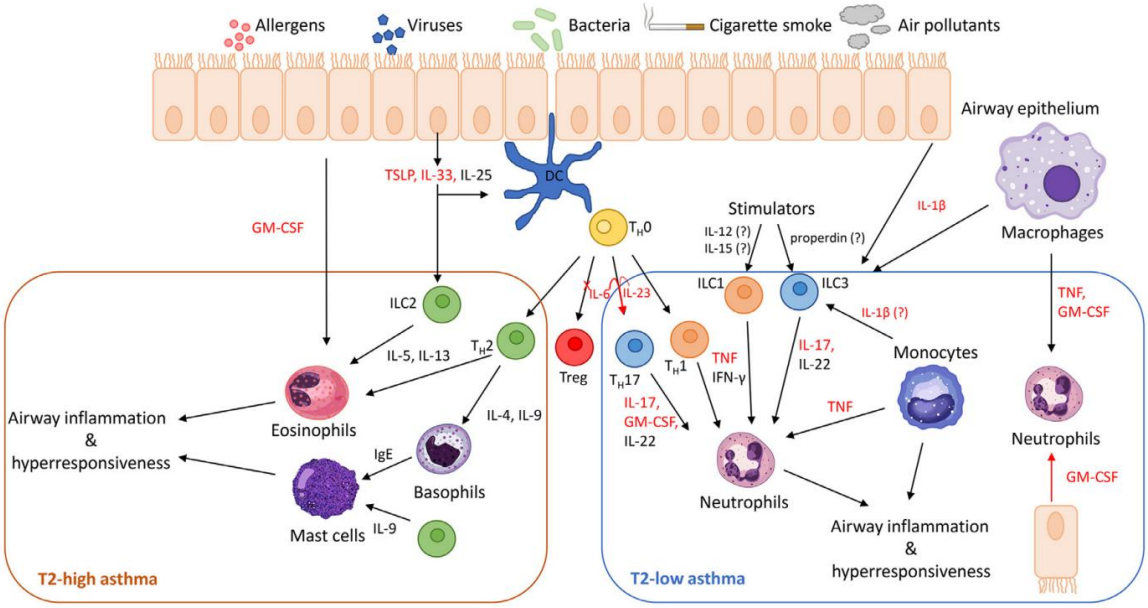


Figure 1.2 Pathophysiology of T2-high and T2-low asthma.

Figure from Niessen *et al* (Niessen et al., 2022). Abbreviations: DC: dendritic cell; GM-CSF: granulocyte-macrophage colony-stimulating factor; IgE: immunoglobulin E; IL: interleukin; Th: T helper cell; TNF: tumour necrosis factor; TSLP: thymic stromal lymphopoietin.

1.2.2 Airways remodelling and other mechanisms

Chronic airways inflammation initiates a cascade of structural and functional alterations in the airway wall, which together define the process of airways remodelling. One of the earliest abnormalities is epithelial barrier dysfunction, characterised by shedding of epithelial cells, impaired barrier integrity, and increased epithelial permeability. These changes facilitate enhanced exposure to allergens, pathogens, and irritants, thereby amplifying airways inflammation and immune activation (Martinez & Vercelli, 2013). In parallel, mucus hypersecretion and airway plugging arise from goblet cell hyperplasia and submucosal gland enlargement, leading to excessive mucus production and accumulation that obstructs the airway lumen and exacerbates airflow limitation (Bush, 2019), as illustrated in Figure 1.3 (Wadsworth et al., 2011). However, the temporal sequence of these abnormalities and whether they precede or follow inflammation remains incompletely understood.

The structural components of remodelling encompass subepithelial fibrosis, thickening of the reticular basement membrane, smooth muscle hypertrophy and hyperplasia, goblet cell metaplasia, angiogenesis, and extracellular matrix deposition. These changes progressively narrow the airway calibre, reduce elasticity, and contribute to persistent airflow limitation even in clinically stable individuals. A cardinal functional correlate of these processes is BHR, which manifests as an exaggerated bronchoconstrictive response to non-specific stimuli, including allergens, cold air, or exercise. Notably, BHR persists despite clinical remission, underscoring its importance as a defining feature of asthma pathophysiology (Nair et al., 2017).

Over time, repetitive cycles of inflammation, epithelial damage, and aberrant repair promote the deposition of extracellular matrix proteins, including collagens and fibronectin, which further thicken the airway wall and contribute to partially irreversible airflow obstruction (Hough et al., 2020). Expansion of airway smooth muscle mass enhances airway contractility, while angiogenesis increases vascularity and promotes wall oedema. In parallel, IL-13 drives goblet cell metaplasia and overproduction of mucins, particularly MUC5AC and MUC5B, resulting in persistent mucus hypersecretion and plugging, which exacerbates obstruction and impairs ventilation homogeneity, particularly during acute exacerbations (Bonser & Erle, 2017). These alterations create a self-perpetuating cycle in which inflammation, barrier dysfunction, mucus overproduction, and structural remodelling reinforce one another, driving disease chronicity and heterogeneity.

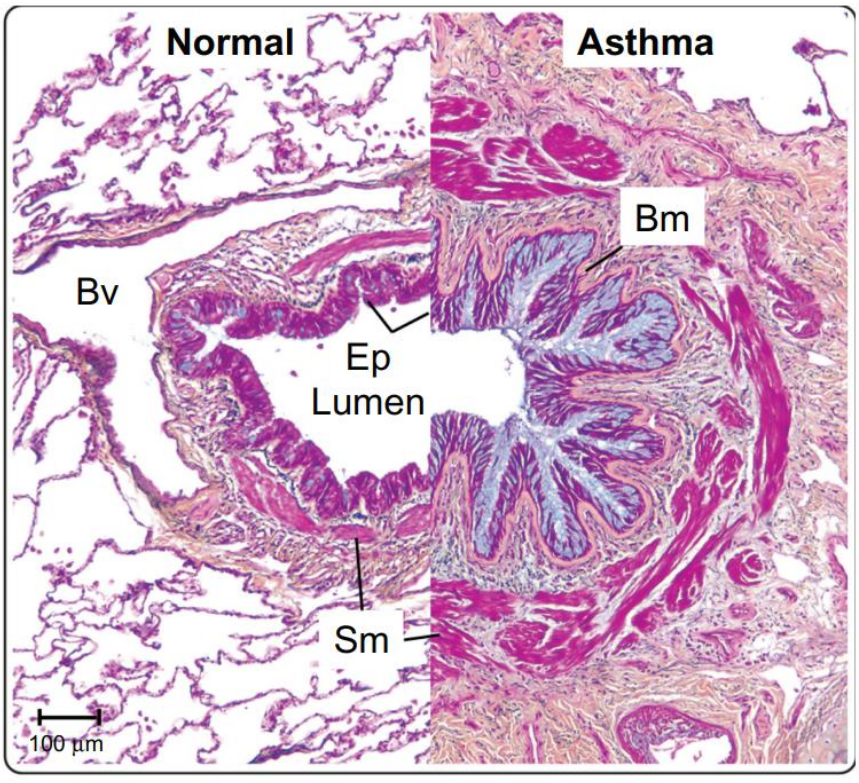


Figure 1.3 Histopathology of airways in asthma.

Figure from Wadsworth *et al*. (Wadsworth et al., 2011) and Holgate *et al*. (Holgate et al., 2015). In asthma, using Movat's pentachrome stain, the epithelium exhibits mucous cell hyperplasia and hypersecretion (blue), accompanied by marked basement membrane (Bm) thickening. Smooth muscle (Sm) volume is increased compared with non-asthmatic tissue. Scale bar = 100 μm. Abbreviations: Bv: blood vessel; Ep: epithelium; Bm: basement membrane; Sm: smooth muscle.

1.3 Genetic and molecular mechanisms of asthma

The genetic and molecular mechanisms underlying asthma remain only partially understood, reflecting the disease's marked heterogeneity and complexity. Early reductionist approaches that focused on individual candidate genes, biomarkers, or isolated signalling pathways proved insufficient to capture the multifactorial nature of asthma. The advent of high-throughput technologies has shifted research towards systems-level analyses, enabling the application of multi-omics approaches that integrate information across molecular layers, including the genome, epigenome, transcriptome, proteome, metabolome, and microbiome (Bunyavanich et al., 2024; Gautam et al., 2022; Ivanova et al., 2019). By unifying these complementary datasets, multi-omics provides a more comprehensive understanding of asthma pathogenesis, offering opportunities to identify novel disease endotypes, discover biomarkers for patient stratification, and highlight potential therapeutic targets.

1.3.1 Genomics and epigenomics

Genomic studies have established asthma as a highly heritable disease, with estimates of heritability ranging between 35% and 90% depending on age and cohort (Hernandez-Pacheco et al., 2019). Genome-wide association studies (GWAS) have been central in identifying susceptibility loci. The GWAS with 10,365 patients demonstrated that asthma is significantly associated with multiple loci, notably *IL18R1*, *IL33*, *SMAD3*, *ORMDL3*, *HLA-DQ*, and *IL2RB* (Moffatt et al., 2010). The most consistently replicated region is the *17q12–21* locus, harbouring genes such as *ORMDL3* and *GSDMB*, which are strongly associated with childhood-onset asthma (Moffatt et al., 2007; Ober & Yao, 2011). Beyond 17q21, large-scale GWAS meta-

analyses have uncovered over 200 loci related to asthma risk, implicating pathways in epithelial barrier function, innate and adaptive immunity, and cytokine signalling (Y. Han et al., 2020). Variants in RUNX1 (Runt-related transcription factor 1) have been linked to enhanced airway hyperresponsiveness and dysregulated immune function (Haley et al., 2011). Genomics-guided discovery methods have been developed for identifying potential drug targets in asthma (El-Husseini et al., 2020). However, many GWAS findings have limited translatability across populations due to the underrepresentation of non-European ancestries (Bunyavanich et al., 2024).

Epigenomic modifications, particularly DNA methylation and histone modifications, bridge the gap between genetic susceptibility and environmental triggers (Gomez, 2019). Epigenome-wide association studies (EWAS) have identified methylation changes in airway epithelial cells and immune cells associated with asthma phenotypes, including Th2 inflammation and corticosteroid response (Arathimos et al., 2017). Recent efforts to design asthma- and allergy-specific methylation arrays revealed functional CpG sites enriched in enhancers and transcription factor binding regions that were underrepresented in commercial arrays (Bunyavanich et al., 2024). Epigenetic regulation also reflects exposure history, including pollutants, tobacco smoke, and microbial stimuli. Long-read sequencing now enables simultaneous detection of host and microbial epigenomes, providing a richer view of host–microbiome interactions (Sheikhpour et al., 2021). Importantly, methylation risk scores have been proposed as an analogue to polygenic risk scores, offering the potential to combine genomic and epigenomic predictors of asthma susceptibility (Stikker et al., 2024). Long-read sequencing improves the detection of methylation patterns and structural variants, advancing asthma research (Bonilla & de Guzman Strong, 2024).

1.3.2 Transcriptomics

Transcriptomics provides dynamic insights into gene activity and cellular responses in asthma (Park & Weiss, 2020). Blood samples mainly reflect systemic inflammation. Bigler *et al.* analysed adult transcriptomes and found that mild/moderate and severe asthma mostly involved the same differentially expressed genes, but the changes were more substantial in severe disease (Jeannette Bigler et al., 2017). In particular, severe asthma was characterised by upregulation of pathways related to chemotaxis, migration, and myeloid trafficking. Consistently, transcriptomic studies of peripheral blood mononuclear cells (PBMCs) among patients with severe asthma have demonstrated enhanced expression of interferon-stimulated genes and innate immune pathways (82). In addition, Bjornsdottir *et al.* reported distinct antigen-independent T-cell activation during exacerbations (Bjornsdottir et al., 2011). Airway samples more directly capture local airways inflammation. Using bronchial brushing transcriptomes from the Severe Asthma Research Program (SARP), Modena *et al.* identified marked downregulation of gene modules associated with epithelial growth, repair, and neuronal function in severe asthma (Modena et al., 2017). Singhania *et al.* observed differential expression of epithelial type 2 and type 17 signatures in type 2 high and in neutrophilic asthma, and found signatures of responses to bacterial infections across tissues (CEACAM5, CD14, and TLR2), including Toll-like receptor signaling in airway T cells, suggesting activated T cells drive neutrophilic inflammation and steroid-insensitive IL-17 response in severe asthma (Singhania et al., 2018). Moreover, Hekking *et al.* showed that gene signatures linked to eosinophilic inflammation and mast cells were more highly expressed in adult-onset severe asthma compared with childhood-onset disease (Hekking et al., 2018).

Transcriptomic profiling of immune cells has provided further mechanistic insights into asthma. Single-cell RNA sequencing (scRNA-seq) is accelerating studies of airway cellular heterogeneity, uncovering rare but functionally essential populations, such as pathogenic Th2 cells co-expressing IL-5 and IL-13, subsets of ILC2 with distinct transcriptional programmes, and dysfunctional epithelial subtypes (Schupp et al., 2021; Vieira Braga et al., 2019; Wang et al., 2010). Furthermore, spatial transcriptomics is providing insights into tissue organisation and cell–cell interactions, revealing pro-inflammatory cellular ecosystems in the asthmatic airways (Joulia et al., 2025).

1.3.3 Other omics

Compared with genomics, epigenomics and transcriptomics, research applying other omics approaches in asthma remains relatively limited. Proteomic analyses have revealed alterations in airway proteins linked to inflammation, remodelling, and oxidative stress, including periostin, YKL-40, and complement proteins (Khezia Asamoah et al., 2024; Gharib et al., 2011; Terracciano et al., 2015). Large asthma cohorts, such as the Wessex Severe Asthma Cohort, SARP and U-BIOPRED cohorts, using BALF and sputum proteomics, as well as multiplex immunoassays, have consistently identified abnormal patterns of inflammatory mediators and remodelling-associated proteins, which have enabled molecular clustering of patients (K. Asamoah et al., 2024; Hinks et al., 2015; T. S. C. Hinks et al., 2016; Takahashi, Pavlidis, Ng Kee Kwong, Hoda, Rossios, Sun, Loza, Baribaud, Chanez, Fowler, Horvath, Montuschi, Singer, Musial, Dahlen, Dahlen, Krug, Sandstrom, Shaw, Lutter, Bakke, Fleming, Howarth, Caruso, Sousa, Corfield, Auffray, De Meulder, Lefaudeux, Djukanovic, Sterk, Guo, Adcock, & Chung, 2018). Metabolomics has highlighted disturbances in lipid

mediators, amino acid metabolism, and energy pathways associated with asthma severity and exacerbation frequency (Kelly et al., 2018; Wang et al., 2021). Changes in lipids have been identified, specifically polyunsaturated fatty acids (PUFAs), which are inversely linked to asthma risk (Lee-Sarwar et al., 2019). The airway and gut microbiomes profoundly influence immune development and asthma risk. Specific bacterial taxa in the gut during early life, such as *Faecalibacterium* and *Bifidobacterium*, have been linked to asthma protection, while airway colonisation by *Moraxella* or *Haemophilus* species has been associated with exacerbations (Fujimura & Lynch, 2015; Jabeen, Sanderson, Tinè, et al., 2024). Research from different omics underscores the heterogeneous nature of asthma and highlights that integrating multi-omics provides deeper insights into pathogenic pathways, disease progression, and potential therapeutic targets.

1.4 Objectives and rationale

A central challenge in asthma research lies in disentangling the overlapping layers of heterogeneity that influence disease course and treatment response (I. D. Pavord et al., 2018). This heterogeneity is determined by both intrinsic and extrinsic factors, including immune pathway activity, host–microbial interactions, and biological sex. Addressing these determinants is essential for uncovering the mechanisms that drive differential asthma phenotypes and for advancing towards a precision medicine framework. In this thesis, I applied a clinical bioinformatics and longitudinal clinical data analysis approach to examine three major domains of asthma heterogeneity: immunological endotypes, chronic bacterial infection and antimicrobial resistance, and sex-related biological differences. These studies provided complementary perspectives that converge on airways inflammation as the unifying hallmark of asthma pathogenesis.

The first project focused on type 2 cytokine activity in defining molecular endotypes of asthma. While corticosteroids and biologics targeting T2 pathways had transformed the management of many patients, a substantial subset remained uncontrolled despite suppression of T2 activity. The mechanisms sustaining airway dysfunction in T2-low asthma were poorly understood, limiting the development of effective therapies. To address this gap, we integrated transcriptomic data from bronchial biopsies and brushings in a multicentre severe asthma cohort with clinical data. This approach enabled delineation of the molecular signatures of T2-high, -intermediate, and -low asthma. By identifying shared and distinct transcriptional pathways and evaluating their independence from corticosteroid exposure, this project sought to elucidate the

mechanistic basis of airway inflammation across endotypes and to inform therapeutic strategies beyond the T2 paradigm.

The second project addressed the contribution of bacterial infection and antimicrobial resistance (AMR) to asthma pathogenesis. Bacterial colonisation of the lower airways had been increasingly recognised as an important driver of asthma exacerbations and disease progression, yet its long-term epidemiological patterns remained poorly characterised. Most existing studies are limited to acute exacerbation events or cross-sectional analyses, leaving significant knowledge gaps regarding the persistence, succession, and resistance profiles of airway pathogens over time. To fill this gap, this thesis undertook an electronic health record (EHR)-based study spanning nearly three decades, analysing large-scale sputum culture and antimicrobial susceptibility data from patients with asthma. This work was designed to characterise the prevalence and diversity of bacterial pathogens in asthma, identify temporal trends and potential microbial succession, quantify the burden and dynamics of AMR, and determine clinical factors independently associated with bacterial isolation, including the role of ICS therapy. These insights provided an evidence base for understanding the interplay between asthma, airway infection, and resistance, with implications for antimicrobial stewardship and the long-term management of asthma.

The third project examined sex-related differences in asthma, a dimension of heterogeneity consistently observed in clinical practice but poorly understood mechanistically. Epidemiological studies have demonstrated that women have a higher prevalence of asthma and tend to experience greater disease severity compared with men. However, the underlying molecular, immunological, and

physiological drivers of these disparities remained poorly understood. To address this, a comprehensive multi-omics approach was employed, integrating transcriptomics, proteomics, metabolomics, microbiomics, and clinical data from a large cohort of adult patients with asthma. The analyses aimed to define sex-specific clinical and physiological features across asthma severities, identify molecular and microbial pathways that distinguish men and women with severe asthma, and validate key findings across independent cohorts. In doing so, this study offered mechanistic insights into the biological basis of sex differences in asthma, with implications for sex-specific treatment strategies.

In summary, this thesis applied a clinical bioinformatics framework to characterise airways inflammation from three complementary perspectives: molecular endotypes shaped by T2 cytokine activity, microbial dynamics of bacterial infection and resistance, and sex-specific mechanisms of disease expression. By using multi-omics analysis and longitudinal clinical data analysis, the thesis advanced a systems-level understanding of asthma heterogeneity, elucidated key pathogenic mechanisms, and provided evidence to inform novel therapeutic targets and personalised treatment strategies.

Chapter 2 Type 2 phenotype in asthma: an airways transcriptomic analysis

Overview

Severe asthma is a heterogeneous disease. The mechanisms driving airway pathology when type 2 (T2) cytokine activity is suppressed remain poorly understood. This study aimed to provide insight by identifying the airway molecular pathways of T2 biomarker-high and -low severe asthma.

Clinical and transcriptomic data from bronchial biopsies and brushes were analysed in the RASP-UK multi-centre severe asthma cohort (18 corticosteroid-resistant T2 biomarker-high [T2-high], 23 T2 biomarker-intermediate [T2-intermediate], 11 T2 biomarker-low [T2-low] patients with severe asthma) together with 20 healthy controls assessed before and after ICS treatment.

Severe asthma, independent of confounding by ICS, was characterised by upregulation of mucins (*MUC5AC*, *MUC2*), lysozyme, *CEACAM5*, *SYNCRIP*, canonical T2-genes (*POSTN*, *IL33*), the transcription factor *FOS*, and the integrin *ITGB8*. T2-high asthma showed upregulation of T2-dependent genes, keratins, adaptive immune responses, impaired ciliary function, and epithelial development. T2-low asthma showed upregulation of Th1- and IL-17-associated genes (*IDO1*, *CXCL10*, *GBP1*, *LAG3*), neuroimmune genes (*SGC2*, *CALCA*), interferon-γ signalling, antigen processing, and enrichment of neutrophils and mast cells. T2-intermediate asthma in part exhibited a mixed molecular profile sharing features of T2-high and T2-low

endotypes, as well as selective expression of the antimicrobial peptide *BPIFA1*, viral response signatures, mast and natural killer (NK) cell enrichment, and activation of transcription factors involved in pathogen defence (*NFKB1*, *TBX21*, *IRF1*, *IRF2*, *BATF*, *STAT2*).

In summary, this study delineated distinct molecular signatures and pathways across three severe asthma phenotypes, independent of corticosteroid effects, thereby providing insights for personalised asthma management and the development of targeted biologic therapies.

2.1 Introduction

2.1.1 Heterogeneity of asthma and type 2 phenotype

Asthma is a chronic inflammatory airway disorder characterised by marked heterogeneity in its clinical presentation, underlying immunopathology, and treatment response. Increasing evidence indicates that asthma comprises a spectrum of phenotypes and endotypes, driven by distinct molecular and cellular pathways. Among these, T2 inflammation represents the most prevalent and well-characterised endotype, which has been illustrated in Chapter 1.2.1.

In clinical practice and research, type 2 biomarkers, including blood and sputum eosinophil counts, FeNO, and serum periostin, have emerged as valuable tools for identifying patients with active T2-driven disease. T2-high asthma often presents with elevated biomarkers and increased corticosteroid responsiveness. By contrast, T2-low asthma is more often linked to obesity, older age of onset, female predominance, fixed airflow limitation, and relative resistance to corticosteroid therapy. Airways remodelling, altered epithelial responses, and dysregulated innate immunity have been proposed as key features of T2-low asthma. However, owing to the limited effective treatment, T2-low asthma is frequently associated with persistent symptoms and increased morbidity (Table 2.1).

Table 2.1 Comparison of type 2 (T2)-high and -low asthma.

	T2-high asthma	T2-low asthma
Immune mechanism	Th2-driven; elevated IL-4, IL-5, IL-13	Non-Th2 inflammation; often neutrophilic, involving Th1 and Th17 cells
Inflammatory cells	Eosinophil-dominant inflammation, mast cells	Neutrophil-dominant or paucigranulocytic, macrophages, Th1 and Th17 cells
Key cytokines	IL-4, IL-5, IL-9, IL-13, IL-25, IL-33, TSLP, GM-CSF	CXCL8, IL-17, IL-22, IL-23, INFγ, TNFα, CXCR2, IL-6
Biomarkers	Elevated FeNO; increased blood/sputum eosinophils	Normal or low FeNO; normal eosinophils; potentially elevated neutrophils
Phenotypes	Allergic asthma, eosinophilic asthma, early-onset asthma	Non-allergic asthma, obesity-related asthma, and late-onset asthma
Treatment response	Good response to ICS and biologics.	Poor response to ICS; limited efficacy of current therapies.
Prognosis	Generally improved with targeted therapy; reduced exacerbations and improved lung function.	Frequently associated with persistent symptoms and increased morbidity.

Information collated from several current reviews of asthma (Carr et al., 2018; Cazzola et al., 2021; C.-Y. Chen et al., 2023; Fahy, 2015; Bart N. Lambrecht & Hamida Hammad, 2015; Saha et al., 2023).

2.1.2 Type 2 phenotype and treatment response

Corticosteroids remain the cornerstone of asthma management, demonstrating high efficacy in reducing airways inflammation, controlling symptoms, and preventing exacerbations, as illustrated in Chapter 1.1.4. Their therapeutic benefits are largely mediated through suppression of type 2 inflammatory pathways, including downregulation of eosinophilic infiltration and cytokine production. Patients with T2-high asthma generally respond well to ICS therapy, showing improvements in lung function, reduced exacerbation frequency, and better overall disease control. In contrast, patients with T2-low asthma often exhibit relative corticosteroid resistance, with persistent symptoms and airways inflammation despite ICS therapy. This differential response underscores the importance of identifying asthma phenotypes and endotypes to optimise treatment strategies and guide the use of additional

targeted therapies.

Blood or airway eosinophilia accompanied by a raised FeNO is found in 50-80% of individuals with mild, corticosteroid-naïve asthma (Berry et al., 2007; McGrath et al., 2012). This eosinophilic phenotype is associated with T2 cytokines, including IL-4- and IL-13–driven airway gene expression and IL-5-dependent tissue eosinophilia (Flood-Page et al., 2003). Monoclonal antibodies targeting the IL-4/-13 (via the IL-4Rα), or IL-5/IL-5Rα, pathways, or the epithelial alarmin TSLP, show greatest efficacy in corticosteroid-resistant severe asthma with high T2 biomarkers (Haldar et al., 2009; Wenzel et al., 2013).

T2-low severe asthma responds poorly to both inhaled corticosteroids (ICS) and T2 biologics, and its molecular basis remains unclear. Transcriptomic studies from airway biopsies have revealed mutually exclusive expression of a typical T2 cytokine-driven and IL-17-driven gene signatures in asthma (D.F. Choy et al., 2015; Ostling et al., 2019). Nevertheless, approximately half of patients with uncontrolled severe asthma receiving high-dose corticosteroid exhibit no evidence of either a T2-high or IL-17-high profile (D.F. Choy et al., 2015; Ostling et al., 2019).

2.1.3 Molecular signatures of type 2 phenotype

Several transcriptomic studies conducted on blood and airway samples have identified molecular signatures of the T2 phenotype (Yue et al., 2025; Zeng et al., 2023). T2-high signature involves IL-13–induced genes, including *POSTN*, *CLCA1*, and *SERPINB2*, which have become hallmark features of this endotype (Peters et al., 2019; Woodruff et al., 2009). Upstream, epithelial alarmins, including IL-33, IL-25, and TSLP,

orchestrate the activation of Th2 cells and ILC2s, driving the production of IL-4, IL-5, and IL-13 (M. E. Kuruvilla et al., 2019). However, T2-low asthma is often characterised by neutrophilic inflammation, with transcriptomic signatures involving chemokine receptors such as *CXCR1* and *CXCR2*, as well as *MMP9*, reflecting activation of neutrophil recruitment and tissue remodelling pathways (Yan et al., 2024). Moreover, sputum transcriptomics of macrophages and neutrophils demonstrated a pro-inflammatory and steroid-resistant phenotype in T2-low asthma (Baines et al., 2011).

2.1.4 Research gaps and rationale

There remain critical research gaps in understanding the molecular basis of the T2 phenotype in asthma. First, while ICS are effective in suppressing airways inflammation, they also induce broad reprogramming of gene expression networks. As such, it is critical to distinguish genuine asthma-associated transcriptional signatures from those driven by ICS exposure, a question that has not been systematically addressed to date. That is to say, the vast majority of published papers on asthma mechanisms have been confounded by the effects of inhaled corticosteroids given to patients with asthma but not to healthy controls. Second, only a limited number of studies have investigated the transcriptomic signatures and molecular pathways underlying T2 biomarkers and T2 phenotypes in airway samples, despite their central role in asthma heterogeneity.

To address these challenges, several complementary strategies were adopted in the study design. First, samples were obtained from participants with objectively confirmed treatment adherence, as verified by FeNO suppression testing. This approach enabled differentiation of corticosteroid-resistant T2-high disease (Heaney et al., 2018).

Second, asthma patients with intermediate T2-biomarkers were managed under biomarker-guided ICS therapy. Their inclusion helped researchers separate true corticosteroid resistance from apparent treatment failure caused by suboptimal therapy. It also helped avoid unnecessary ICS escalation, a common limitation of symptom-directed treatment. Third, asthma patients with low T2-biomarkers were included in this study to explore the genes involved in T2-low inflammation. Finally, to account for ICS-related transcriptional effects, I included a comparator group of healthy participants, both with and without ICS treatment.

To conclude, our study cohort comprised patients with corticosteroid-resistant T2-high asthma, corticosteroid-partially responsive T2-intermediate asthma, and T2-low asthma, alongside healthy participants with and without ICS treatment. Through this design, this study aimed to explore the airway transcriptome across the extremes of the T2 biomarker spectrum and to identify the molecular signatures underlying T2-high, T2-intermediate, and T2-low severe asthma.

2.2 Objectives

The primary objective of this study was to identify the distinct molecular and cellular processes underpinning severe asthma phenotypes within a multi-centre UK cohort. Through a biomarker-driven treatment optimisation study design and FeNO suppression testing, steroid-resistant T2-high, T2-intermediate, and T2-low asthma patients were identified. Specifically, this study aimed to:

1. Describe the clinical characteristics of T2-high, -intermediate, and -low severe asthma.
2. Characterise the airway transcriptomic signatures of severe asthma independent of ICS use, by including a healthy control group exposed to ICS.
3. Examine the airway transcriptomic correlates of established type 2 biomarkers.
4. Define the molecular pathways underlying T2-high, -intermediate, and -low severe asthma.
5. Identify shared and distinct cellular and transcriptional mechanisms across type 2 phenotypes.

By addressing these aims, this study provided a robust assessment of airway transcriptome signatures in asthma and elucidated the molecular mechanisms underlying type 2 phenotypes. The findings were expected to inform novel therapeutic targets and support personalised asthma management.

2.3 Methods

2.3.1 Study population

The United Kingdom Refractory Asthma Stratification Programme (RASP) bronchoscopy study was a multicentre investigation that prospectively recruited 52 patients with severe asthma, classified into 18 T2-high, 23 T2-intermediate, and 11 T2-low phenotypes (Khalfaoui et al., 2022b). Eligible participants were aged between 18 and 70 years, current non-smokers with a smoking history of <15 pack-years, and not receiving biologics. Clinical data were collected, and bronchoscopies were performed. Recruitment was based on predefined inclusion and exclusion criteria (Khalfaoui et al., 2022b):

(i) T2-high, defined as individuals with FeNO ≥ 45 ppb and blood eosinophils ≥ 0.3 $\times 10^9$/L and failed the FeNO suppression test (Boddy et al., 2020; Heaney et al., 2019; McNicholl et al., 2012).

(ii) T2-low, defined as FeNO ≤ 30 ppb and blood eosinophils ≤ 0.2 $\times 10^9$/L.

(iii) T2-intermediate, defined as patients with intermediate biomarker levels and underwent biomarker-driven ICS therapy (Heaney et al., 2020).

The Leicester Healthy Volunteer Study (HVS) included 20 healthy participants receiving inhaled fluticasone propionate 500 mcg bd daily for 4 weeks (Marchi et al., 2024). Clinical data were collected at baseline. Bronchoscopies were performed before and after 4 weeks of ICS therapy. Biopsy and brush samples from both RASP and HVS were collected using identical standard operating procedures.

2.3.2 Bronchoscopy and sample collection

The respiratory system is conventionally divided into upper and lower tracts. The upper tract consists of the nasal cavity, pharynx, and larynx, whereas the lower tract comprises the trachea, bronchi, and lungs (Ionescu, 2013). Biopsies and brushings were collected from 2nd-5th generation bronchi, which represent accessible conducting airways and are commonly affected in asthma (Criner et al., 2020).

Bronchoscopy, performed according to British Thoracic Society (BTS) guidelines, is a standard procedure for airway examination (Du Rand et al., 2013). Bronchial biopsies were taken from the segmental carinae using sterile biopsy forceps, with tissue samples directly transferred into RNAlater and stored at 4 °C for 24 hours before being moved to −80 °C until RNA extraction. Bronchial brushings were obtained from segmental and subsegmental bronchi using a sterile single-use cytology brush. The brushes were immediately immersed in ice-cold RNA stabilisation medium to preserve cellular integrity and nucleic acid quality. All procedures were conducted by experienced bronchoscopists, with continuous monitoring of oxygen saturation and haemodynamic stability. All centres had expertise in conducting research bronchoscopies in asthma. RASP-UK and Leicester HVS followed identical standard operating procedures for tissue collection and processing.

2.3.3 RNA sequencing

Total RNA and DNA were extracted separately using the QIAgen AllPrep DNA/RNA/miRNA Universal Mini Kit following the manufacturer's instructions. Ribosomal RNA was depleted with the RiboZero Magnetic Gold kit, and RNA

sequencing libraries were generated with the Illumina TruSeq Stranded Total RNA protocol. Libraries were sequenced in single-end mode. RNA-seq data are publicly available in the Gene Expression Omnibus (GEO) under accession numbers GSE242048 and GSE301357.

For RNA-seq analysis, reads in FASTQ format (single- and paired-end) were aligned to the human reference genome GRCh38 using the STAR aligner (v2.7.1a). Gene-level quantification was performed with the *Rsubread* package (Foundation). RASP and some HVS samples were sequenced using a single-end strategy, which is cost-effective and sufficient for most expression studies, but may introduce alignment ambiguity, particularly in regions with overlapping genes on the same strand, as is observed in 10x Genomics data. To mitigate this, we used optimised reference annotations provided by the Pool lab (https://www.thepoollab.org/resources), specifically designed to reduce such ambiguities and improve gene quantification accuracy.

2.3.4 Bioinformatic and computational analysis

Raw count data were initially pre-processed and quality-filtered using the edgeR package to remove lowly expressed genes and to stabilise variance across samples. For normalisation, the variance stabilising normalisation (VSN) method was applied via the *VSN* package to account for differences in library size and sequencing depth. To mitigate potential batch effects, data integration across experimental batches was first performed using the ComBat algorithm implemented in the *sva* package (Johnson et al., 2007). To ensure robustness against residual batch effects and variability, differential expression was also independently evaluated using a non-parametric,

rank-based approach provided by the *RankProd* package (Hong et al., 2006).

Differential expression analysis was implemented using the *DESeq2* package (Love et al., 2014), which models raw read counts under a negative binomial distribution framework and applies shrinkage estimation for dispersion and fold-change parameters. Genes with a Benjamini–Hochberg adjusted p-value (false discovery rate, FDR) < 0.05 were considered significantly differentially expressed. For downstream exploratory analyses, count data were further transformed using the variance stabilising transformation (VST) procedure, which provides homoscedastic expression values suitable for clustering and correlation analysis.

To identify molecular correlates of inflammatory biomarkers, the limma-voom pipeline was applied, incorporating covariate adjustments for potential confounders such as age and sex. In this framework, each gene's expression was modelled as a continuous predictor of biomarker levels, allowing the identification of biomarker-associated gene signatures. Pathway-level analyses were performed using Gene Ontology (GO) enrichment via the *clusterProfiler* package (Yu et al., 2012), with gene sets sourced from the GO Consortium. Additionally, Gene Set Enrichment Analysis (GSEA) (Subramanian et al., 2005) was conducted to investigate pathway perturbations across T2 phenotypes, using curated immune-related pathways obtained from the ImmPort database (Bhattacharya et al., 2014).

Network-based approaches were applied to characterise higher-order co-expression structures. Specifically, Weighted Gene Co-expression Network Analysis (WGCNA) (Langfelder & Horvath, 2008a) was used to construct co-expression modules, with

module eigengenes subsequently correlated against clinical metadata to assess module–trait relationships. For unsupervised clustering of the global gene expression matrix, K-means clustering was performed using the *ClusterGVis* package (Zhang, 2022), with the optimal number of clusters determined by the silhouette method.

To gain insight into upstream regulatory drivers, transcription factor (TF) activity was inferred using DoRothEA (Garcia-Alonso et al., 2019), which integrates curated TF–target regulons with gene expression data to estimate TF activity scores. Finally, reference-based cell type deconvolution was performed with CIBERSORTx (Newman et al., 2015) and the Travaglini lung cell atlas (Travaglini et al., 2020), enabling estimation of relative proportions of epithelial, immune, and stromal cell subsets in each sample.

2.3.5 Statistical analysis

Categorical variables were presented as frequencies and percentages, and continuous variables were reported as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on distribution. Comparisons of multiple groups were conducted using Chi-square tests for categorical variables, and ANOVA or Kruskal-Wallis tests for continuous variables, as appropriate. Correlation between clinical characteristics was assessed using Spearman's rank correlation coefficient. All statistical analyses were performed using R software (version 4.4.0), with statistical significance defined as $p < 0.05$.

2.3.6 Ethics and consent

The United Kingdom Refractory Asthma Stratification Programme (RASP) bronchoscopy study was a multicentre study that prospectively recruited individuals with severe asthma according to predefined inclusion and exclusion criteria. The study was approved by the East Midlands – Leicester South Research Ethics Committee (REC; reference 16/EM/0260) and registered at ClinicalTrials.gov (NCT02883530) (Khalfaoui et al., 2022a). In addition, biopsy and brush samples collected under the same standard operating procedure were obtained from the pre-intervention arms of a second single-centre bronchoscopy study in adult healthy volunteers investigating the effects of inhaled corticosteroids (ICS), hereafter referred to as Leicester HVS (NCT02476825, REC approval 15/EM/0313) (Marchi et al., 2024). Both pre- and post-intervention biopsies from Leicester HVS were included. All participants provided written informed consent. The Leicester HVS study was conducted concurrently with RASP and employed the same standard operating procedure and bronchoscopist (PB) across both studies.

2.4 Results

2.4.1 Cohort characteristics

The clinical characteristics of the predefined bronchoscopy study groups are summarised in Table 2.2. As previously reported (Khalfaoui et al., 2022a), FeNO and blood eosinophil levels were markedly elevated in the T2-high asthma group, consistent with its entry criteria. Sputum eosinophils were also highest in T2-high patients, whereas T2-low patients exhibited the greatest symptom burden.

Table 2.2 Participant characteristics.

Characteristics	T2-high (n=18)	T2-intermediate (n=23)	T2-low (n=11)	Healthy controls (n=20)	p-value
Demographics					
Age, years	56 [50, 62]**	55 [46, 62]**	53 [35, 56]	38 [24, 51]	0.001
Sex, Females, %	9 (50%)	12 (52%)	4 (36%)	12 (60%)	0.7
Ethnicity, Caucasian, %	18 (100%)	19 (83%)	11 (100%)	17 (85%)	0.2
BMI, kg/m^2	31 ± 7.4**	33 ± 7.9***	31 ± 6.8**	25 ± 3.6	0.002
Ex-smoker, %	5 (28%)	7 (30%)	2 (18%)	4 (20%)	0.8
Asthma history					
Age onset, years	28 ± 19	27 ± 20	20 ± 18	N/A	0.5
Asthma duration, years	27 ± 20	27 ± 15	26 ± 14	N/A	1
Annual severe exacerbations	2 [1.0, 3.0]	1 [0.0, 2.0]	4 [0.5, 6.0]	N/A	0.042
Annual A&E attendances	0 [0.0, 0.8]	0 [0.0, 0.0]	0 [0.0, 2.5]	N/A	0.03
Lung function & symptom control					
FEV1, % pred	67.5 [57.1, 80.3]***	77.9 [72.1, 92.9]***	86.3 [75.8, 97.1]***	104.0 [95.5, 110.3]	<0.001
FEV1/FVC, %	59.8 [56.9, 67.3]***	70.0 [62.6, 76.7]***	70.3 [60.3, 78.3]***	82.0 [79.5, 85.3]	<0.001
ACQ-5	1.6 [0.6, 2.2]	1.2 [0.6, 1.9]	1.8 [1.1, 2.3]	N/A	0.4
Biomarkers					
Total IgE, kU/L	129 [86, 231]§	110 [20, 287]	231 [86, 297]	Not done	0.5
FeNO, ppb	71.5 [46.3, 97.0]***/###/§§§	23 [15.8, 33.1]§	15 [10.5, 17.0]	18.0 [12.0, 23.0]	<0.001
Blood eosinophils, ×10^9/L	0.37 [0.23, 0.55]***/##/§§§	0.17 [0.07, 0.29]§	0.07 [0.05, 0.14]	0.1 [0.07, 0.16]	<0.001
Sputum eosinophils, %	10.7 [2.3, 32.8]	1.2 [0.1, 5.1]	0.3 [0.2, 0.4]	Not done	0.06
Sputum neutrophils, %	17.1 [2.1, 31.3]§	40 [14.3, 78.9]	68.8 [42.4, 80.0]	Not done	0.07
Inflammatory cells in lamina propria					
Eosinophils, cells/mm^2	22.6 [8.7, 33.7]	17.8 [2.9, 29.3]	15.8 [2.7, 28.2]	21.0 [11.9, 29.8]	0.7
Neutrophils, cells/mm^2	47.5 [20.1, 51.5]*	39.1 [29.0, 52.3]**	40.2 [36.5, 53.7]*	67.0 [44.6, 110.2]	0.003
Chymase+ mast cells, cells/mm^2	8.2 [3.4, 26.3]***	13.3 [3.4, 23.5]**	15.2 [0.9, 25.4]**	34.0 [21.9, 43.9]	<0.001
Tryptase+ mast cells, cells/mm^2	40.4 [13.2, 49.3]**	38.3 [28.7, 48.1]**	25.3 [12.0, 36.4]***	57.0 [43.5, 68.3]	0.001

Characteristics	T2-high (n=18)	T2-intermediate (n=23)	T2-low (n=11)	Healthy controls (n=20)	p-value
Chymase: Tryptase ratio	0.39 [0.25, 0.57]*	0.40 [0.20, 0.54]*	0.49 [0.10, 0.69]	0.60 [0.52, 0.69]	0.046
Remodelling features					
Epithelium, % biopsy area	6.2 [3.3, 8.1]	5.0 [3.1, 8.6]	7.5 [3.4, 13.9]	5.7 [4.0, 8.5]	0.7
Airway smooth muscle, % biopsy area	10.8 [4.4, 15.0]*	9.7 [6.5, 13.6]**	8.3 [5.8, 15.9]*	4.6 [2.8, 10.1]	0.02
MUC5AC, % epithelial area	6.8 [3.6, 9.8]***	5.2 [2.8, 6.7]***	6.7 [5.2, 10.6]***	2.0 [1.3, 3.3]	<0.001
Reticular basement membrane, µM	5.2 [5.1, 6.4]	5.0 [4.3, 5.5]	4.4 [3.8, 5.3]	5.2 [4.6, 5.9]	0.2
Medications					
ICS, %	18 (100%)	23 (100%)	11 (100%)	N/A	N/A
ICS dose, mcg BDP eq	2000 [2000, 2000]	1800 [1000, 2000]	1600 [1600, 2000]	N/A	0.2
Maintenance OCS, %	6 (33%)	8 (35%)	4 (36%)	N/A	1
OCS dose, mg	10 [6.3, 10.0]	8.8 [5.0, 12.5]	8.8 [6.9, 10.0]	N/A	0.9
LABA, %	18 (100%)	23 (100%)	11 (100%)	N/A	N/A
LAMA, %	9 (50%)	8 (35%)	7 (64%)	N/A	0.3
LTRA, %	10 (56%)§	12 (52%)	1 (9%)	N/A	0.03
THEO, %	8 (44%)#	3 (13%)	1 (9%)	N/A	0.02

Continuous variables are presented as mean ± SD or median (interquartile range).

For continuous variables, the ANOVA or the Kruskal–Wallis test was used across applicable groups. For categorical variables, the Chi-squared test was used across applicable groups. *p < 0.05, **p < 0.01, ***p < 0.001 compared with healthy control subjects. #p < 0.05, ##p < 0.01, ###p < 0.001 compared with T2- intermediate. §p < 0.05, §§p < 0.01, §§§p < 0.001 compared with T2- low.

Abbreviations: ACQ-5: Asthma Control Questionnaire - 5; BDP eq: beclometasone dipropionate equivalent; BMI: body mass index; FeNO: Fractional concentration of exhaled nitric oxide; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; ICS: inhaled corticosteroids; Ig: immunoglobulin; IQR: interquartile range; LABA: long-acting Beta2 agonist; LAMA: long-acting muscarinic antagonist; LTRA: leukotriene receptor antagonist; OCS: oral corticosteroids; SD: standard deviation; THEO: theophylline.

Correlations between clinical parameters were assessed in patients with asthma. In line with previous reports, FEV_1% predicted was strongly and positively correlated with the forced expiratory ratio (FER). Moderate positive correlations were identified between BMI and ACQ-5 symptom score (Figure 2.1), and between FeNO and blood eosinophils ($p=0.55$, $p=2.2\times10^{-5}$). In contrast, age showed negative correlations with both IgE levels and FER.

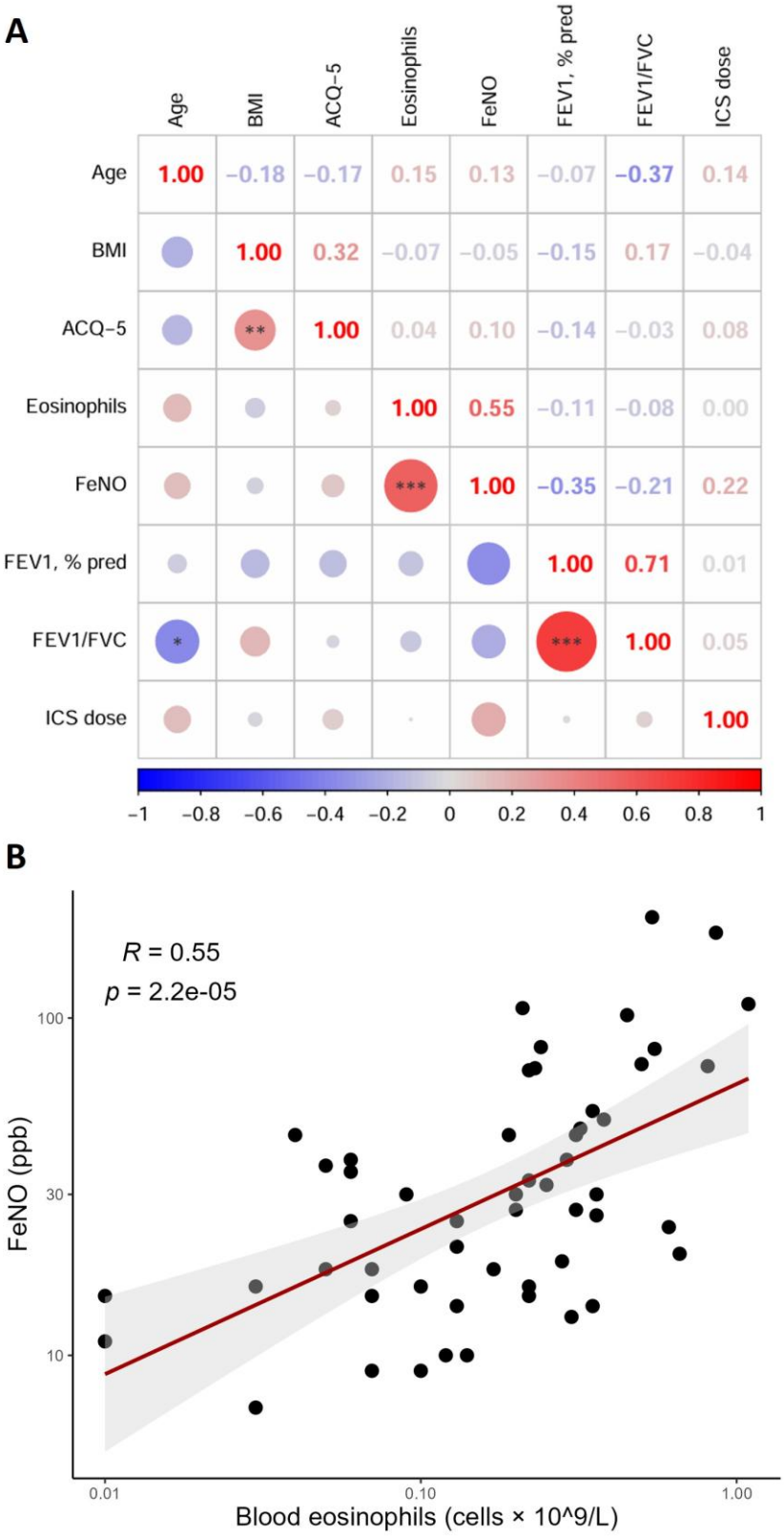


Figure 2.1 Correlations among clinical characteristics in patients with asthma.

(A) Correlation matrix of clinical variables. Spearman correlation matrix showing pairwise associations between clinical characteristics, including age, blood eosinophil counts, fractional

exhaled nitric oxide (FeNO), Asthma Control Questionnaire score (ACQ-5), total immunoglobulin E (IgE), body mass index (BMI), inhaled corticosteroid (ICS) dose, and lung function measures (forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC) and percent predicted FEV_1 (FEV_1% predicted). Circle colour indicates the direction of correlation (red = positive, blue = negative), and circle size reflects the correlation strength. Stars indicate statistical significance of the correlation: $p < 0.05$: *, $p < 0.01$: **, $p < 0.001$: ***.
(B) Scatter plot of the correlation between FeNO levels and blood eosinophil counts (cells × 10^9/L) in patients with asthma. Both axes are log-transformed. A linear regression line (red) with a 95% confidence interval (grey) is fitted to the data. (Spearman's R = 0.45, p = 0.013), indicating a moderate, positive correlation between FeNO levels and blood eosinophil counts in this patient population.

2.4.2 Transcriptomic signatures of asthma independent of ICS therapy

Transcriptomic signatures of asthma

Differentially expressed genes (DEGs) between severe asthma and healthy individuals not receiving ICS were assessed in bronchial biopsies (Table S2.1 in appendix and Figure 2.2). Overall, 947 DEGs were identified, with 192 upregulated and 755 downregulated. Asthma was associated with significant upregulation of canonical type 2 inflammatory genes such as the IL-13–responsive gene *CLCA1* (Woodruff et al., 2007) and the eosinophil chemokine *CCL26* (eotaxin-3) (Couillard et al., 2021). Several additional genes previously implicated in asthma were also upregulated, including the epithelial receptor *CEACAM5* (Singhania et al., 2018), *ALOX15B* (encoding arachidonate 15-lipoxygenase type B), *PHACTR3* (Siddiqui et al., 2018), and the mucin gene *MUC6* (Singhania et al., 2017). *ALOX15B* is essential for ferroptosis in epithelial cells and contributes to the production of pro-resolving lipid mediators (Nagasaki et al., 2022). There was also upregulation of *EREG* (encoding the growth factor epiregulin), which is known to induce TGFα and AREG. In biopsies,

the most highly upregulated gene was *CYP1A1*, a dioxin-inducible cytochrome P450 aryl hydrocarbon hydroxylase implicated in inducing epithelial alarmins during oxidative stress (Wang et al., 2019).

Consistently, each of these genes was also significantly upregulated in bronchial brushings from all asthma patients compared to healthy controls not receiving ICS (Table S2.2 in appendix). In bronchial brushings, the predominant signal was one of transactivation, with 214 genes upregulated and only 72 significantly downregulated. Additional genes upregulated in asthma in brushings specifically included the cystatins *CST1*, *CST2*, *CST4* (type-2 immunomodulatory genes (Singhania et al., 2018)); *FETUB*, a neprilysin inhibitor; *PPBP*, a potent neutrophil chemoattractant and activator (Takahashi, Pavlidis, Ng Kee Kwong, Hoda, Rossios, Sun, Loza, Baribaud, Chanez, Fowler, Horvath, Montuschi, Singer, Musial, Dahlen, Dahlen, Krug, Sandstrom, Shaw, Lutter, Bakke, Fleming, Howarth, Caruso, Sousa, Corfield, Auffray, De Meulder, Lefaudeux, Djukanovic, Sterk, Guo, Adcock, et al., 2018); the eosinophil product *CLC*, the defensins (*DEFA1B, DEFA1, DEFA3*), and several chemokines (*CCL7, CCL13/MCP-4, CCL24/eotaxin-2*).

Amongst downregulated genes in bronchial biopsies in asthma *versus* healthy controls not receiving ICS, there were many (269) immunoglobulin genes, an expected consequence of therapeutic ICS (Marchi et al., 2024). Several other genes were markedly downregulated in both bronchial biopsies and brushings, and are not typically considered steroid-sensitive. These included *WNT2* (Wnt signalling), *KIR2DL4* (an inhibitory receptor on NK and $CD8^+$ T cells), *GABRG1* (a GABAergic inhibitory receptor), and *CNTNAP5* (a contactin-associated gene previously linked to

asthma exacerbations in GWAS). In biopsies only, the most strongly downregulated gene was *AGTR2* (angiotensin II receptor), which has been shown in murine models to protect against airway inflammation and hyperreactivity (Patel et al., 2019). Other notable downregulated genes included *PLA2G2D* (a pro-resolving phospholipase), *MARCO* (a macrophage scavenger receptor), and *CCL19* (a B-cell chemoattractant).

Gene Ontology (GO) analysis of biopsy genes showed enrichment of 'biological process' GO terms for genes upregulated in asthma versus healthy not receiving ICS, including several processes related to epithelial barrier function: 'homophilic cell adhesion via plasma membrane adhesion molecules'; 'keratinocyte differentiation'; and 'ERBB2 signalling', a pathway associated with airway epithelial barrier dysfunction, mucus hypersecretion, remodelling, and delayed epithelial repair in asthma (Inoue et al., 2019) (Figure 2.2). Downregulated GO pathways included terms likely secondary to therapeutic steroids – 'immunoglobulin production'; 'antigen-receptor mediated signalling'; 'leukocyte mediated immunity'; 'leukocyte cell-cell adhesion'; 'response to molecule of bacterial origin' – as well as 'extracellular matrix organization' and 'collagen metabolic process'.

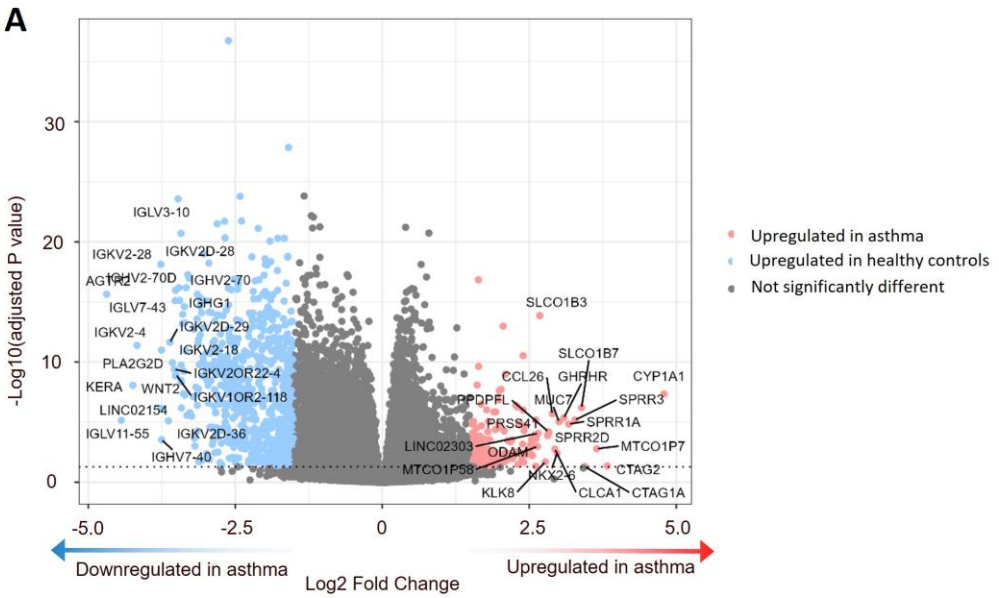

A
-Log10(adjusted P value)
30
20
10
0
IGLV3-10
IGKV2-28
IGKV2D-28
AGTR2
IGHV2-70D
IGHV2-70
IGLV7-43
IGHG1
IGKV2-4
IGKV2D-29
IGKV2-18
PLA2G2D
IGKV2OR22-4
KERA
WNT2
IGKV1OR2-118
LINC02154
IGLV11-55
IGKV2D-36
IGHV7-40
SLCO1B3
SLCO1B7
CCL26
GHRHR
CYP1A1
PPDPFL
MUC7
SPRR3
PRSS41
SPRR1A
SPRR2D
MTCO1P7
LINC02303
ODAM
MTCO1P58
NKX2-6
CTAG2
KLK8
CLCA1
CTAG1A
-5.0
-2.5
0
2.5
5.0
Downregulated in asthma
Log2 Fold Change
Upregulated in asthma
Upregulated in asthma
Upregulated in healthy controls
Not significantly different
B
olefinic compound metabolic process
monocarboxylic acid transport
keratinocyte differentiation
digestion
homophilic cell adhesion via plasma membrane adhesion molecules
negative regulation of myotube differentiation
ERBB2 signaling pathway
response to corticosteroid
protein localization to extracellular region
immunoglobulin production
antigen receptor-mediated signaling pathway
leukocyte mediated immunity
leukocyte cell-cell adhesion
extracellular matrix organization
positive regulation of cytosolic calcium ion concentration
collagen metabolic process
response to molecule of bacterial origin
Upregulated in asthma
Downregulated in asthma
p.adjust
0.025
0.020
0.015
0.010
0.005
GeneRatio
0.025
0.050
0.075
C
Log2 Fold Change
CPA4
GHRHR
KLK8
SLCO1B7
CSN3
CST1
SPRR3
SPRR1A
CST2
MTCO1P7
CLCA1
CPA4
GHRHR
NKX2-6
SLCO1B7
SPRR3
ECEL1P1
SLCO1B1
CST1
ACTBP12
SFTPA2
SFTPA1
GHRHR
CPA4
SLCO1B3
SLCO1B7
SLCO1B1
SPRR1A
SLCO1B3-SLCO1B7
PPDPFL
T2-high asthma
vs
Healthy controls
T2-interminate asthma
vs
Healthy controls
T2-low asthma
vs
Healthy controls
IGHV3OR16-6
IGHV2-70D
LINC02154
IGHV3-49
IGLV3-22
ARL14EPL
IGLV11-55
KERA
IGKV5-2
AGTR2
KERA
ARHGAP36
IGLV4-60
LINC02154
IGHV7-40
IGKV2OR22-4
AGTR2
MTCYBP31
IGLV11-55
IGKV2-4
SPIB
KERA
TRAV26-2
CLEC4C
IGKV2-4
IGKV7-3
PLA2G2D
IGLV11-55
AGTR2
WNT2
Upregulated
Downregulated

Figure 2.2 Differential gene expression and pathway enrichment between severe asthma and healthy individuals not receiving inhaled corticosteroids, in bronchial biopsies.

(A) Volcano plot of differentially expressed genes (DEGs). The x-axis shows $\log_2$ fold change and the y-axis shows $-\log_{10}$ adjusted p-value. Significantly upregulated genes ($\log_2$FC > 1.5, padj < 0.05) are highlighted in red, downregulated genes ($\log_2$FC < –1.5, padj < 0.05) in blue, and non-significant genes in grey. The top 20 upregulated and top 20 downregulated genes (ranked by fold change) are labelled.

(B) Gene Ontology (GO) biological process enrichment of genes differentially regulated in biopsies in asthma and healthy controls not receiving ICS. Dot size indicates gene ratio, and dot colour indicates adjusted p-value. GO terms enriched in each group are plotted on the corresponding side of the x-axis.

(C) Differential gene expression across asthma phenotypes versus healthy controls. All displayed genes are significantly differentially expressed (padj < 0.05). Each point represents a gene's log2 fold change, with red indicating log2 fold change > 1, blue indicating log2 fold change < -1, and grey indicating -1 ≤ log2 fold change ≤ 1.

Transcriptomic signatures of asthma independent of ICS

To distinguish immunopathogenic processes intrinsic to asthma from those secondary to corticosteroid therapy, differential gene expression analysis was performed between patients with treatment-optimised asthma and healthy controls after 4 weeks of fluticasone 500 µg twice daily. Using *DESeq2* and *ComBat* for batch correction, in bronchial biopsies, 184 genes were upregulated and 44 downregulated (Table S2.1 in appendix), while in bronchial brushings, 116 were upregulated and 93 downregulated (Table S2.2 in appendix).

There was significant overlap between biopsies and brushes, with 16 of the top 20 upregulated genes in biopsies also upregulated in brushings. These included the RNA binding protein *SYNCRIP*, the most highly upregulated gene in both; *PINX1*, a gene required for TNFα-induced chemokine expression in ASM cells (Deacon & Knox,

2018); the carboxypeptidase *CPA4*; *CLDN2* which encodes the tight junction protein claudin-2; *B3GNT 6*, an IL-13 inducible gene involved in mucin biosynthesis (Macowan et al., 2025) and several genes also identified in the preceding analysis, including *CST1*, *CST2*, *CST4*, *CEACAM5*, *CLCA1*, and *FETUB*. Other genes of interest upregulated in both brushes and biopsies were genes linked to airways mucin *MUC5AC*, *ITLN1*, and the antigen-presenting molecule *CD1A*. Highly downregulated genes in both biopsies and brushings included *SCGB1A1* (uteroglobin, CC16), consistent with prior reports (Singhania et al., 2017; Singhania et al., 2018; Woodruff et al., 2007). Other highly downregulated genes included *SCGB3A1*, *HIF3A*, and *SST*, which encodes the neuropeptide somatostatin.

To confirm these results, I applied a non-parametric rank product approach, which is robust to batch effects and expression variability (Table 2.3 and Figure 2.3). Amongst the 20 most highly upregulated genes, this confirmed the significance of *MUC5AC*, *SYNCRIP*, and *CEACAM5*, and additionally identifying significant upregulation of *LYZ* (lysozyme), *POSTN* (periostin), *IL33* (alarmin IL-33), *MUC2*, *FOS*, and *ITGB8* (integrin β8, a regulator of TGFβ activation and airway remodelling).

GO analysis of biological processes was performed on bronchial biopsies from patients with asthma compared with healthy controls receiving ICS. The processes most enriched in asthma were related to adaptive and T cell–mediated mucosal immune activation, including 'adaptive immune response', 'regulation of lymphocyte activation', 'positive regulation of immune response', and 'T cell activation' (Table 2.4). Conversely, the processes most enriched in healthy controls were related to mitochondrial function and aerobic respiration, typically associated with anti-

inflammatory immunometabolic cell states. In focused gene set enrichment analysis (GSEA) of immune response GO pathways, asthma was associated with pathways related to T cell-stimulated mast cells, IFN-α-stimulated dendritic cells, virus-stimulated immune cells, and suppression of pathways related to immunoregulatory T cells (Table 2.5).

Table 2.3 Top differentially expressed genes between patient with asthma and healthy controls with ICS in bronchial biopsies.

Top 20 most upregulated and 20 most downregulated genes in bronchial biopsies using a rank product approach comparing all participants with asthma versus healthy control participants receiving inhaled fluticasone propionate 500 mcg bd daily for 4 weeks.

	Biopsies			
	Gene	**RP/Rsum**	**PFP**	**Comments**
Increased in asthma	MUC5AC	13	1.6E-38	Major airway mucin; contributing to mucus hypersecretion.
	MT-ATP8	27	6.7E-33	Mitochondrial ATP synthase component; may reflect metabolic stress.
	LYZ	92	1.2E-23	Encodes lysozyme; elevated in airway inflammation and innate immune response.
	CEACAM5	156	4.6E-20	Cell adhesion molecule; implicated in epithelial remodelling and neutrophilic asthma.
	SYNCRIP	217	7.4E-18	RNA-binding protein; may regulate inflammation-related transcripts in airway cells.
	POSTN	240	3.3E-17	Periostin: biomarker of type 2 inflammation, associated with IL-13-driven airway remodelling.
	IL33	252	6.8E-17	Alarmin cytokine; key initiator of type 2 immunity and eosinophilic inflammation.
	MUC2	253	7E-17	Mucin involved in barrier function; may contribute to mucus overproduction.
	FOS	369	1.3E-14	Immediate early gene; involved in pro-inflammatory signaling and epithelial activation.
	CLCA2	371	1.4E-14	Chloride channel regulator; linked to goblet cell function and mucus production.
	LINC00342	380	1.9E-14	Long noncoding RNA; potential regulatory role in airway inflammation.
	SLC6A14	401	3.9E-14	Amino acid transporter; upregulated in type 2-high asthma and linked to epithelial activation.
	DMBT1	418	6.6E-14	Host defense protein; involved in mucosal immunity and epithelial differentiation.
	EGR1	436	1.2E-13	Transcription factor; mediates inflammatory and stress responses in airway epithelium.
	SLC12A2	504	7.9E-13	Ion transporter (NKCC1); affects airway surface hydration and mucus properties.
	ITGB8	521	1.1E-12	Integrin beta-8; regulates TGF-β activation and airway remodelling.
	ADAM9	539	1.8E-12	Metalloproteinase; involved in epithelial repair and tissue remodelling.
	TMED7	625	1.2E-11	Vesicle trafficking protein; may modulate cytokine signaling pathways.
	TCN1	701	5E-11	Vitamin B12-binding protein; associated with neutrophilic inflammation.
	LYPLA1	712	6.1E-11	Lysophospholipase; potentially linked to lipid metabolism and epithelial homeostasis.
Decreased in asthma	SERPING1	811	1.99E-10	Inhibits complement cascade; downregulation may enhance airway inflammation.
	TUBB4A	796	1.58E-10	Cytoskeletal stability; reduced expression may reflect epithelial structural disruption.
	ADH1B	735	5.92E-11	Metabolizes alcohols and aldehydes; lower levels may impair detoxification in airway cells.
	HBB	717	4.47E-11	Hemoglobin beta; downregulation may indicate reduced oxygen transport or erythroid content.
	TIMP3	585	3.68E-12	Inhibits matrix metalloproteinases; reduced expression may promote airway remodelling and fibrosis.
	GPX3	393	2.19E-14	Antioxidant enzyme; downregulation increases susceptibility to oxidative stress.
	APOD	391	2.15E-14	Lipid transporter; loss may disrupt epithelial lipid homeostasis and repair mechanisms.
	A2M	383	1.64E-14	Broad-spectrum protease inhibitor; reduced levels may enhance proteolytic tissue damage.
	FTH1P16	322	1.59E-15	Pseudogene; possible role in gene regulation through ceRNA networks. Downregulation may impact iron homeostasis.
	TSC22D3	299	6.18E-16	Anti-inflammatory, glucocorticoid-induced gene; lower expression suggests impaired corticosteroid response.
	BPIFA1	231	1.64E-17	Innate immune protein; decreased levels reduce epithelial antimicrobial defense.
	CTSD	190	9.84E-19	Lysosomal protease; downregulation may alter cell turnover or mucus processing.
	CES1	183	5.77E-19	Detoxification enzyme; reduced expression limits epithelial clearance of xenobiotics.
	ALDH3B1	145	1.80E-20	Aldehyde detoxification; lower expression may increase oxidative injury.
	MIR12136	140	1.19E-20	microRNA; downregulation may dysregulate immune and epithelial gene expression networks.
	PSCA	118	8.66E-22	Surface antigen; limited known function in airways, but loss may affect epithelial cell signaling.
	CAPS	56	9.28E-27	Calcium-binding protein; decreased levels may impair vesicle trafficking and secretion.
	CYP4B1	44	1.22E-28	Xenobiotic-metabolizing enzyme; downregulation limits defense against inhaled toxins.
	SCGB3A1	39	1.89E-29	Secretoglobin family; lower levels may impair epithelial repair and immune modulation.
	SCGB1A1	6	1.65E-45	Club cell secretory protein; reduced expression with impaired epithelial protection and anti-inflammatory response.

Abbreviations: RP/Rsum: Rank product/rank sum; PFP: percentage of false positive predictions.

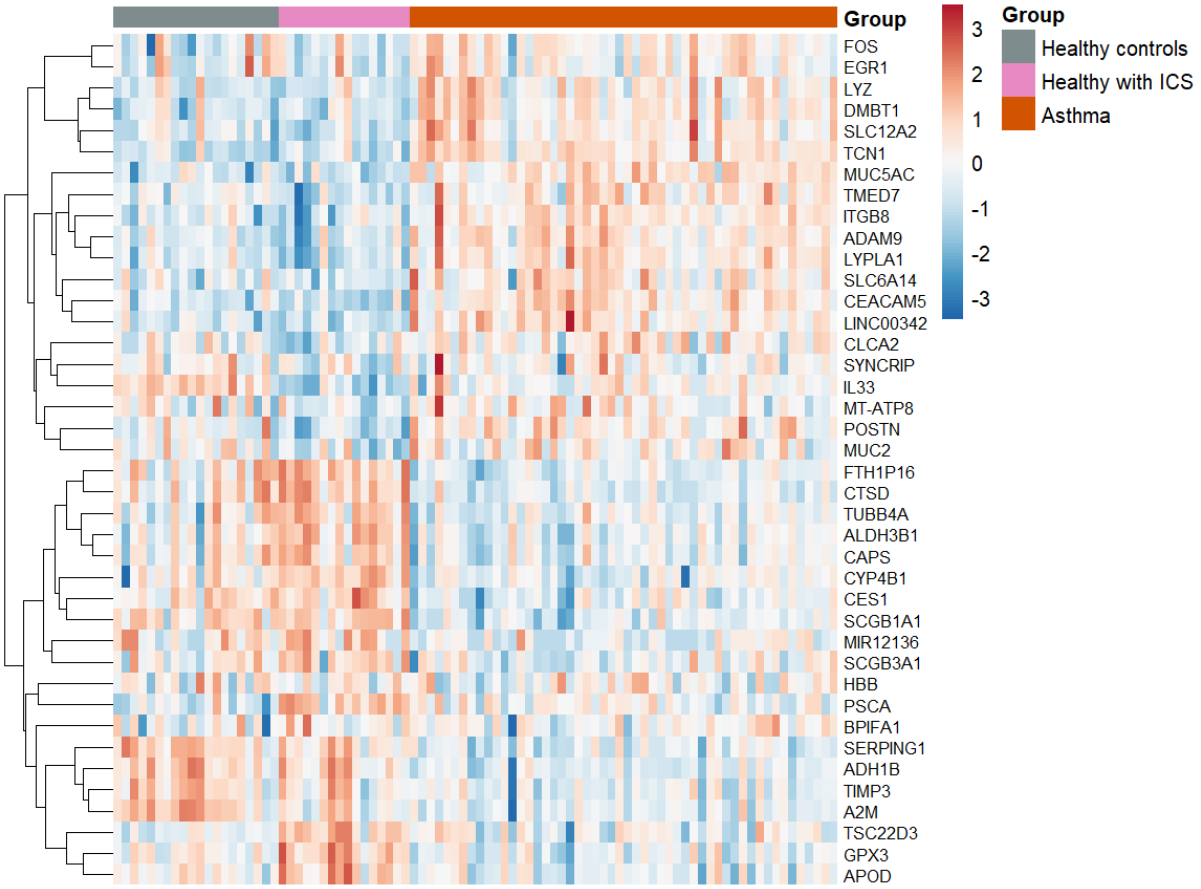


Figure 2.3 Heatmap of differentially expressed genes across patients with asthma and healthy controls with or without ICS.

The heatmap displays the expression levels of selected asthma signature genes across three groups: asthma patients (orange), healthy controls using inhaled corticosteroids (ICS; pink), and healthy controls not using ICS (grey). Each row represents one gene, and each column represents one individual sample. Gene expression values are z-score normalised across samples, with red indicating higher expression and blue indicating lower expression. Genes were selected based on established asthma signatures from Table 2.2.

Table 2.4 Gene Ontology Biological Process pathways between patients with asthma and healthy controls with ICS in bronchial biopsies.

Gene Ontology (GO) Biological Process pathways significantly enriched between asthma patients and healthy controls receiving inhaled corticosteroids were identified using rank product analysis. P-values (p) and adjusted p-values (p adj) are shown, with NES denoting the normalised enrichment score. Pathways shown are ordered by p-value. The x-axis in the figure represents approximate gene ranks.

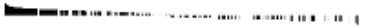
Biopsies	GO Pathway	Gene ranks	NES	p	p adj
Increased in asthma	GOBP_ADAPTIVE_IMMUNE_RESPONSE		4.05	4.3E-128	1.7E-75
	GOBP_REGULATION_OF_LYMPHOCYTE_ACTIVATION		3.56	3.1E-78	3.1E-69
	GOBP_POSITIVE_REGULATION_OF_IMMUNE_RESPONSE		3.44	1.1E-69	1.4E-66
	GOBP_T_CELL_ACTIVATION		3.40	2.2E-67	2.1E-64
	GOBP_IMMUNE_RESPONSE_REGULATING_SIGNALING_PATHWAY		3.46	3.4E-66	2.0E-63
	GOBP_LEUKOCYTE_MEDIATED_IMMUNITY		3.47	8.4E-65	5.1E-62
	GOBP_IMMUNE_RESPONSE_REGULATING_CELL_SURFACE_RECEPTOR_SIGNALING_PATHWAY		3.66	1.8E-65	1.0E-62
	GOBP_POSITIVE_REGULATION_OF_CELL_ACTIVATION		3.49	1.8E-64	8.9E-62
	GOBP_POSITIVE_REGULATION_OF_IMMUNE_RECEPTORS_BUILT_FROM_IMMUNOGLOBULIN_SUPERFAMILY_DOMAINS		3.60	2.3E-63	1.0E-61
	GOBP_LYMPHOCYTE_MEDIATED_IMMUNITY		3.60	7.9E-63	3.1E-60
Reduced in asthma	GOBP_MITOCHONDRION_ORGANIZATION		-1.72	5.3E-08	6.7E-07
	GOBP_MITOCHONDRIAL_TRANSLATION		-2.33	1.9E-08	2.7E-07
	GOBP_FATTY_ACID_METABOLIC_PROCESS		-1.86	1.5E-08	2.2E-07
	GOBP_CILIUM_ORGANIZATION		-1.83	1.0E-08	1.5E-07
	GOBP_AEROBIC_RESPIRATION		-2.11	4.9E-09	7.7E-08
	GOBP_NCRNA_PROCESSING		-1.92	8.2E-10	1.5E-09
	GOBP_TRNA_METABOLIC_PROCESS		-2.21	3.8E-10	7.2E-09
	GOBP_CELLULAR_RESPIRATION		-2.10	3.7E-10	7.0E-09
	GOBP_MITOCHONDRIAL_GENE_EXPRESSION		-2.40	7.7E-11	1.7E-09
	GOBP_MITOCHONDRIAL_RESPIRATORY_CHAIN_COMPLEX_ASSEMBLY		-2.52	1.4E-11	3.5E-10
		0 5000 10000 15000			

Table 2.5 Immune response pathways between patients with asthma and healthy controls with ICS in bronchial biopsies.

Immune response pathways significantly enriched between asthma patients and healthy controls receiving inhaled corticosteroids were identified using rank product analysis. P-values (p) and adjusted p-values (p adj) are shown, with NES denoting the normalised enrichment score. Pathways shown are ordered by p-value. The x-axis in the figure represents approximate gene ranks. NES, normalised enrichment score.

	Biopsies	Immune Signature Geneset	Gene ranks	NES	p	p adj	Comment on Gene Set
Increased in asthma	GSE19888_ADENOSINE_A3R_INH_PRETREAT_AND_ACT_BY_A3R_VS_TCELL_MEMBRANES_ACT_MAST_CELL_UP			3.40	6.3E-40	9.3E-37	Up-regulated in mast cell lines incubated with the peptide ALL1 versus stimulation by T cell membranes.
	GSE7509_UNSTIM_VS_IFNA_STIM_IMMATURE_DC_DN			3.46	2.3E-40	9.3E-37	Down-regulated in immature dendritic cells: untreated versus interferon alpha
	GSE42021_TREG_PLN_VS_CD24INT_TREG_THYMUS_DN			3.34	1.3E-35	2.2E-32	Down-regulated in T reg: peripheral lymph nodes versus thymic CD24 int
	GSE42021_CD24HIL_VS_CD24INT_TREG_THYMUS_DN			3.24	5.1E-34	6.5E-31	Down-regulated in thymic T reg: CD24 high versus CD24 int
	IL_INACT_MONOV_INFLUENZA_A_INDONESIA_05_2005_H5N1_AGE_18_49YO_1DY_UP			2.87	1.6E-34	1.6E-31	Up-regulated in neutrophils 1d after exposure to i.m. inactivated monovalent influenza A vaccine
	POST_VS_0D_PREIMM_MF59_ADJUVANTED_1DY_GENES_IN_BTM_M40_AND_M53_UP			3.57	6.9E-31	5.8E-28	Up-regulated in PBMC 1d postboost with Fluad influenza A vaccine
	GSE7509_UNSTIM_VS_FCGRIIB_STIM_DC_DN			3.19	1.3E-30	9.4E-27	Down-regulated in dendritic cells: untreated versus anti-FcgRIIB
	GSE37533_PPARG1_FOXP3_VS_FOXP3_TRANSDUCED_CD4_TCELL_DN			3.11	4.4E-30	2.5E-27	Down-regulated in CD4 over-expressing: FOXP3 and PPARg1 form of PPARG versus FOXP3
	GSE40685_TREG_VS_FOXP3_KO_TREG_PRECURSOR_DN			3.10	4.3E-29	2.5E-27	Down-regulated in CD4: FOXP3+ T reg versus FOXP3 knockout T reg precursor
	GSE42021_TREG_PLN_VS_TREG_PRECURSORS_THYMUS_DN			3.11	1.6E-29	8.4E-05	Down-regulated in T reg from: peripheral lymph nodes versus thymic precursors
Reduced in asthma	ADENOSINE_A3R_ACT_VS_A3R_ACT_WITH_A3R_INH_PRETREATMENT_IN_MAST_CELL_UP			-1.82	9.5E-06	4.9E-05	Up-regulated in mast cell lines treated with Cl-IB-MECA versus incubated with the ALL1 peptide followed by treatment with Cl-IB-MECA
	GSE35543_IN_VIVO_NTREG_VS_CONVERTED_EX_ITREG_UP			-1.81	8.4E-06	4.4E-05	Up-regulated in comparison between in vivo derived natural T reg and converted induced T reg that lost FOXP3 expression
	GSE23505_UNTREATED_VS_4DAY_IL6_IL1_TREATED_CD4_TCELL_UP			-1.77	8.1E-06	4.2E-05	Up-regulated in CD4 T cells: untreated versus IL1B and IL6
	GSE18791_CTRL_VS_NEWCASTLE_VIRUS_DC_6H_UP			-1.81	6.0E-06	3.3E-05	Up-regulated in comparison of control cDCs versus cDCs infected with Newcastle disease virus at 6 h
	GSE18893_TCONV_VS_TREG_24H_TNF_STIM_UP			-1.79	5.0E-06	2.7E-05	Up-regulated in T conv versus T reg cells treated with TNF for 24h
	GSE31082_DN_VS_DP_THYMOCYTE_UP			-1.77	4.7E-06	2.6E-05	Up-regulated in comparison of CD4-CD8- thymocytes versus CD4+ CD8+ thymocytes
	GSE24210_CTRL_VS_IL35_TREATED_TCONV_CD4_TCELL_DN			-1.81	4.0E-06	2.2E-05	Down-regulated in T conv cells: control versus treated with IL35
	GSE41867_DAY6_VS_DAY8_LCMV_ARMSTRONG_EFFECTOR_CD8_TCELL_DN			-1.78	3.5E-07	2.0E-06	Down-regulated in CD8 T effector cells, acute infection with LCMV-Armstrong: day 6 versus day 8
	CD4_TCELL_BALBC_VS_TH17_ENRI_CD4_TCELL_SKG_PMA_IONO_STIM_FR4NEG_UP			-1.88	7.3E-07	4.8E-06	Up-regulated in FOLR4- CD4 T cells treated by PMA&ionomycin: BALB/c versus SKG mice
	GSE17974_CTRL_VS_ACT_IL4_AND_ANTI_IL12_24H_CD4_TCELL_DN			-1.90	2.1E-07	1.6E-06	Down-regulated in comparison of untreated CD4 T cells at 0 h versus treated with IL4 and anti-IL12 at 24 h
			0 5000 10000 15000				

2.4.3 Transcriptome profiles associated with type 2 biomarkers

Both shared and distinct gene expression patterns of key inflammatory biomarkers in asthma were identified. Several genes were significantly associated with blood eosinophil counts and FeNO, including *CCL26* and *MMP1*. Some genes were associated only with eosinophils, such as *EDN2*, whereas others showed strong associations exclusively with FeNO, such as *POSTN* and *CST1* (Figure 2.4A). Distinct gene signatures were linked to sputum eosinophils and sputum neutrophils. Genes like *CXCL10 (IP-10)* and *STC2* were associated with eosinophils, whereas *PRH2* and *SYT10* were linked to neutrophils (Figure 2.4B).

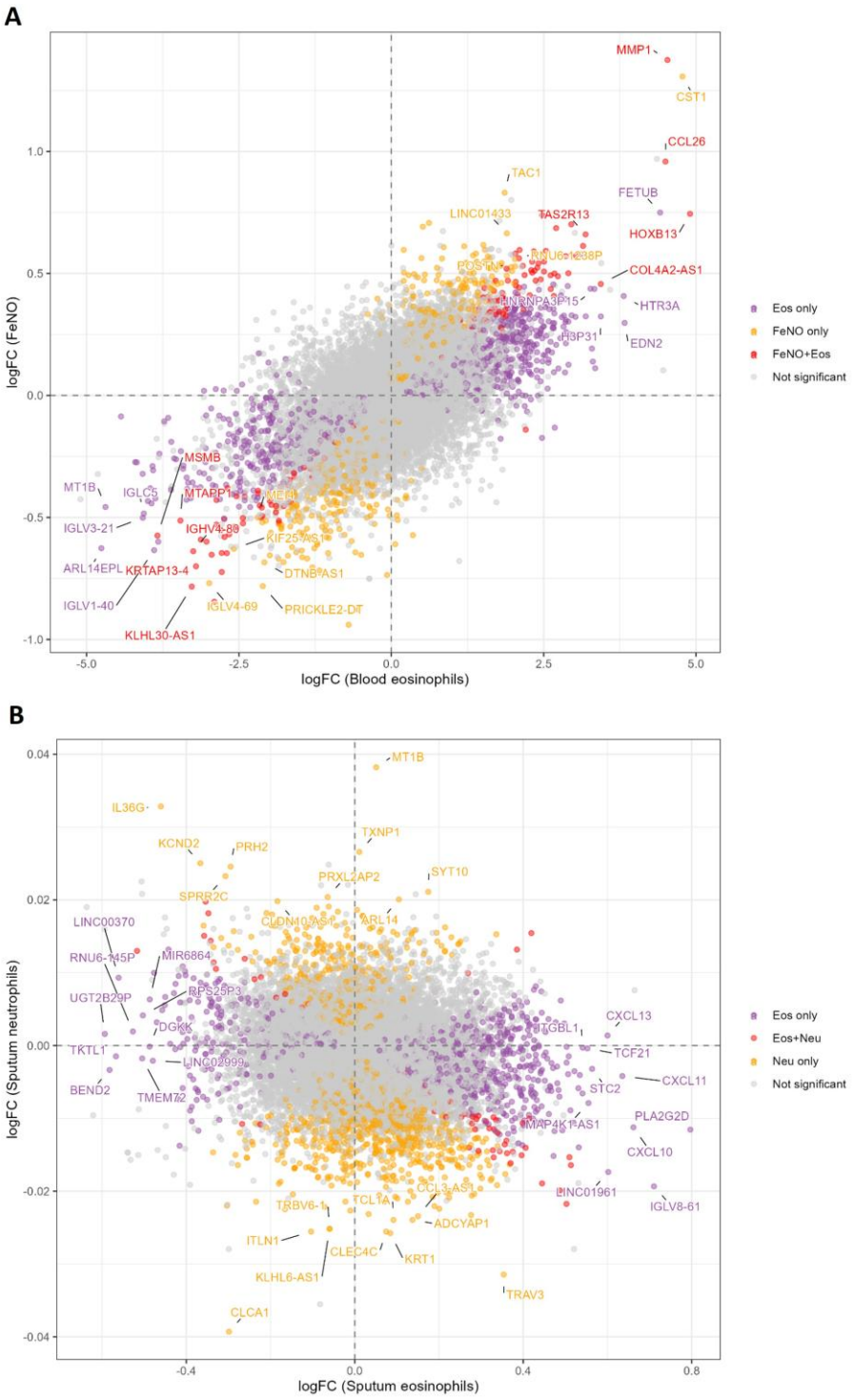


Figure 2.4 Transcriptomic signatures associated with key inflammatory biomarkers of asthma in bronchial biopsies.

(A) Transcriptomic signatures associated with blood eosinophils and FeNO in severe asthma. **(B)** Transcriptomic signatures associated with sputum eosinophils and sputum neutrophils in severe asthma.

2.4.4 Molecular pathways across type 2 phenotypes

Type-2 high asthma

Differential expression between T2-high, T2-intermediate, and T2-low asthma was further assessed using *DESeq2* and *ComBat.* There were 115 genes upregulated, and 38 genes downregulated in T2-high *versus* T2-low asthma (Table S2.3 in appendix and Figure 2.5). T2-high asthma, defined as corticosteroid-resistant, was characterised by strong upregulation of *CST1, CST2, CST4,* and *CLC*. Other upregulated genes included canonical markers of type 2 inflammation (*CLCA1, FETUB, POSTN, SERPINB2, CCL26* and its receptor *CCR3*). In addition, keratin family genes (*KRT1, KRT4*) and genes related to epithelial structure and barrier function (*PRB1, PRB2, ROR1*) were upregulated, suggesting enhanced epithelial remodelling and dysfunction (Schiavone et al., 2020). Additional highly upregulated genes included *CTSG* (cathepsin G, a secreted neutrophil serine protease), *NTRK2* (TrkB, a neurotrophic receptor implicated in airway neuroplasticity) (Dragunas et al., 2020), *CD36* (a phosphorylcholine receptor associated with allergic airway disease in murine models) (Patel & Kearney, 2017), and *CD44* (which promotes antigen-specific Th2 responses in murine models) (Katoh, 2021).

Gene set enrichment analysis (GSEA) of bronchial biopsies revealed that, compared with T2-low and T2-intermediate phenotypes, T2-high asthma was associated with enrichment of adaptive B- and T-cell immune pathways, including 'somatic diversification of immune receptors', 'B-cell differentiation', 'B-cell activation', 'humoral immune response mediated by circulating immunoglobulin', 'T-cell differentiation', and 'negative regulation of activation-induced T-cell death' (Figure 2.5). T2-high asthma was also associated with increased type I interferon signalling, including 'activation of

innate immune response' and 'antiviral mechanisms by IFN-stimulated genes'. Rank product analysis further demonstrated upregulation of signatures for activated effector $CD8^+$ T cells, activated NK cells, and $CD4^+$ Th17 cells (Table 2.7). In addition, GO analysis revealed enrichment of biological processes related to cell proliferation and histone modification (Table 2.6).

Type-2 low asthma

Type-2 low asthma was characterised by strong upregulation of Th1- and IL-17-associated genes, such as *IDO1* (Table S2.3). *IDO1* encodes indoleamine 2,3-dioxygenase 1, an enzyme induced by TLRs, IFNs, and IL-10 that suppresses Th2 responses. *CXCL10* was also upregulated in T2-low asthma (Figure 2.5; Table 2.8). CXCL10 is known to be induced by IFN-γ, to activate Th1 cells, and to function as a mast cell chemoattractant secreted by airway smooth muscle (D. F. Choy et al., 2015). It has also been shown to enhance airway hyperresponsiveness, eosinophilia, IL-4 production, and $CD8^+$ T-cell responses (Gourley et al., 1999) (Figure 2.5 and Table 2.8). Additional upregulated genes included *GBP1* (guanylate binding protein 1, mediating IFN-γ responses) and the neuroimmune genes *SCG2* (secretogranin II) and *CALCA*.

In GSEA of bronchial biopsies, T2-low asthma was associated with enrichment of pathways involved in mucosal pathogen defence, including antigen processing, TCR activation, CD28 co-stimulation, interferon-γ signalling, and complement activation (Figure 2.5). Rank product analysis further revealed that T2-low asthma was associated with upregulation of signatures corresponding to Treg cells and activated mast cells (Table 2.7).

Type-2 intermediate asthma

Only a few genes were upregulated in T2-intermediate asthma compared with T2-high asthma using DESeq2: 8 in biopsies and 6 in brushings (Table S2.3). These included T2-low–associated genes such as *GBP1* and complement factors *C4A* and *C4B* in biopsies, and *CXCL10* in brushings. More specific to T2-intermediate asthma were *IFIT3* (interferon-inducible protein) and *BPIFA1* (*SPLUNC1*), the most strongly upregulated gene in this phenotype. *BPIFA1* is an antimicrobial protein secreted in the airway, whose expression is induced by CC16 and which inhibits IL-13–induced *CCL26* expression.

In GSEA of bronchial biopsies, T2-intermediate asthma was particularly associated with enrichment of myeloid leukocyte pathways, including 'production of molecular mediators of the immune response', 'leukocyte-mediated immunity', 'leukocyte chemotaxis', 'immunoregulatory interactions between lymphoid and non-lymphoid cells', and 'neutrophil degranulation' (Figure 2.5). Rank product analysis further showed that T2-intermediate asthma was associated with upregulation of signatures related to leukocyte responses to viral infection, including virally activated neutrophils (Table 2.8). GO analysis also revealed enrichment of biological processes involving activated leukocytes, lymphocytes, and adaptive T-cell responses (Table 2.9).

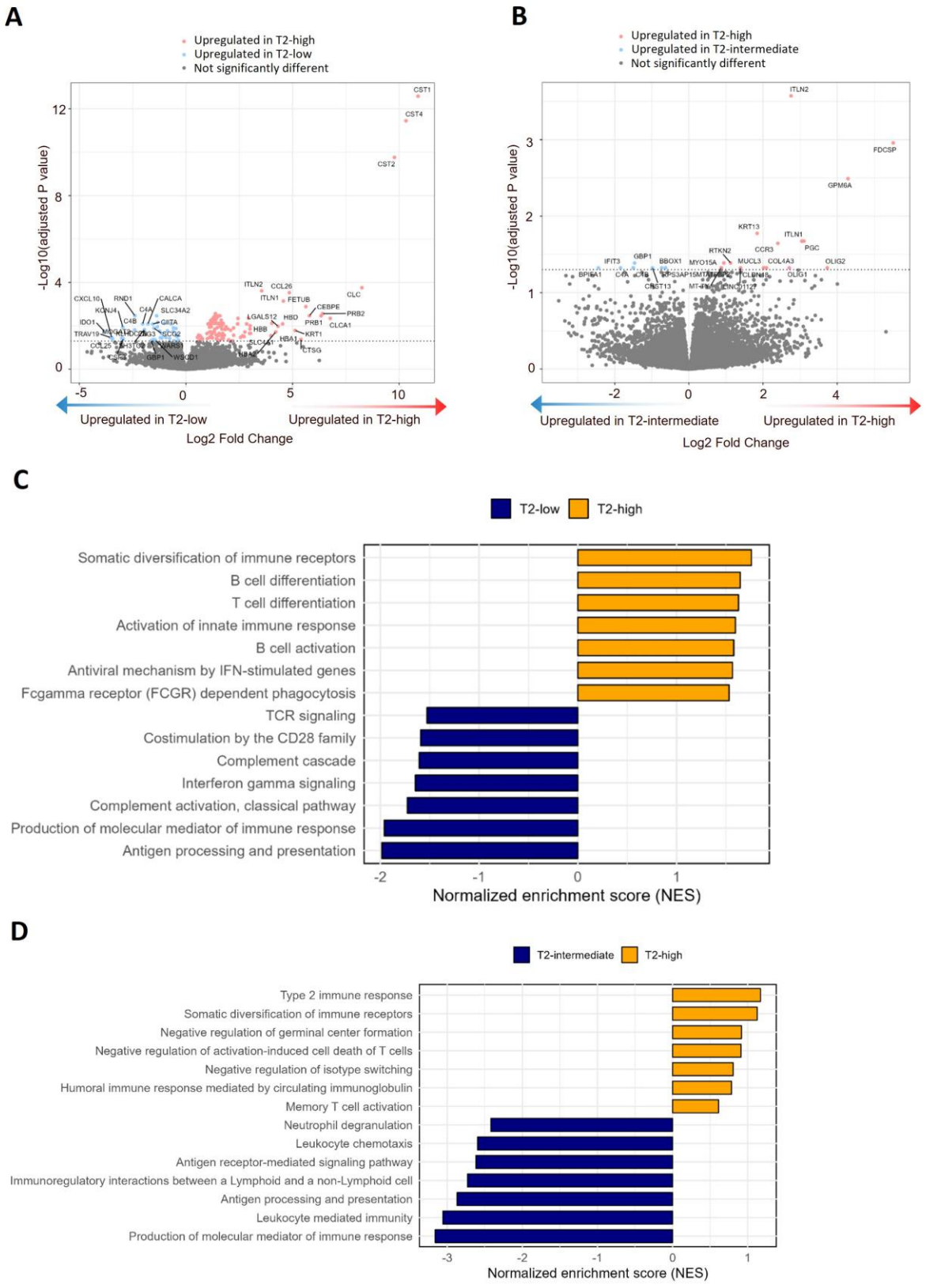


Figure 2.5 Differential gene expression and immune pathway enrichment in bronchial biopsies from T2-high, T2-intermediate, and T2-low asthma.

(A) Volcano plot showing differentially expressed genes between T2-high and -low asthma.

Upregulated genes are shown in red (T2-high) and blue (T2-low); selected genes are labelled. **(B)** Volcano plot showing differentially expressed genes between T2-high and -intermediate asthma. Upregulated genes are shown in red (T2-high) and blue (T2-intermediate); selected genes are labelled.
(C) Gene set enrichment analysis (GSEA) of immune pathways comparing T2-high *versus* T2-low asthma. The normalised enrichment score (NES) is shown for selected pathways, with blue bars indicating enrichment in T2-low and orange bars indicating enrichment in T2-high.
(D) Gene set enrichment analysis (GSEA) of immune pathways comparing T2-high *versus* T2-intermediate asthma. The normalised enrichment score (NES) is shown for selected pathways, with blue bars indicating enrichment in T2-intermediate and orange bars indicating enrichment in T2-high.

Table 2.6 Gene Ontology Biological Process pathways enriched between T2-high and T2-low asthma in bronchial biopsies.

Gene Ontology Biological Process pathways significantly enriched between T2-high and T2-low asthma were identified using rank product analysis. P-values (p) and adjusted p-values (p adj) are shown, with NES denoting the normalised enrichment score. Pathways shown are ordered by p-value. The x-axis in the figure represents approximate gene ranks. NES, normalised enrichment score.

Biopsies	GO Pathway	Gene ranks	NES	p	p adj
Increased in T2-high	GOBP_RIBOSOME_BIOGENESIS		2.43	5.6E-18	4.4E-15
	GOBP_CELL_CYCLE_PHASE_TRANSITION		2.18	4.0E-17	2.2E-14
	GOBP_RIBONUCLEOPROTEIN_COMPLEX_BIOGENESIS		2.19	1.6E-16	7.2E-14
	GOBP_DNA_REPLICATION		2.32	1.3E-15	5.1E-13
	GOBP_MITOTIC_CELL_CYCLE_PHASE_TRANSITION		2.15	5.6E-15	1.8E-12
	GOBP_CHROMOSOME_SEGREGATION		2.20	2.8E-14	7.9E-12
	GOBP_RRNA_METABOLIC_PROCESS		2.27	3.1E-13	7.5E-11
	GOBP_NCRNA_PROCESSING		2.08	5.8E-13	1.3E-10
	GOBP_DNA_CONFORMATION_CHANGE		2.18	6.5E-13	1.4E-10
	GOBP_HISTONE_MODIFICATION		1.96	3.0E-12	5.9E-10
Increased in T2-low	GOBP_PROTEIN_LOCALIZATION_TO_CILIUM		-2.79	2.1E-12	4.4E-10
	GOBP_MICROTUBULE_BASED_TRANSPORT		-2.44	1.5E-13	3.8E-11
	GOBP_AXONEMAL_DYNEIN_COMPLEX_ASEMBLY		-3.07	8.2E-15	2.5E-12
	GOBP_INTRACILIARY_TRANSPORT		-3.11	1.5E-15	5.4E-13
	GOBP_CILIUM_OR_FLAGELLUM_DEPENDENT_CELL_MOTILITY		-2.86	1.5E-16	7.2E-14
	GOBP_MICROTUBULE_BUNDLE_FORMATION		-2.92	2.8E-17	1.8E-14
	GOBP_CILIUM_MOVEMENT		-3.06	2.0E-24	2.0E-21
	GOBP_MICROTUBULE_BASED_MOVEMENT		-2.63	3.5E-25	4.6E-22
	GOBP_AXONEME_ASSEMBLY		-3.53	9.6E-30	1.9E-26
	GOBP_CILIUM_ORGANIZATION		-3.17	7.8E-46	3.1E-42
		0 5000 10000 15000			

Table 2.7 Immune response pathways enriched between T2-high and T2-low asthma in bronchial biopsies.

Immune response pathways significantly enriched between T2-high and T2-low asthma were identified using rank product analysis. P-values (p) and adjusted p-values (p adj) are shown, with NES denoting the normalised enrichment score. Pathways shown are ordered by p-value. The x-axis in the figure represents approximate gene ranks. NES, normalised enrichment score.

	Biopsies	Immune Signature Geneset	Gene ranks	NES	p	p adj	Comment on Gene Set
Increased in T2-high		GSE15750 _DAY6_VS_DAY10_TRAF6KO_EFF_CD8_TCELL_UP		2.94	4.7E-26	2.0E-22	Up-regulated in acute CD8 T_{EFF} cell activation
		GSE13547_CTRL_VS_ANTI_IGM_STIM_BCELL_12H_UP		2.98	7.9E-26	2.0E-22	Up-regulated in B lymphocytes: control versus stimulated by anti-IgM for 12h
		GSE15750_DAY6_VS_DAY10_EFF_CD8_TCELL_UP		2.89	1.4E-24	2.3E-21	Up-regulated in acute CD8 T_{EFF} cell activation
		HOWARD_NK_CELL_INACT_MONOV_INFLUENZA_A_INDONESIA_05_2005_H5N1_AGE_18_49YO_3DY_UP		3.24	6.2E-23	7.9E-20	Up-regulated in natural killer cells 3d after influenza vaccination
		GSE14415_NATURAL_TREG_VS_TCONV_DN		2.85	2.5E-21	2.5E-18	Down-regulated in natural T reg versus T conv
		VAN_DER_BIGGELAAR_PBMC_PREVNAT_9MO_INFANT_STIMULATED_VS_UNSTIMULATED_9MO_UP		2.90	3.5E-19	2.9E-16	Up-regulated in PBMC after vaccination
		GSE18281_SUBCAPSULAR_CORTICAL_REGION_VS_WHOLE_MEDULLA_THYMUS_UP		2.71	4.8E-19	3.3E-16	Up-regulated in thymus subcapsular cortical region versus the whole medulla
		GSE39110_DAY3_VSDAY6_POST_IMMUNIZATION_CD8_TCELL_DN		2.69	5.2E-19	3.3E-16	Down-regulated in CD8 T cells after immunization: day 3 versus day 6
		GSE36476_CTRL_VS_TSST_ACT_40H_MEMORY_CD4_TCELL_YOUNG_DN		2.69	6.2E-19	3.5E-16	Down-regulated in comparison of untreated CD4 memory T cells versus those treated with TSST
		GSE27241_WT_VS_RORGT_KO_TH17_POLARIZED_CD4_TCELL_UP		2.71	2.1E-17	1.0E-14	Up-regulated in polarizing CD4 Th17 cells: wildtype versus RORC knockout
Increased in T2-low		GSE27896_HDAC6_KO_VS_WT_TREG_DN		-1.87	6.0E-06	2.3E-04	Down-regulated in T reg: HDAC6 knockout versus wildtype
		GSE1432_1H_VS_24H_IFNG_MICROGLIA_DN		-1.88	1.7E-06	7.6E-05	Down-regulated microglia cells 1 h after stimulation with IFNG
		GSE42021_TREG_PLN_VS_CD24INT_TREG_THYMUS_DN		-1.88	1.3E-06	6.1E-05	Down-regulated in T reg: peripheral lymph nodes versus thymic CD24 int
		19888_ADENOSINE_A3R_INT_PRETREAT_AND_ACT_BY_A3R_VS_TCELL_MEMBRANES_ACT_ACT_MAST_CELL_UP		-1.89	1.1E-06	5.4E-05	Up-regulated in HMC-1 (mast leukemia) cells incubated with an adenosine receptor agonist
		GSE42021_CD24HI_VS_CD24LOW_TCONV_THYMUS_DN		-1.91	8.5E-07	4.4E-05	Down-regulated in thymic T conv: CD24 high versus CD24 low
		GSE6674_ANTI_IGM_VS_ANTI_IGM_AND_CPG_STIM_BCELL_UP		-1.95	2.3E-07	1.4E-05	Up-regulated in B lymphocytes: anti IgM versus anti IgM and CpG
		THAKAR_PBMC_INACTIVATED_INFLUENZA_AGE_21_30YO_RESPONDERS_28DY_UP		-1.74	1.5E-07	1.0E-05	Up-regulated in PBMC 28d after influenza vaccine
		GSE40685_TREG_VS_FOXP3_KO_TREG_PRECURSOR_UP		-1.98	1.4E-07	9.4E-06	Up-regulated in CD4: FOXP3+ T reg versus FOXP3 knockout T reg precursor
		SCHERER_PBMC_APSV_WETVAX_AGE_18_32YO_5_TO_7DY_UP		-1.89	1.0E-07	7.1E-06	Up-regulated in PBMC at 5 to 7d after exposure to smallpox vaccine
		GSE40685_TREG_VS_FOXP3_KO_TREG_PRECURSOR_DN		-1.99	9.0E-08	6.4E-06	Down-regulated in CD4: FOXP3+ T reg versus FOXP3 knockout T reg precursor.
			0 5000 10000 15000				

Table 2.8 Gene Ontology Biological Process pathways between T2-high and T2-intermediate asthma in bronchial biopsies.

Gene Ontology Biological Process pathways significantly enriched between T2-high and T2-intermediate asthma were identified using rank product analysis. P-values (p) and adjusted p-values (p adj) are shown, with NES denoting the normalised enrichment score. Pathways shown are ordered by p-value. The x-axis in the figure represents approximate gene ranks. NES, normalised enrichment score.

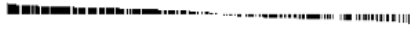
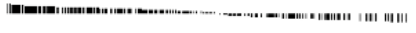
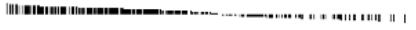
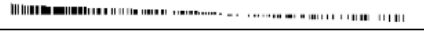
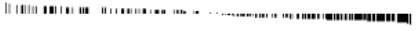
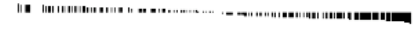
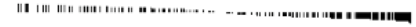
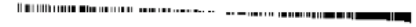
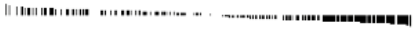
Biopsies	GO Pathway	Gene ranks	NES	p	p adj
Increased in T2-high	GOBP_DNA_REPLICATION		2.39	3.8E-17	4.0E-15
	GOBP_DNA_DEPENDENT_DNA_REPLICATION		2.52	8.5E-15	7.0E-13
	GOBP_CHROMOSOME_SEGREGATION		2.15	5.0E-15	3.1E-11
	GOBP_DNA_CONFORMATION_CHANGE		2.09	8.1E-12	4.1E-10
	GOBP_CELL_CYCLE_CHECKPOINT_SIGNALING		2.29	1.9E-11	9.2E-10
	GOBP_DNA_REPAIR		1.89	2.4E-11	1.1E-09
	GOBP_RNA_SPLICING		2.00	3.4E-11	1.5E-09
	GOBP_RIBONUCLEOPROTEIN_COMPLEX_BIOGENESIS		1.95	3.5E-11	1.5E-09
	GOBP_MRNA_PROCESSING		1.92	6.3E-11	2.6E-09
	GOBP_NUCLEAR_CHROMOSOME_SEGREGATION		2.12	6.7E-28	2.8E-25
Increased in T2-intermediate	GOBP_LYMPHOCYTE_MEDIATED_IMMUNITY		-2.86	2.8E-29	1.1E-26
	GOBP_ACTIVATION_OF_IMMUNE_RESPONSE		-2.85	6.7E-30	2.9E-26
	GOBP_IMMUNE_RESPONSE_REGULATING_CELL_SURFACE_RECEPTOR_SIGNALING_PATHWAY		-2.99	5.4E-30	2.7E-27
	GOBP_IMMUNE_RESPONSE_REGULATING_SIGNALING_PATHWAY		-2.73	1.6E-30	9.2E-28
	GOBP_T_CELL_ACTIVATION		-2.71	3.3E-31	2.2E-30
	GOBP_REGULATION_OF_LYMPHOCYTE_ACTIVATION		-2.81	3.5E-35	2.7E-32
	GOBP_POSITIVE_REGULATION_OF_CELL_ACTIVATION		-2.96	3.1E-35	2.7E-32
	GOBP_LEUKOCYTE_MEDIATED_IMMUNITY		-2.95	1.6E-37	2.0E-34
	GOBP_POSITIVE_REGULATION_OF_IMMUNE_RESPONSE		-2.85	4.2E-38	8.2E-35
	GOBP_ADAPTIVE_IMMUNE_RESPONSE		-3.23	9.3E-56	3.7E-52
		0 5000 10000 15000			

Table 2.9 Immune response pathways enriched between T2-high and T2-intermediate asthma in bronchial biopsies.

Immune response pathways significantly enriched between T2-high and T2-low asthma were identified using rank product analysis. P-values (p) and adjusted p-values (p adj) are shown, with NES denoting the normalised enrichment score. Pathways shown are ordered by p-value. The x-axis in the figure represents approximate gene ranks. NES, normalised enrichment score.

	Biopsies Immune Signature Geneset	Gene ranks	NES	p	p adj	Comment on Gene Set
Increased in T2-high	GSE15750_DAY6_VS_DAY10_TRAF6KO_EFF_CD8_TCELL_UP		2.73	4.2E-22	6.4E-20	Up-regulated in acute CD8 TEFF cell activation
	HOWARD_NK_CELL_INACT_MONOV_INFLUENZA_A_INDONESIA_05_2005_H5N1_AGE_18_49YO_3DY_UP		3.07	9.3E-21	1.2E-18	Up-regulated in natural killer cells 3d after influenza vaccination
	GSE13547_CTRL_VS_ANTI_IGM_STIM_BCELL_12H_UP		2.66	1.6E-19	1.6E-17	Up-regulated in B lymphocytes: control versus stimulated by anti-IgM for 12h
	GSE15750_DAY6_VS_DAY10_EFF_CD8_TCELL_UP		2.59	1.0E-18	8.9E-17	Up-regulated in acute CD8 TEFF cell activation
	GSE18893_TCONV_VS_TREG_24H_TNF_STIM_UP		2.41	2.8E-15	1.5E-15	Up-regulated in comparison of wild type CD8 effector T cells at day 6 versus those at day 10.
	GSE39556_CD8A_DC_VS_NK_CELL_MOUSE_3H_POST_POLYIC_INJ_UP		2.35	9.8E-14	4.3E-12	Up-regulated after poly(IC) injection: CD8A dendritic cells versus NK cells
	GSE27241_WT_VS_RORGT_KO_TH17_POLARIZED_CD4_TCELL_UP		2.45	1.3E-13	5.6E-12	Up-regulated in polarizing CD4 Th17 cells: wildtype versus RORC knockout
	GSE19888_ADENOSINE_A3R_ACT_VS_TCELL_MEMBRANES_ACT_AND_A3R_INH_PRETREAT_IN_MAST_CELL_DN		2.36	4.9E-13	1.8E-11	Down-regulated in HMC-1 (mast leukemia) cells: Cl-IB-MECA versus incubated with the peptide ALL1
	GSE25088_WT_VS_STAT6_KO_MACROPHAGE_IL4_STIM_DN		2.29	2.0E-12	7.0E-11	Down-regulated in bone marrow-derived macrophages treated with IL4: wildtype versus STAT6 knockout
	GSE21546_UNSTIM_VS_ANTI_CD3_STIM_DP_THYMOCYTES_DN		2.28	4.0E-12	1.3E-10	Down-regulated in double positive thymocytes: untreated versus stimulated by anti-CD3
Increased in T2-intermediate	GAUCHER_PBMC_VF_VAX_STAMARIL_UNKNOWN_AGE_30YO_UP		-3.21	4.7E-32	2.4E-29	Up-regulated in PBMC after exposure to YF-Vax/Stamaril
	GSE42021_TREG_PLN_VS_CD24INT_TREG_THYMUS_DN		-3.31	2.1E-34	1.2E-31	Down-regulated in T reg: peripheral lymph nodes versus thymic CD24 int
	GSE37533_PPARG1_FOXP3_VS_FOXP3_TRANSDUCED_CD4_TCELL_DN		-3.32	1.0E-34	6.4E-31	Down-regulated in CD4 over-expressing: FOXP3 and PPARg1 form of PPARG versus FOXP3
	GSE42021_TREG_VS_TCONV_PLN_UP		-3.38	5.0E-37	3.7E-34	Up-regulated in cells from peripheral lymph nodes: T reg versus T conv
	HOWARD_NEUTROPHIL_INACT_MONOV_INFLUENZA_A_INDONESIA_05_2005_H5N1_AGE_18_49YO_1DY_UP		-2.99	9.7E-38	8.2E-35	Up-regulated in neutrophils 1d after exposure to inactivated monovalent influenza A split-virus vaccine
	GAUCHER_PBMC_VF_VAX_STAMARIL_UNKNOWN_AGE_7DY_UP		-3.23	8.1E-39	8.2E-36	Up-regulated in PBMC after exposure to YF-Vax/Stamaril
	GSE42021_CD24HIL_VS_CD24INT_TREG_THYMUS_DN		-3.43	5.1E-39	6.5E-36	Down-regulated in thymic T reg: CD24 high versus CD24 int
	GSE19888_ADENOSINE_A3R_INH_PRETREAT_AND_ACT_BY_A3R_VS_TCELL_MEMBRANES_ACT_MAST_CELL_UP		-3.49	4.5E-48	7.6E-47	Up-regulated in HMC-1 cells: incubated with peptide ALL then with Cl-IB-MECA versus stimulation by T cell membranes
	OWARD_PBMC_MONOV_INFLUENZA_A_INDONESIA_05_2005_H5N1_AGE_19_39YO_A503_ADJUVANT_VS_BUFFER_1DY_UP		-3.20	6.2E-45	1.6E-41	Up-regulated in PBMC vaccinated with AS03 adjuvant vs PBS after exposure to inactivated influenza A vaccine
	SCHERER_PBMC_APSV_WETVAX_AGE_18_32YO_5_TO_7DY_UP		-3.49	5.8E-48	2.9E-44	Up-regulated in PBMC at 5 to 7d after exposure to smallpox vaccine
		0 5000 10000 15000				

2.4.5 Cluster and network analyses across type 2 phenotypes

K-means clustering identified six gene clusters (C1–C6) that distinguished T2-high, -intermediate, and -low phenotypes (Figure 2.6). T2-high asthma featured upregulation of C1 (epidermal development) and C5 (cytoplasmic translation, viral process), and downregulation of C2 (cilial organisation), which showed increasing expression from T2-intermediate to T2-low asthma and health. T2-low asthma specifically upregulated C3 (gland development, fatty acid metabolism). T2-intermediate asthma uniquely showed the highest expression of C4 (immune mediator, immunoglobulin production) and C6 (leukocyte migration).

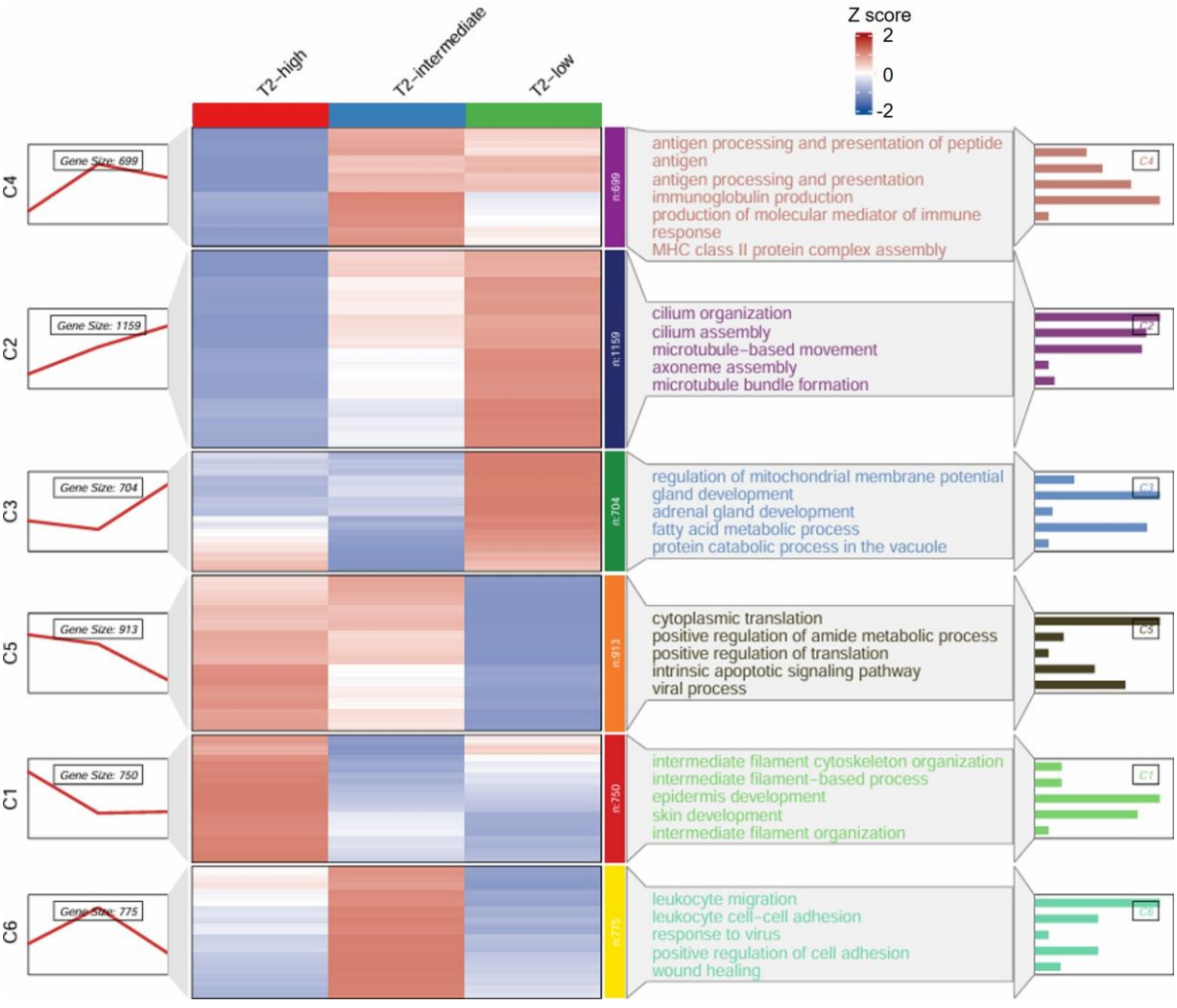
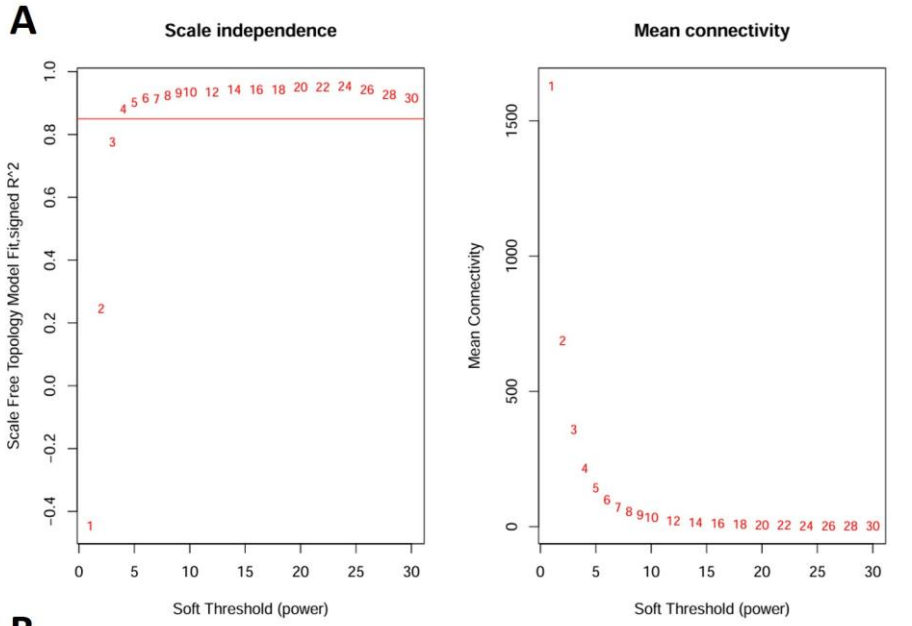


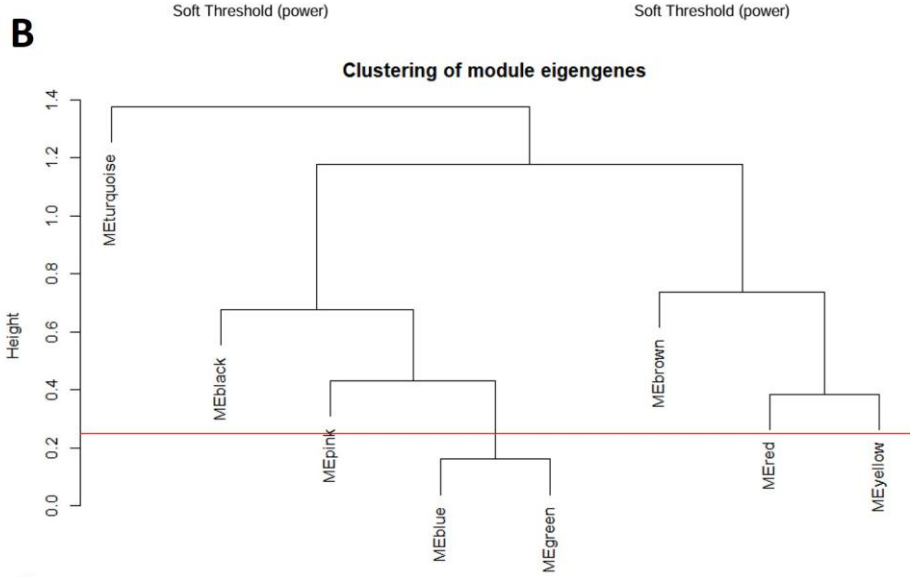
Figure 2.6 Gene cluster in bronchial biopsies across T2-high, -intermediate, and

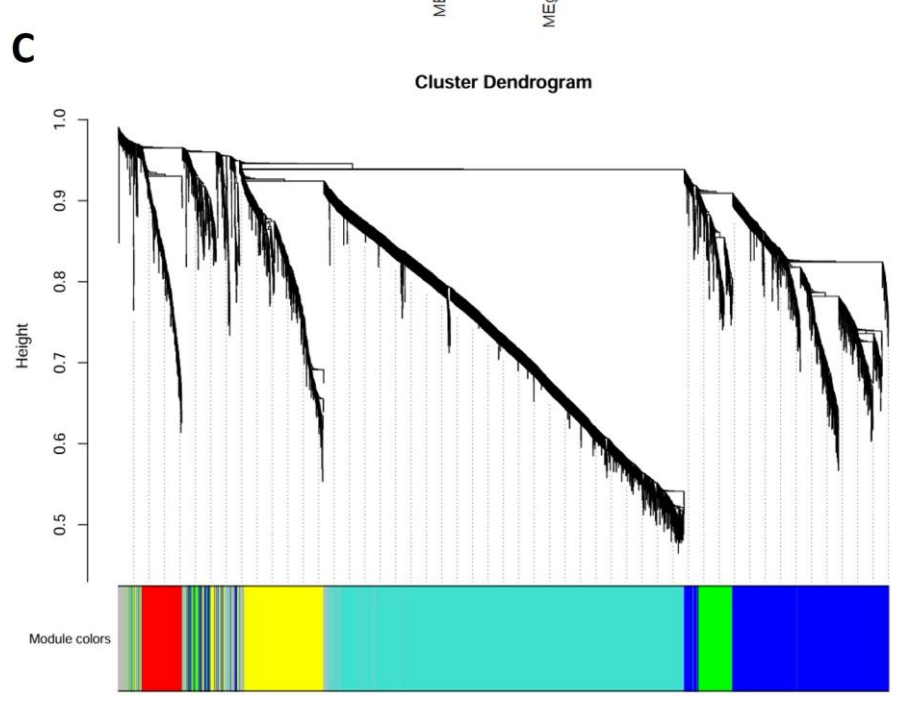
–low asthma.

Heatmap of gene clusters showing pathway-level transcriptomic differences across T2-high, -intermediate, and -low asthma in bronchial biopsies. Genes were grouped into distinct co-expression clusters (C1–C6) using *k*-means clustering based on shared expression profiles. The line plots on the left show the average expression profile of each gene cluster across the T2 asthma phenotype. The heatmap displays Z-score-normalised gene expression values with rows corresponding to genes and columns grouped by T2 asthma phenotype. Red indicates higher expression and blue indicates lower expression. The top enriched Gene Ontology (GO) biological process terms for each cluster are shown on the right. Coloured bars reflect the statistical significance of enrichment.

To explore associations between specific gene modules and clinical traits, weighted gene co-expression network analysis (WGCNA) was performed (Figure 2.8). The most significant module–trait relationships were observed for T2-intermediate asthma. It was positively associated with the 'immune response' module, enriched for GO terms including 'immune response-regulating signaling pathway', 'leukocyte mediated immunity', 'regulation of effector process', 'leukocyte proliferation' and 'lymphocyte proliferation'; T2-intermediate asthma was also positively associated with a 'Pathogen defence' module, enriched for responses to viruses, symbionts and type II interferon. This module was negatively associated with FeNO levels, consistent with the mutually exclusive association previously described between FeNO and *Haemophilus influenzae* (Jabeen, Sanderson, Tine, et al., 2024). A 'Development and metabolism' module was associated positively with male sex and negatively with symptoms (ACQ-5), whereas an 'organic metabolism' module, enriched for xenobiotic metabolic processes, was strongly associated with female sex.


A
Scale independence
Scale Free Topology Model Fit,signed R^2
Soft Threshold (power)
Mean connectivity
Mean Connectivity
Soft Threshold (power)


B
Clustering of module eigengenes
Height
MEturquoise
MEblack
MEpink
MEblue
MEgreen
MEbrown
MEred
MEyellow


C
Cluster Dendrogram
Height
Module colors

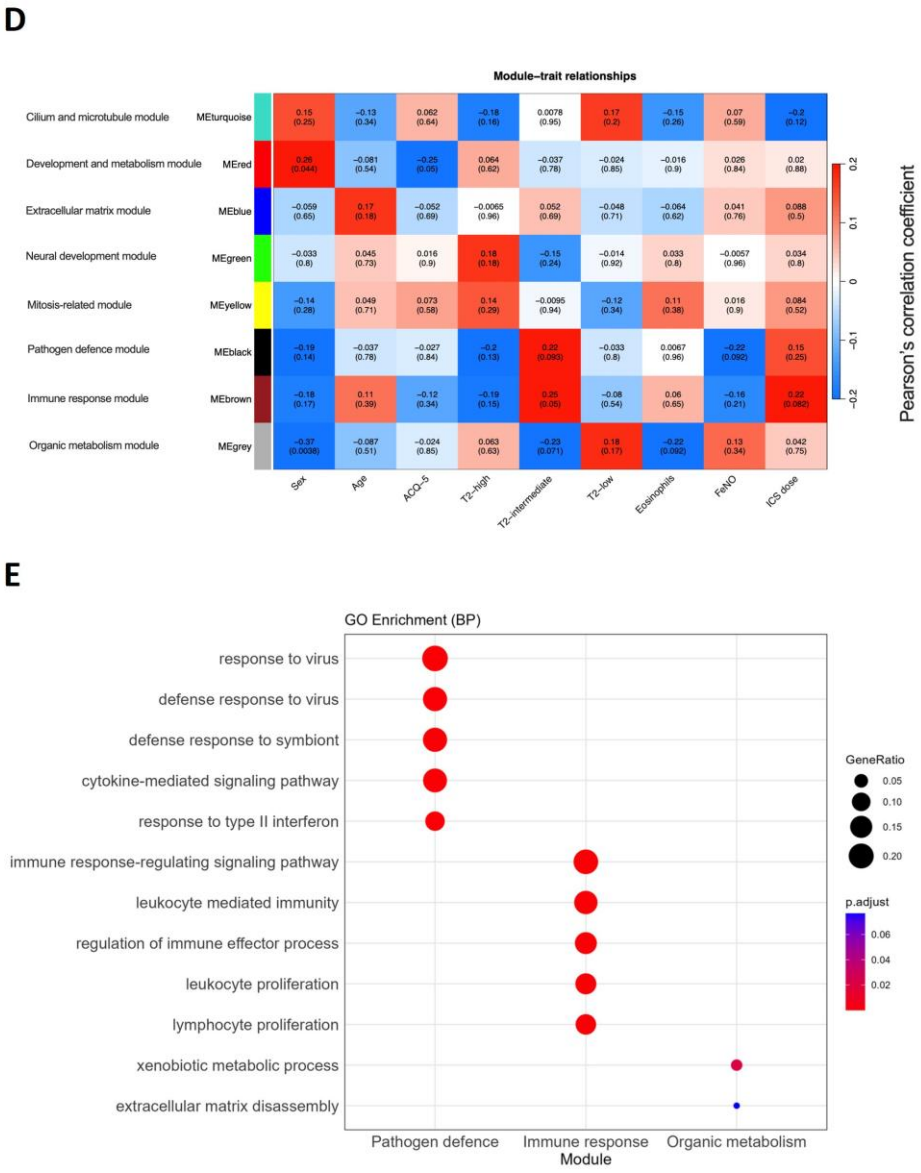


Figure 2.8 Weighted gene co-expression network analysis (WGCNA) of differentially expressed genes in bronchial biopsies from patients with asthma.

(A) Analysis of network topology for various soft-thresholding powers. The left panel shows the scale-free topology fit index (R^2) as a function of the soft-thresholding power, while the right panel shows the mean connectivity. A soft-thresholding power was chosen to balance scale independence and mean connectivity.

(B) Clustering of module eigengenes based on their correlation. Modules with similar expression profiles across samples are grouped, indicating higher-order relationships among co-expression modules.

(C) Gene clustering dendrogram based on topological overlap, with assigned module colours displayed below. Each branch corresponds to a gene cluster (module) identified by dynamic tree cutting, with colour assignments representing distinct modules.

(D) Modules were named according to their top significantly enriched Gene Ontology (GO) biological process term. Each cell displays the correlation coefficient (top) and the corresponding p-value (bottom, in parentheses). Blue indicates negative correlations, and red indicates positive correlations; more saturated colours represent stronger associations.
(E) Gene Ontology (GO) enrichment analysis of selected WGCNA modules. The dot plot displays representative GO biological process terms that are enriched in the selected modules. Dot size reflects the proportion of genes in each module associated with the GO term (GeneRatio), and dot colour represents the adjusted p-value. ME: module eigengene. For sex, females were compared with males.

2.4.6 Cell composition across type 2 phenotypes

Healthy controls not receiving ICS were associated with pathways reciprocal to those described above, and with downregulation of $CD4^+$ T-cell activation and virally activated $CD8^+$ T cells. In cell deconvolution analysis of bronchial biopsy transcriptomic data using CIBERSORTx, asthma was enriched for mast cells, activated dendritic cells (DCs), and activated natural killer (NK) cells, but depleted in plasma cells, naïve B cells, $CD8^+$ T cells, and regulatory T cells (Tregs) (Figure 2.9). An alternative approach, comparing with single-cell signatures from a lung cell atlas (Travaglini et al., 2020), confirmed enrichment of NK cells and plasmacytoid DCs in asthma, and additionally identified enrichment of lung B cells, naïve and effector memory $CD4^+$ T cells, naïve $CD8^+$ T cells, classical monocytes, adventitial fibroblasts, and natural killer T cells (Table 2.10). Cell deconvolution revealed distinct immune cell enrichments across asthma subtypes, with naïve lung CD8 T cells and NK cells increased in T2-high asthma, mast cells and NK cells enriched in T2-intermediate asthma, and mast cells increased in T2-low asthma (Table 2.11 and Table 2.12).

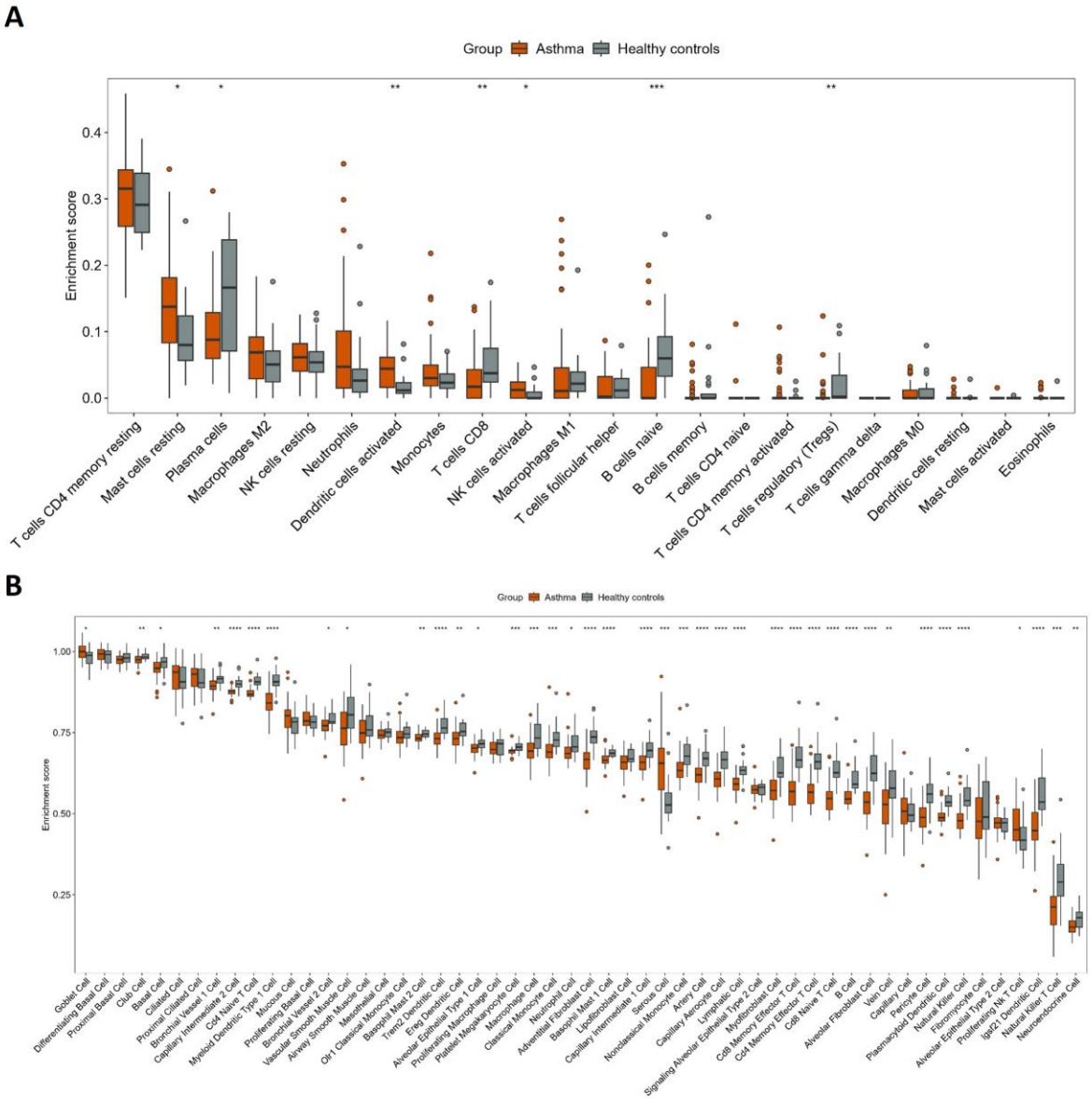


Figure 2.9 Cell type composition of patients with asthma and healthy controls in bronchial biopsies.

Cell type deconvolution was performed using CIBERSORTx (A) and the Travaglini Lung Atlas (B), comparing asthma patients with healthy controls not receiving ICS. Boxplots display median and interquartile ranges. Statistical significance was assessed using the Mann–Whitney U test (A) and ANOVA (B).

(A) Immune cell type composition of patients with asthma and healthy controls.

(B) Lung cell type composition of patients with asthma and healthy controls.

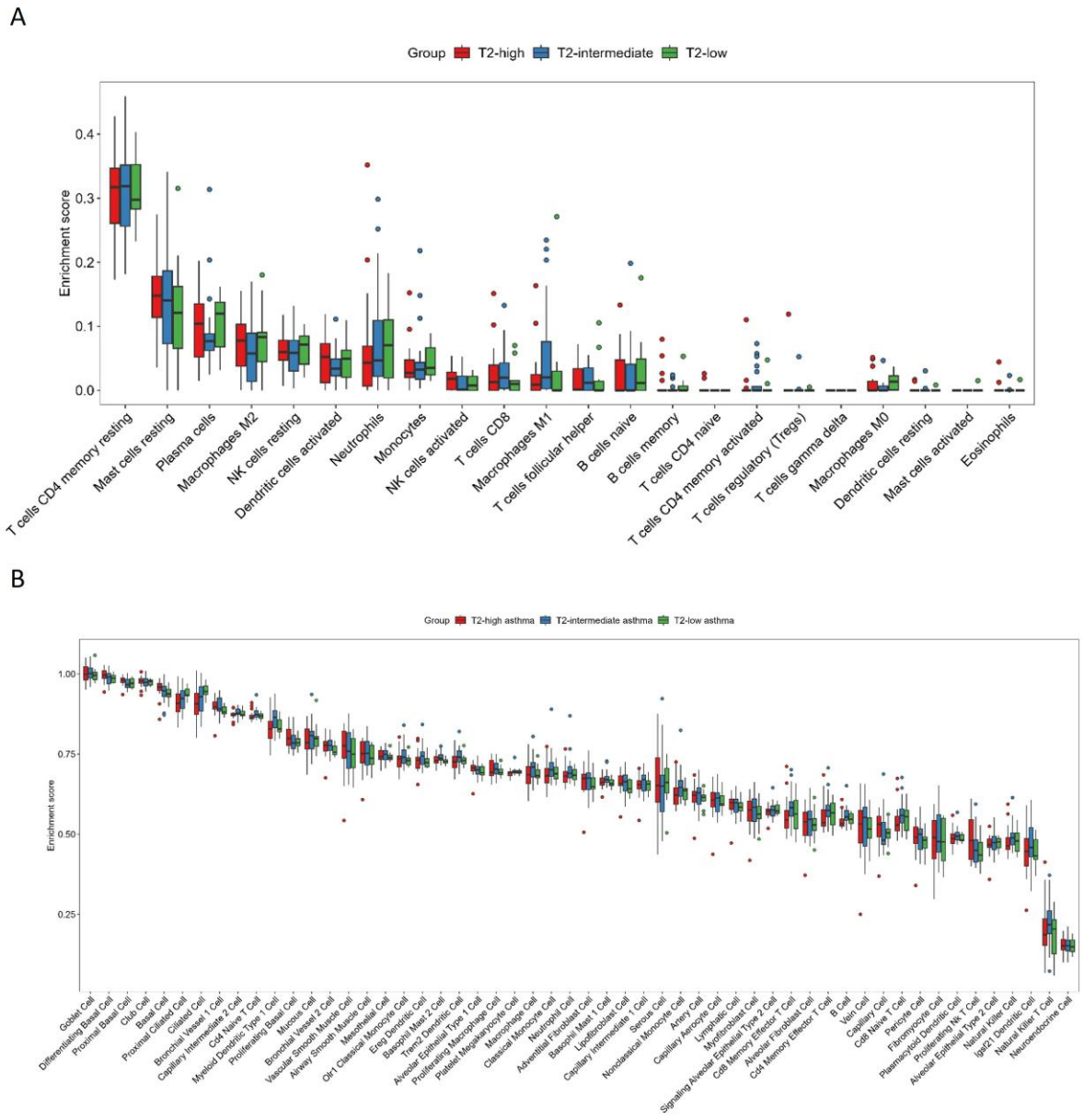


Figure 2.10 Cell type composition across type 2 phenotype in bronchial biopsies.

Cell type deconvolution was performed using CIBERSORTx (A) and the Travaglini Lung Atlas (B), comparing T2-high, T2-intermediate, and T2-low asthma phenotypes. Boxplots display median and interquartile ranges. Statistical significance was assessed using the Mann–Whitney U test (A) and ANOVA (B).

(A) Immune cell type composition across T2-high, -intermediate, and -low asthma groups.

(B) Lung cell type composition across T2-high, -intermediate, and -low asthma groups.

Table 2.10 Lung cell type pathways between patients with asthma and healthy controls with ICS in bronchial biopsies.

Lung cell type pathways significantly enriched between asthma patients and healthy controls receiving inhaled corticosteroids were identified using rank product analysis. P-values (p) and adjusted p-values (p adj) are shown, with NES denoting the normalised enrichment score. Pathways shown are ordered by p-value. The x-axis in the figure represents approximate gene ranks. NES, normalised enrichment score.

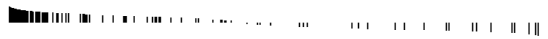
	Biopsies	Predicted cell type	Gene ranks	NES	p	p adj
Increased in asthma		LUNG_CD8_NAIVE_T_CELL		3.37	5.4E-34	2.3E-32
		LUNG_NATURAL_KILLER_CELL		3.04	1.7E-23	3.7E-22
		LUNG_CD4_MEMORY_EFFECTOR_T_CELL		3.21	5.2E-23	7.5E-22
		LUNG_B_CELL		2.94	2.4E-22	2.5E-21
		LUNG_CLASSICAL_MONOCYTE_CELL		2.61	3.6E-20	3.1E-19
		LUNG_CD4_NAIVE_T_CELL		2.54	3.0E-13	2.1E-12
		LUNG_ADVENTITIAL_FIBROBLAST_CELL		2.15	7.5E-12	4.6E-11
		LUNG_CD8_MEMORY_EFFECTOR_T_CELL		2.66	8.5E-10	4.6E-10
		LUNG_PLASMACYTOID_DENDRITIC_CELL		2.40	1.1E-10	5.2E-10
		LUNG_NATURAL_KILLER_T_CELL		2.65	1.6E-10	6.7E-10
			0 5000 10000 15000			

Table 2.11 Lung cell type pathways enriched between T2-high and T2-low asthma in bronchial biopsies.

Lung cell type pathways significantly enriched between T2-high and T2-low asthma were identified using rank product analysis. P-values (p) and adjusted p-values (p adj) are shown, with NES denoting the normalised enrichment score. Pathways shown are ordered by p-value. The x-axis in the figure represents approximate gene ranks. NES, normalised enrichment score.

	Biopsies	Predicted cell type	Gene ranks	NES	p	p adj
Increased in T2-high		LUNG_PROLIFERATING_NK_T_CELL		2.67	1.1E-16	4.8E-15
		LUNG_BASAL_CELL		2.38	8.1E-14	1.7E-12
		LUNG_DIFFERENTIATING_BASAL_CELL		2.13	3.3E-09	4.7E-08
		LUNG_BRONCHIAL_VESSEL_1_CELL		1.80	2.6E-06	2.8E-05
		CD4_NAIVE_T_CELL		1.68	5.3E-04	3.2E-03
		LUNG_GOBLET_CELL		1.46	6.8E-03	3.3E-02
Increased in T2-low		LUNG_INTERMEDIATE_2_CELL		-1.30	6.2E-03	3.3E-02
		LUNG_BASOPHIL_MAST_1_CELL		-1.56	4.2E-04	3.0E-03
		LUNG_BASOPHIL_MAST_2_CELL		-1.49	8.9E-05	7.6E-04
			0 5000 10000 15000			

Table 2.12 Lung cell type pathways enriched between T2-high and T2-intermediate asthma in bronchial biopsies.

Lung cell type pathways significantly enriched between T2-high and T2-intermediate asthma were identified using rank product analysis. P-values (p) and adjusted p-values (p adj) are shown, with NES denoting the normalised enrichment score. Pathways shown are ordered by p-value. The x-axis in the figure represents approximate gene ranks. NES, normalised enrichment score.

	Biopsies	Predicted cell type	Gene ranks	NES	p	p adj
Increased in T2-high		LUNG_PROLIFERATING_NK_T_CELL		2.49	1.9E-13	9.0E-13
		LUNG_BASAL_CELL		1.98	1.4E-07	4.6E-07
		LUNG_DIFFERENTIATING_BASAL_CELL		1.57	1.3E-03	2.4E-03
Increased in T2-intermediate		LUNG_NEUTROPHIL_CELL		-2.11	1.4E-12	5.6E-12
		LUNG_CD4_MEMORY_EFFECTOR_T_CELL		-2.86	2.8E+13	1.2E-12
		LUNG_B_CELL		-2.63	9.3E-15	5.0E-14
		LUNG_MACROPHAGE_CELL		-2.54	3.6E-15	2.2E-14
		LUNG_BASOPHIL_MAST_1_CELL		-2.41	4.7E-16	3.4E-15
		LUNG_NATURAL_KILLER_CELL		-2.87	9.5E-18	8.2E-17
		LUNG_NONCLASSICAL_MONOCYTE_CELL		-2.76	1.2E+18	1.3E-17
		LUNG_BASOPHIL_MAST_2_CELL		-2.22	4.0E-19	5.8E-18
		LUNG_CD8_NAIVE_T_CELL		-3.02	6.5E-21	1.4E-19
		LUNG_CLASSICAL_MONOCYTE_CELL		-2.85	7.7E-26	3.3E-24
			0 5000 10000 15000			

2.4.7 Transcription factors across type 2 phenotypes

The activity of the 20 most differentially expressed transcription factors across phenotypes was inferred (Figure 2.11). T2-high and T2-intermediate asthma were characterised by common upregulation of eight transcription factors, including *MYC, HIF1A,* and *STAT1*, whereas strong upregulation of *ATF3* and *ZBTB7A* was specific to T2-low asthma. T2-intermediate asthma was uniquely characterised by upregulation of nine transcription factors, including *NFKB1, TBX21, IRF1, IRF2, BATF,* and *STAT2*.

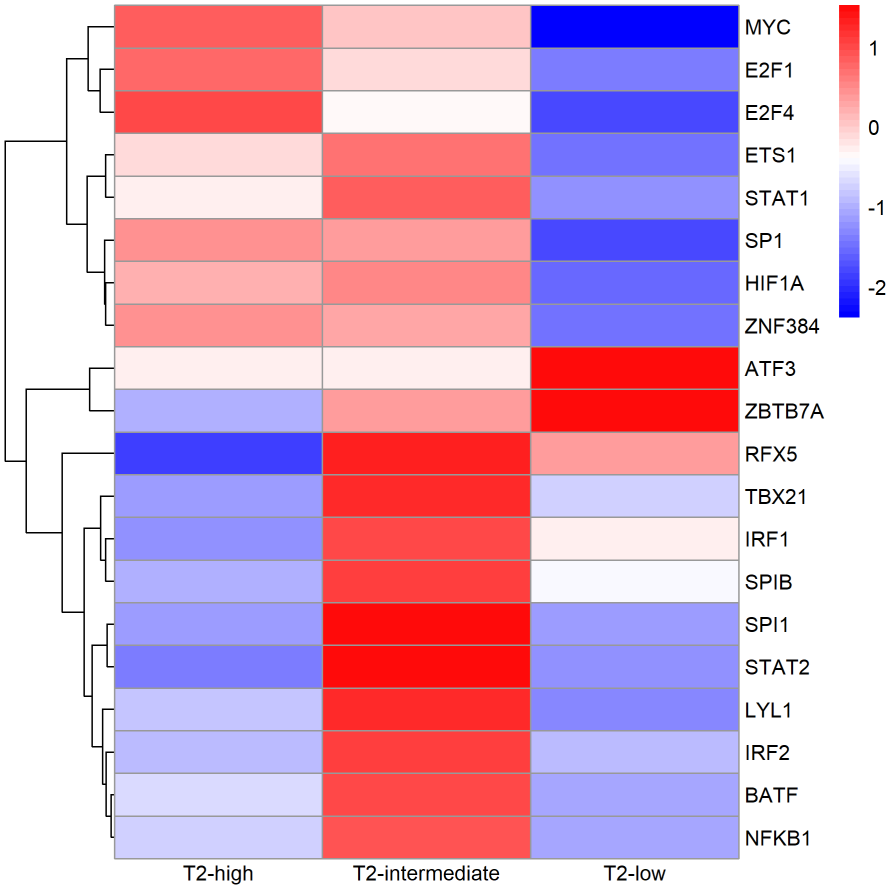


Figure 2.11 Transcription factor activity across T2-high, -intermediate, and –low asthma in bronchial biopsies.

Transcription factor (TF) enrichment across T2-high, -intermediate, and -low asthma in

bronchial biopsies. The heatmap displays inferred TF activity scores based on the expression of their target genes. Rows represent transcription factors, and columns represent T2 asthma phenotype. Red indicates higher inferred activity and blue indicates lower activity.

2.4.8 Type 2 and IL-17 signature expression across type 2 phenotypes

Previous transcriptomic analyses of airway biopsies had identified T2 cytokine- and IL-17-dependent gene signatures (D.F. Choy et al., 2015; Ostling et al., 2019) (Figure 2.12). In a gene set variation analysis of 57 bronchial brushings, significant enrichment of the T2-high gene score was observed in participants, with a similar but non-significant trend in bronchial biopsies. In contrast, a significant increase in the IL-17 score was observed in T2-low asthma in bronchial brushings, but not in biopsies.

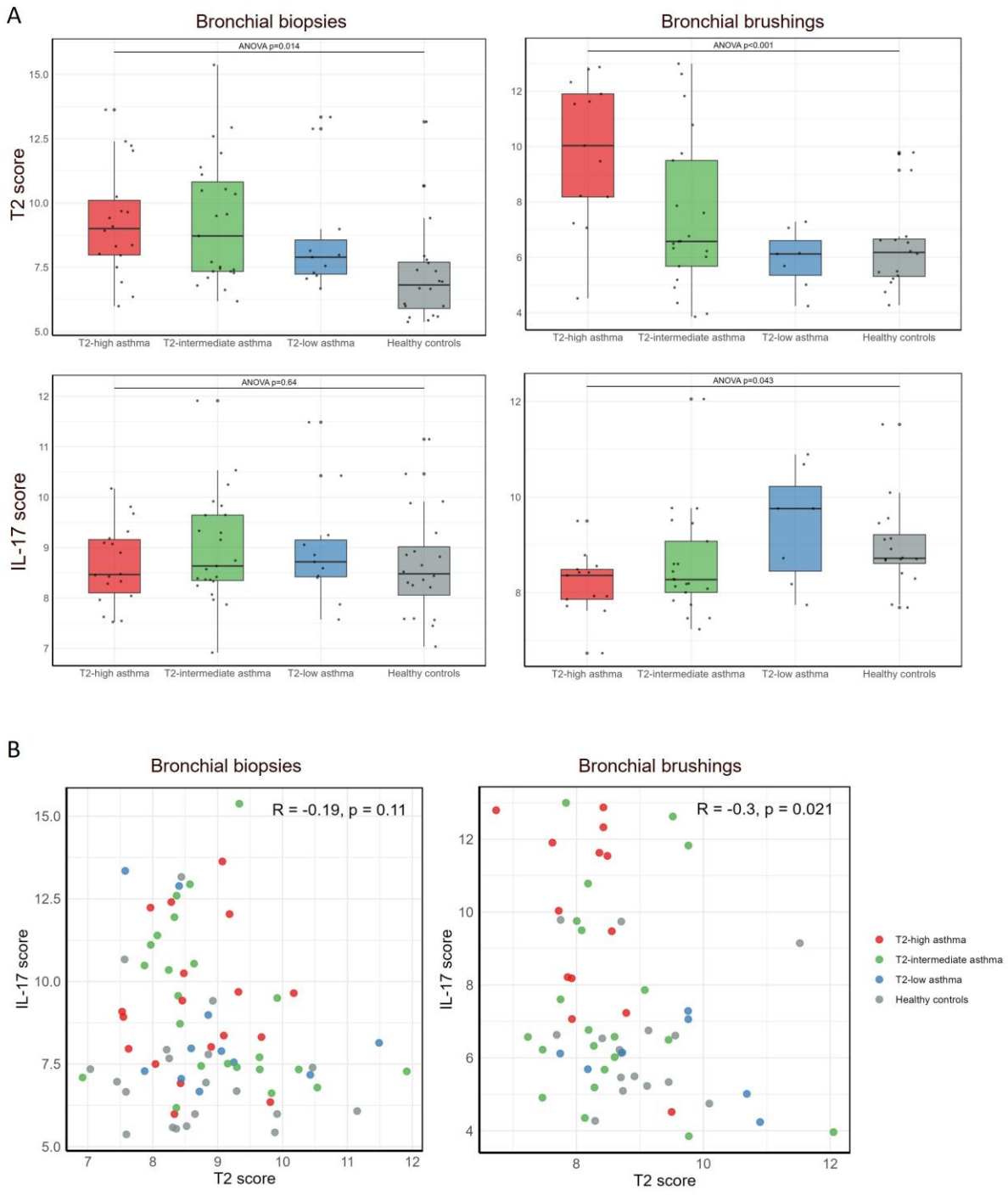


Figure 2.12 Type 2 (T2) and IL-17 signature expression across T2-high, -intermediate, and -low asthma, and healthy controls in bronchial biopsies and brushings.

T2 and IL-17 signature scores were calculated using IL-13–induced genes (*POSTN, SERPINB2,* and *CLCA1*) and IL-17–induced chemokines (*CXCL1, CXCL2, CXCL3, IL8,* and

CSF3), respectively, to estimate T2 and IL-17 immune activity. Healthy controls did not receive ICS. Signature scores were derived by averaging $\log_2$-transformed gene expression counts, normalised by library size, with 1 added to account for zero counts.

2.5 Discussion

To our knowledge, this is the first study to assess transcriptomic differences between severe asthma and health independent of confounding from therapeutic corticosteroid use, since all participants received high-dose ICS. Furthermore, stratified analyses of T2-high, T2-intermediate, and T2-low severe asthma were performed, incorporating patients managed with protocolised, biomarker-guided treatment optimisation.

2.5.1 Core transcriptomic signatures of asthma

When compared with ICS-treated healthy controls, severe asthma was characterised by strong upregulation of mucins (*MUC5AC, MUC2*), canonical T2-related genes, lysosomal genes, *CEACAM5*, and *SYNCRIP*. *CEACAM5* is a surface receptor on bronchial epithelial cells identified as upregulated in several severe asthma cohorts (Mumby et al., 2022; Shikotra et al., 2017; Singhania et al., 2018). It is upregulated by T2 cytokines and may mediate a subset of the IL-13 transcriptional epithelial signature, most notably modulation of type I interferon signalling (Mumby et al., 2022). *EACAM5* is also upregulated by IFN-γ and common respiratory viruses, and serves as an adhesion factor for *Haemophilus influenzae* and *Moraxella catarrhalis* (Klaile et al., 2013), suggesting a potential role in increased bacterial infection in severe asthma (Jabeen, Sanderson, Tine, et al., 2024). These data support further investigation of CEACAM5 as a drug target with the CEACAM5-targeting antibody labetuzumab.

Cytochrome P450 1A1 (*CYP1A1*) was the most differentially expressed gene (DEG)

in bronchial biopsies from patients with asthma compared with healthy controls not receiving ICS, but this was not attributable to therapeutic ICS, as no significant *CYP1A1* induction was observed in healthy controls treated with ICS (Marchi et al., 2024). Long-term increases in *CYP1A1* expression are attributable to hypomethylation of *CYP1A1* DNA from cigarette smoke, including personal, passive early-life or *in utero* exposures. Furthermore, *CYP1A1* is a marker of activation of the aryl hydrocarbon receptor, which is induced by aeroallergens, as well as oxidative stress, personal or maternal smoking, activated eosinophils, diesel exhaust, and other airway pollutants (Wang et al., 2019). The aryl hydrocarbon receptor is a critical regulator of innate and adaptive immune responses, controlling expression of IL-10, type I interferons, IL-12, IL-17, and TGF-β. A pathogenic role of *CYP1A1*, or upstream aryl hydrocarbon receptor, is therefore implied, as they are closely linked to mediating allergen-induced and ROS-dependent degranulation and IgE-mediated mast cell activation, and production of the epithelial alarmins TSLP, IL-33, and IL-25 (Wang et al., 2019).

2.5.2 Mechanistic insights into the type 2 biomarkers

For the T2 biomarkers, patients with high FeNO and high blood eosinophils shared common transcriptomic signatures related to T2 inflammation (e.g., *CCL26*) and airways remodelling (e.g., *MMP1*). However, some genes were associated only with eosinophils, such as *EDN2* (endothelin), which may play a role in eosinophil recruitment and activation (George et al., 2020), whereas others were only associated with FeNO, such as *POSTN* and *CST1*, which are linked to IL-13-dependent pathways

in airway epithelial cells (Diver et al., 2022). This study further demonstrated distinct gene expression patterns between eosinophilic and neutrophilic inflammation. Eosinophilic inflammation genes (e.g., *CXCL10* and *STC2*) and neutrophilic inflammation genes (e.g., *PRH2* and *SYT10*) were consistent with previous studies (Pelaia et al., 2015).

2.5.3 Molecular mechanisms of type 2 phenotypes

The RASP-UK collaboration has previously validated the a priori classification of these T2-high, -intermediate and -low groups based on recorded biomarkers and sputum cytokines (Khalfaoui et al., 2022b). In this study, airway T2- and IL-17–dependent gene signatures further substantiated the T2 status of these groups. Although these clinical phenotypes appear remarkably similar histologically (Khalfaoui et al., 2022b), dysregulated group-specific inflammatory and remodelling pathways were revealed at the molecular level. Comparisons between phenotypes revealed a striking suppression of ciliary gene sets and pathways in T2-high asthma, whereas T2-intermediate and T2-low asthma exhibited relatively preserved profiles, consistent with recent findings from U-BIOPRED (Devilliers et al., 2024). Ciliary dysmotility is described in severe asthma, but the underlying mechanism remains unknown. Ciliary dysmotility has been described in severe asthma, although its underlying mechanism remains unclear. One plausible explanation is epithelial metaplasia, characterised by the replacement of ciliated epithelial cells with goblet cells. This process is accompanied by altered expression of structural proteins (e.g., keratins) and adhesion

molecules (e.g., CEACAM5), together with pathological mucus overproduction and shifts in microbial colonisation, which may collectively impair mucociliary clearance.

A substantially different repertoire of genes was found to be upregulated in T2-low and intermediate asthma, including *CXCL10*, a chemokine induced by IFN-γ that preferentially recruits activated Th1 cells and mast cells via CXCR3 (Brightling et al., 2005). In murine models, *CXCL10* was shown to be upregulated following allergen challenge, contributing to AHR, eosinophilia, and the recruitment of IL-4 producing and $CD8^+$ T cells (Medoff et al., 2002). In humans, *CXCL10* is linked to a mast cell signature and is a marker for exacerbation risk (Gauthier et al., 2017) and a predictor of response to omalizumab (Akenroye et al., 2024), suggesting an important pathogenic role in T2-low disease.

Another interesting feature of T2-low asthma was upregulation of neuroimmune genes, notably *SCG2* and *CALCA*, implicating pathogenic neuroimmune pathways. Secretogranin II (*SCG2*, chromogranin-C) is a neuropeptide chemotactic to eosinophils (Atanasova & Reznikov, 2018). Pulmonary expression of both *SCG2* and *CALCA* is highly specific to ionocytes. *CALCA* encodes three peptide hormones: calcitonin, katacalcin and calcitonin gene-related peptide (CGRP) by tissue-specific alternative mRNA splicing. In the lungs, aeroallergens induce CGRP release from pulmonary neuroendocrine cells (Mann-Nuttel et al., 2025). These cells lie in close apposition to CGRP-receptor-expressing ILC2, DCs, basal epithelial cells, and

neurons. CGRP can promote Th2 and Th9 responses and has been associated with sensory neuroplasticity and fatal asthma (Dragunas et al., 2025).

Transcriptomic analysis revealed that the T2-intermediate phenotype was not simply positioned between T2-high and T2-low asthma, but instead was distinguished by distinct virus-activated signatures, including mast cell and NK cell signatures, a pathogen defence gene module and nine distinct transcription factors. The gene most specifically upregulated in this phenotype was BPI Fold Containing Family A Member I (*SPLUNC1*). This epithelial protein reduces epithelial surface tension, enhances mucus clearance, and inhibits Gram-negative bacterial biofilms. *BPIFA1* inhibits IL-13-mediated eotaxin-3 and ASM contraction. However, it is markedly induced by exposure to epoxy chemicals in sensitised individuals, and by secretoglobin (*SCGB1A1*, *CC16*), recruiting macrophages and neutrophils, implying a proinflammatory role (Lindahl et al., 2001).

2.5.4 Research limitations

This study has several limitations. First, bronchoscopies were conducted only during stable disease states and not during exacerbations, which may have constrained the ability to capture dynamic airway changes. Second, although the inclusion of healthy controls treated with high-dose ICS represents a distinctive strength, this short-term intervention is unlikely to fully reflect the consequences of long-term corticosteroid exposure in asthma. Third, despite the application of a deconvolution approach, the

use of bulk RNA sequencing precluded direct measurement of individual cell types, as well as cell type specific gene expression or spatial information. Finally, patients fulfilling the T2-low definition were relatively uncommon in our severe asthma clinics, resulting in a modest sample size. Nevertheless, this group was characterised by the identification of highly significant molecular features.

2.5.5 Clinical implications

The findings of this study have important implications for the clinical management of severe asthma. First, the identification of distinct molecular signatures across T2-high, T2-intermediate, and T2-low phenotypes provides a mechanistic basis for phenotype-driven treatment strategies. In T2-high asthma, the dominance of IL-13–responsive and epithelial remodelling genes reinforces the rationale for targeting type 2 pathways with biologics such as anti–IL-4Rα or anti–IL-13 therapies. In contrast, the upregulation of Th1- and IL-17–associated pathways in T2-low asthma underscores the need to develop and trial novel therapeutics, including agents that modulate interferon-γ and neutrophil-driven inflammation. Integrating phenotypes into clinical practice may improve patient stratification, inform the selection of targeted biologics, and ultimately reduce the burden of treatment-refractory disease. These insights also provide a framework for biomarker discovery, paving the way for more precise monitoring of therapeutic response and disease progression.

2.6 Conclusions

In summary, three major asthma phenotypes have been characterised, independent of confounding by therapeutic corticosteroids, with distinct gene sets and pathways identified for each. Corticosteroid-resistant T2-high severe asthma characterised by upregulated T2-high genes, epithelial barrier genes and keratins; T2-low severe asthma was characterised by Th1- and IL-17-associated genes, neuroimmune genes, and mast cell enrichment; steroid-responsive T2-intermediate asthma was characterised by signatures of pathogen defence, virus activation, enrichment for mast and NK cells, and six distinct transcription factors. These insights should guide future efforts to identify novel treatment targets for these less well-studied phenotypes.

Chapter 3 Bacteria and antibiotic resistance in asthma: a 27-year longitudinal analysis

Overview

Bacterial airways infection contributes to the pathogenesis of asthma exacerbations. However, the long-term epidemiology of respiratory pathogens and antimicrobial resistance (AMR) in asthma remains poorly characterised.

An electronic health record study was conducted using data from Oxfordshire, UK, including results including 4,350 sputum cultures with 11,101 antimicrobial susceptibility test results from 1,106 patients with asthma between 1998 and 2024. Factors independently associated with positive cultures were assessed using logistic regression.

A total of 31.4% of sputum cultures were positive. The most frequently isolated pathogens in initial cultures were *Haemophilus influenzae* (14.5%), *Pseudomonas aeruginosa* (6.7%), *Moraxella catarrhalis* (4.0%), *Staphylococcus aureus* (3.3%), *Streptococcus pneumoniae* (2.4%), and non-tuberculous mycobacteria (2.4%). In 16.0% of positive cultures, multiple species were isolated, most frequently *P. aeruginosa* and *S. aureus* (2.0%). Serial sampling within individuals revealed a decline over time in *H. influenzae* and a marked increase in *P. aeruginosa*, suggesting microbial succession over time. 45.8% of isolates were resistant to at least one antibiotic, with multi-drug

resistance widespread in *S. aureus* and *E. coli*. High resistance rates to narrow-spectrum β-lactams were seen in *M. catarrhalis* (90.9%), *S. aureus* (50.0%), and *H. influenzae* (30.1%). Between 2010 and 2014, resistance to quinolones, monobactams, and carbapenems declined significantly. Factors independently associated with positive sputum cultures included older age, higher neutrophils, and use of fluticasone propionate, but not beclomethasone or budesonide.

To conclude, potentially pathogenic bacteria were isolated from approximately one-third of asthma patients with sputum culture, and are associated with older age, neutrophilic inflammation, and use of inhaled fluticasone.

3.1 Introduction

3.1.1 Lung bacteria in asthma

Asthma is a common chronic respiratory disease, primarily characterised by chronic airways inflammation, airway hyperresponsiveness, and reversible airflow limitation. Although traditionally regarded as an inflammatory airway disorder, increasing evidence implicates bacterial infections in asthma pathophysiology (Campbell et al., 2023). Bacterial infections are associated with more severe respiratory symptoms, increased risk of exacerbations, and reduced responsiveness to corticosteroid therapy (Huang et al., 2015). Consequently, antibiotics are frequently prescribed in clinical practice, often empirically, to manage these episodes.

Reported culture positivity rates in asthma were estimated from 7% to 27% (Cazzola et al., 1991; X. Chen et al., 2023; Zhang et al., 2016), especially among patients with neutrophilic airways inflammation (Jabeen et al., 2022). The most frequently detected pathogens include *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Moraxella catarrhalis*, and *Staphylococcus aureus* (Green et al., 2014; Jabeen et al., 2022; Simpson et al., 2016; Q. Zhang et al., 2012).

Several small-scale cross-sectional studies have identified potentially pathogenic bacteria (PPB) in a subset of patients with severe asthma, using sputum culture or sequencing approaches. Zhang *et al.* identified *H. influenzae, P. aeruginosa*, and *S. aureus* as the most common strains in the sputum of 56 patients with stable severe

asthma, with isolation being associated with longer asthma duration and exacerbations (Qingling Zhang et al., 2012). Simpson *et al.* reported *Tropheryma* in 40% of 30 participants and *H. influenzae* in 76% of participants with poorly controlled asthma (Simpson et al., 2016). Jabeen *et al.* found that 23% of 81 patients with severe asthma were dominated by a single respiratory pathogen, including *H. influenzae*, *M. catarrhalis*, *S. pneumoniae* and *P. aeruginosa*, and further explored associated inflammatory pathways (Jabeen, Sanderson, Tinè, et al., 2024). While antibiotic resistance is well-characterised in chronic obstructive pulmonary disease (COPD) (Smith et al., 2021) and cystic fibrosis (Vitiello et al., 2024), studies in asthma are sparse.

3.1.2 Antibiotic use and resistance in asthma care

Antibiotics have been used in the management of asthma for several decades (Vanderweil et al., 2008). However, current clinical guidelines advise against the empirical use of antibiotics during acute asthma exacerbations without clear clinical evidence of bacterial infection, due to a lack of evidence of efficacy (Normansell et al., 2018; Ramakrishnan & Couillard, 2021). More recently, interest has shifted towards their potential immunomodulatory and anti-inflammatory properties. Both macrolides and inhaled antibacterial agents have been investigated for their ability to reduce airways inflammation and improve clinical outcomes (Kwok et al., 2025). Among them, the synthetic macrolide antibiotic azithromycin has demonstrated efficacy in reducing exacerbation rates in patients with severe asthma, regardless of their type 2

inflammation status (Brusselle et al., 2013; Peter G Gibson et al., 2017). It is recommended as an add-on to optimal therapy (Hinks et al., 2021; Smith et al., 2020).

At the same time, repeated antibiotic exposure in asthma can exert selective pressure, promoting the emergence and persistence of drug-resistant bacterial strains, which can complicate the management of future exacerbations and limit therapeutic options. The English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR) has repeatedly highlighted rising resistance among key respiratory pathogens and emphasised the importance of antimicrobial stewardship (Diane Ashiru-Oredope et al., 2023). Therefore, it is essential to investigate antibiotic resistance in the context of asthma.

3.1.3 Bacterial infection, airways inflammation, and corticosteroid treatment

Bacterial infection is recognised as an important contributor to airways inflammation in asthma, and bacterial colonisation of the airways can modulate both innate and adaptive immune responses. *Haemophilus*, *Pseudomonas, and Moraxella* are more prevalent in neutrophilic inflammation (Green et al., 2014), characterised by increased production of pro-inflammatory cytokines (e.g., IL-8, TNF-α), and steroid resistance (T. F. C. Fraga-Silva et al., 2023; Goleva et al., 2013). However, *Streptococcus* and *Staphylococcus* may be more prevalent in eosinophilic inflammation (Marathe et al., 2022). These organisms may sustain inflammation via innate immune activation and

impair the resolution of airway injury.

Chronic or recurrent bacterial infection may also alter airway microbiome composition, leading to persistent immune activation and airway remodelling (Taylor et al., 2018). For example, *H. influenzae* colonisation has been linked to impaired epithelial barrier function of the lower respiratory tract, enhanced Th17 responses, and reduced corticosteroid responsiveness (T. F. d. C. Fraga-Silva et al., 2023). Moreover, *H. influenzae* interacts synergistically with rhinoviruses through upregulation of ICAM-1 and by fostering a neutrophil-rich inflammatory milieu, to which it is particularly adapted (Brown et al., 2022). Conversely, commensal organisms such as *Streptococcus salivarius* may exert protective effects by limiting pneumococcal binding to pharyngeal epithelial cells (Manning et al., 2016).

Furthermore, ICS, a cornerstone of asthma treatment, may alter the airway microbiome, impair host defence mechanisms, and promote colonisation with PPB, potentially increasing resistance risk (T. S. Hinks et al., 2016; Marri et al., 2013; Martin et al., 2020). Previous studies have found that ICS therapy was linked to an increased risk of pneumonia in asthma (Kim et al., 2019). There was a dose-dependent increase in the risk of *M. catarrhalis* infection associated with ICS exposure (Johnsen et al., 2023). Moreover, different types of ICS have varying effects and patients with fluticasone have a higher infection rate (Choi et al., 2021). However, the direct impact of different ICS types on airway microbiota in asthma remains poorly understood.

3.1.4 Research gaps and rationale

Several important research gaps remain in understanding the role of bacterial infection and resistance in asthma. Existing studies are constrained by small sample sizes, cross-sectional designs, and a lack of longitudinal follow-up, which limits the ability to characterise temporal trends in bacterial infections and resistance. Antibiotic resistance profiles in asthma remain incompletely characterised, despite the widespread use of antibiotics in clinical practice. Likewise, the impact of ICS on airway bacterial communities and the dynamics of resistance has not been systematically investigated. Electronic health records (EHRs) offer a powerful means to address these gaps. Linked, longitudinal datasets combine demographic and clinical information with microbiology results, including sputum cultures and susceptibility profiles. Such resources enable time-series analyses that capture temporal trends and seasonal variation. For asthma, a condition marked by recurrent exacerbations, frequent healthcare utilisation, and substantial exposure to corticosteroids, EHR-based approaches are particularly well-suited to disentangling the interplay between host, pathogen, treatment, and resistance over time. Leveraging these data provides a robust framework to fill key knowledge gaps and to guide more targeted clinical and public health strategies.

3.2 Objectives

The primary objective of this study was to address these gaps by using 27 years of electronic health record (EHR) data from one UK region to characterise the prevalence, temporal trends, resistance profiles, and risk factors of sputum bacteria in asthma. Specifically, this study aimed to:

1. Describe the characteristics of the study participants.
2. Identify the sputum bacterial pathogens in individuals with asthma.
3. Determine the antibiotic resistance patterns of bacterial pathogens in asthma.
4. Examine the temporal trends of bacterial pathogens and their resistance patterns.
5. Assess hospital antibiotic prescribing practices and their relationship with resistance patterns.
6. Identify sociodemographic and clinical factors associated with culture positivity.

By addressing these aims, this study provided one of the most comprehensive longitudinal assessments of sputum bacteria in asthma to date. The findings were intended to inform clinical management and antimicrobial stewardship.

3.3 Methods

3.3.1 Patients and setting

This study used de-identified electronic health records (EHRs) from the Infections in Oxfordshire Research Database (IORD). IORD contains data from the Oxford University Hospitals (OUH) National Health Service (NHS) Foundation Trust, which consists of four teaching hospitals in Oxfordshire, UK, with a total of ~1,100 beds, and is the only provider of acute hospital services in the region for ~755,000 people. IORD includes information about admissions, as well as results from all blood and microbiological samples submitted from both primary care and OUH and tested in OUH laboratories (Finney et al., 2011).

Patients with a primary ICD-10 diagnosis code of asthma in any inpatient admission between January 1998 and July 2024 were included in the study. Individuals with primary or secondary diagnosis codes for other respiratory conditions, such as bronchiectasis or COPD, were excluded (Figure 3.1a). All sputum cultures obtained after the first primary asthma diagnosis were analysed. Patient characteristics were defined at the time of the first hospital admission. To avoid duplication from repeated sampling, only the first isolate was included when multiple cultures were obtained from the same patient within a 30-day window (Figure 3.1b).

3.3.2 Sputum culture and antimicrobial susceptibility testing

Sputum samples were collected as part of routine clinical care from patients attending

secondary or tertiary care for asthma management. Samples were processed in accredited hospital microbiology laboratories following standard operating procedures. Following a macroscopic assessment of sample quality, the sputum was homogenised and inoculated onto both selective and non-selective culture media. Bacterial isolates were identified using conventional microbiological methods and, in later years, automated platforms such as matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (Ahmed et al., 2014). Bacterial species isolated from sputum were grouped into clinically relevant taxonomic categories (Table S3.1 in appendix).

Antimicrobial susceptibility testing (AST) was performed on clinically significant isolates using standardised disc diffusion or automated broth microdilution methods, in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (Zhang et al., 2025). Susceptibility profiles were reported for a panel of commonly prescribed antibiotics, and antibiotics were categorised into predefined classes based on AST and prescription records (Table S3.2 in appendix).

3.3.3 Outcomes and covariates

Demographics, inpatient episode information (including diagnostic codes), haematological and biochemical parameters, microbiological findings, AST results, and treatment records were extracted from EHRs (prescription data only available from 2016). Comorbidities of asthma patients were identified based on secondary

diagnostic codes. Hospital prescribing data in patients with a previous primary asthma diagnosis code were compared with the antibiotic resistance rate in pathogens isolated from sputum cultures during the same period at the population level (community prescribing data were not available in IORD).

Sputum cultures were considered positive if they yielded at least one potentially pathogenic bacterium (PPB). The prevalence of PPB was defined as the proportion of positive cultures among all submitted sputum samples. Resistance rates were calculated as the proportion of resistant pathogens amongst all PPB. Based on analysis suggesting bimodal distributions in the frequencies of pathogen isolation (Figure S1), persistent isolation was defined as detection of the same initial PPB in ≥40% of subsequent serial sputum cultures. Co-isolation was defined as the presence of two or more distinct bacterial species from the same culture. Co-resistance was defined as resistance to ≥2 antibiotic classes, and multi-drug resistance (MDR) as resistance to ≥3 classes within a single bacterial pathogen.

3.3.4 Statistical analysis

Categorical variables were summarised as frequencies and percentages, and continuous variables were reported as means ± standard deviation (SD) or medians with interquartile ranges (IQR), depending on distribution. Categorical variables were conducted using Chi-square or Fisher's exact tests, while continuous variables were compared using ANOVA, Mann–Whitney U test, or Kruskal–Wallis test as appropriate.

Exact binomial tests were used to assess whether observed co-isolation rates deviated significantly from expectations. Multivariable logistic regression on complete cases was also used to estimate odds ratios (ORs) for the independent associations between patient characteristics and the risk of positive sputum cultures. Non-linear effects of continuous variables were modelled using natural cubic splines, with the number of knots determined by minimisation of the Bayesian information criterion (BIC). All analyses were conducted in R (version 4.4.0).

3.3.5 Ethics and consent

Ethical approval for the use of IORD as a de-identified research database was obtained from the South Central Research Ethics Committee (REC; 19/SC/0403) and the Health Research Authority Confidentiality Advisory Group (19/CAG/0144). As a de-identified database, individual patient consent was not required. The analysis conducted was reviewed and approved by the IORD oversight panel, which includes patient and public representatives. All data were handled in accordance with applicable data protection and confidentiality standards.

3.4 Results

3.4.1 Cohort characteristics

Between January 1998 and July 2024, 8,998 patients were admitted to Oxford University Hospitals (OUH) with a primary diagnosis of asthma, of whom 7,132 (79.3%) had no other chronic respiratory conditions recorded in diagnostic codes (Figure 3.1).

A total of 4,350 sputum cultures were obtained after exclusion of 142 repeat samples collected within 30 days. The majority of cultures were negative (2,984; 68.6%), while 1,366 (31.4%) yielded bacterial growth. From these positive cultures, 1,625 potentially pathogenic organisms were identified. Antimicrobial susceptibility testing (AST) was performed on these isolates, generating 11,101 individual susceptibility results (Figure 3.1).

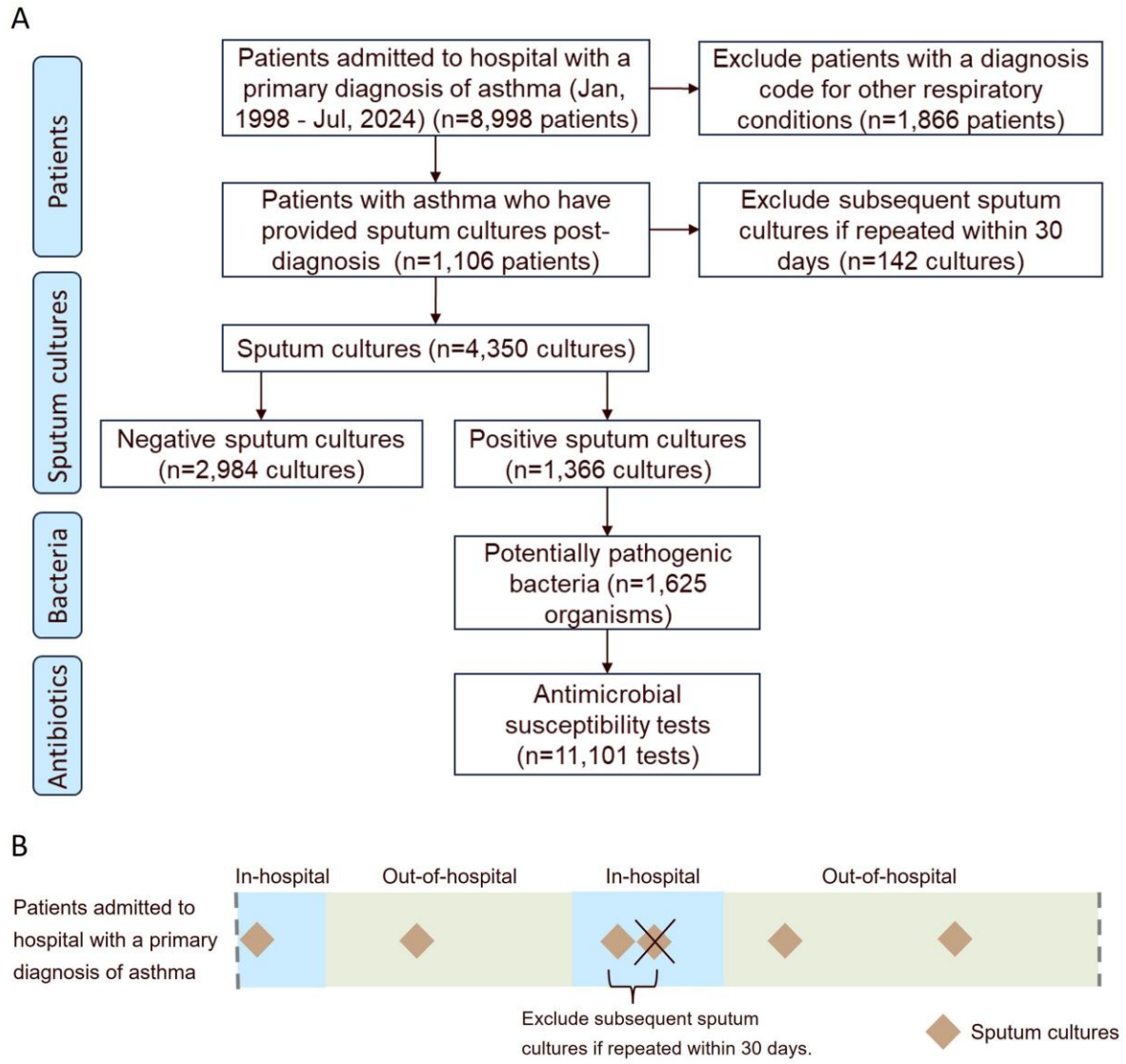


Figure 3.1 Study cohort selection and sputum sampling strategy.

(A) Cohort flow diagram.

(B) Schematic representation of the timeline and criteria for selecting sputum culture episodes. IORD contains results from all sputum samples submitted for testing at OUH, whether they were taken during or outside of an admission.

Among the 7,132 eligible patients, 1,106 (15.5%) submitted one or more sputum samples for culture after their first primary asthma diagnosis code during the study

period. The median time from asthma diagnosis to first sputum sample was 0.94 years (IQR 0.01–5.28) (Table 3.1). Of those who underwent sputum culture, 393 (35.5%) patients had at least one positive result. Compared with patients with only negative cultures, patients with positive cultures were significantly older (54 [41, 67] vs. 50 [33, 65] years, p = 0.002), more likely to be of Caucasian ethnicity (273/293 (93.2%) vs. 446/503 (88.7%), p = 0.04), came from less deprived areas (p = 0.002), and more likely to have cardiovascular diseases (p < 0.001) (Table 3.1).

Moreover, patients with positive cultures exhibited higher blood neutrophil counts (7.6 [6.2, 10.6] vs. 6.9 [5.0, 10.2] $x10^9$/L, p = 0.009) and greater proportion of high C-reactive protein (CRP) level patients (>10 mg/L) (104/166 (62.7%) vs. 177/410 (43.2%), p < 0.001) and were more likely to have used antibiotics in the past year (p < 0.001) compared with patients with only negative cultures (Table 3.1). Conversely, eosinophil counts were significantly lower in the culture-positive group (0.06 [0.01, 0.14] vs. 0.13 [0.02, 0.35] × 10^9/L, p < 0.001), as were blood basophil counts (0.04 [0.02, 0.06] vs. 0.05 [0.03, 0.07] $x10^9$/L, p < 0.001). ICS therapy and distribution of the calendar year of the first PPB isolate differed significantly across the groups (p < 0.001) (Table 3.1).

Table 3.1 Characteristics of patients with asthma at first hospital admission.

Characteristics	Ever positive culture (n=393)	Never positive culture (n=713)	No culture (n=6,206)	p-value (ever *vs.* never)	p-value (3 groups)
Age, years	54 [41, 67] (n=393)	50 [33, 65] (n=713)	27 [8, 49] (n=6,206)	0.002	<0.001
Sex, male, %	141/393 (35.9%)	256/713 (35.9%)	3342/6206 (53.9%)	1.00	<0.001
Ethnic, Caucasian, %	273/293 (93.2%)	446/503 (88.7%)	3374/4189 (80.5%)	0.04	<0.001
BMI, kg/m^2*	29.2 ± 1.2 (n=35)	29.6 ± 0.73 (n=93)	29.8 ± 0.42 (n=410)	0.64	0.92
IMD score	10.6 [6.2, 16.6] (n=390)	10.9 [6.9, 19.3] (n=706)	10.7 [6.4, 18.1] (n=5,888)	0.002	0.07
Comorbidity					
Cardiovascular disease, %	71/393 (18.0%)	135/731 (18.5%)	635/6026 (10.5%)	0.78	<0.001
Type 2 diabetes, %	22/393 (5.6%)	32/731 (4.4%)	197/6026 (3.3%)	0.50	0.02
Blood test					
Leukocytes, 10^9/L	10.4 [8.3, 13.3] (n=151)	10.2 [8.0, 13.2] (n=370)	10.1 [7.9, 12.9] (n=2,096)	0.26	0.02
Neutrophils, 10^9/L	7.6 [6.2, 10.6] (n=151)	6.9 [5.0, 10.2] (n=370)	7.1 [5.1, 9.6] (n=2,096)	0.009	0.008
Lymphocytes, 10^9/L	1.7 [1.1, 2.2] (n=151)	1.7 [1.1, 2.8] (n=370)	1.7 [1.1, 2.4] (n=2,096)	0.09	0.07
Eosinophils, 10^9/L	0.06 [0.01, 0.14] (n=151)	0.13 [0.02, 0.35] (n=370)	0.12 [0.02, 0.37] (n=2,096)	<0.001	<0.001
Basophils, 10^9/L	0.04 [0.02, 0.06] (n=151)	0.05 [0.03, 0.07] (n=370)	0.04 [0.03, 0.07] (n=2,096)	<0.001	<0.001
Monocytes, 10^9/L	0.71 [0.51, 0.93] (n=151)	0.67 [0.47, 0.89] (n=370)	0.66 [0.49, 0.87] (n=2,096)	0.62	0.69
High CRP value, %	104/166 (62.7%)	177/410 (43.2%)	1044/2247 (46.5%)	<0.001	<0.001
ICS usage*				<0.001	<0.001
No ICS therapy, %	34/126 (27.0%)	56/265 (21.1%)	1029/2,382 (43.2%)		
Beclomethasone dipropionate, %	47/126 (37.3%)	128/265 (48.3%)	1014/2,382 (42.6%)		
Budesonide, %	16/126 (12.7%)	39/265 (14.7%)	170/2,382 (7.1%)		
Fluticasone propionate, %	29/126 (23.0%)	42/265 (15.8%)	169/2,382 (7.1%)		
Prior antibiotic use in the past year*	63/113 (55.7%)	73/230 (31.7%)	954/2,641 (36.1%)	<0.001	<0.001

Characteristics	Ever positive culture (n=393)	Never positive culture (n=713)	No culture (n=6,206)	p-value (ever *vs.* never)	p-value (3 groups)
Calendar year				<0.001	<0.001
1998-2004	156/393 (40.0%)	210/713 (29.5%)	1,638/6,206 (26.4%)		
2005-2009	92/393 (23.4%)	160/713 (22.4%)	1,211/6,206 (19.5%)		
2010-2014	52/393 (13.2%)	113/713 (15.8%)	962/6,206 (15.5%)		
2015-2019	59/393 (15.0%)	119/713 (16.7%)	1,188/6,206 (19.1%)		
2020-2024	34/393 (8.7%)	111/713 (15.6%)	1,027/6,206 (16.6%)		

Data are presented as mean ± SD, median [IQR] or n/N (%), unless otherwise specified.

*Data on BMI, ICS usage, and prior antibiotic use within the past year were available only from 2016 onwards in the electronic hospital records.

Abbreviations: BMI: Body mass index; CRP: C-reactive protein; ICS: Inhaled corticosteroid; IMD: Index of Multiple Deprivation score (lower values indicate less deprivation).

A high CRP value indicates a CRP level greater than 10 mg/L.

3.4.2 Prevalence of bacterial pathogens

Among the 4,350 sputum cultures collected, 1,106 were initial cultures and 3,244 were follow-up cultures. Among them, 393 (35.5%) initial cultures and 973 (30.0%) follow-up cultures were positive for at least one PPB ($p = 0.001$) (Table 3.2). In initial cultures, the most frequently identified organisms were 160 (14.5%) *Haemophilus influenzae*, 74 (6.7%) *Pseudomonas aeruginosa*, 44 (4.0%) *Moraxella catarrhalis*, 37 (3.3%) *Staphylococcus aureus*, 27 (2.4%) *Streptococcus pneumoniae*, 26 (2.4%) non-tuberculous *mycobacteria* (NTM), and 23 (2.1%) *Escherichia coli*. Less frequently isolated organisms included 9 (0.8%) Klebsiella species, 4 (0.4%) *Proteus mirabilis*, 4 (0.4%) *Stenotrophomonas maltophilia*, 3 (0.3%) β-haemolytic streptococci, 3 (0.3%) *Serratia marcescens*, 2 (0.2%) *Enterobacter cloacae*, and 2 (0.2%) *Mycobacterium tuberculosis*. Most species, such as *H. influenzae* and *M. catarrhalis*, were more frequently isolated in initial cultures ($p < 0.001$), whereas *P. aeruginosa* was more common in subsequent follow-up cultures ($p < 0.001$) (Table 3.2).

Table 3.2 Distribution of bacterial pathogens isolated in sputum cultures from patients with asthma

Bacterial pathogen	Number isolated from initial cultures (%) (n=1,106)		Number isolated from following cultures (%) (n=3,244)		p-value†
Any bacteria	393	(35.5%)	973	(30.0%)	<0.001
Haemophilus influenzae	160	(14.5%)	157	(4.8%)	<0.001
Pseudomonas aeruginosa	74	(6.7%)	533	(16.4%)	<0.001
Moraxella catarrhalis	44	(4.0%)	54	(1.7%)	<0.001
Staphylococcus aureus	37	(3.3%)	94	(2.9%)	0.51
Other bacteria	35	(3.2%)	88	(2.7%)	0.50
Streptococcus pneumoniae	27	(2.4%)	54	(1.7%)	0.13
Non-tuberculous *mycobacteria*	26	(2.4%)	71	(2.2%)	0.84
Escherichia coli	23	(2.1%)	58	(1.8%)	0.62
Klebsiella species	9	(0.8%)	12	(0.4%)	0.11
Proteus mirabilis	4	(0.4%)	7	(0.2%)	0.49
Stenotrophomonas maltophilia	4	(0.4%)	25	(0.8%)	0.10
β-haemolytic streptococci	3	(0.3%)	6	(0.2%)	0.70
Serratia marcescens	3	(0.3%)	8	(0.2%)	1.00
Enterobacter cloacae	2	(0.2%)	5	(0.2%)	1.00
Mycobacterium tuberculosis	2	(0.2%)	0	(0.0%)	0.06

† Fisher's exact test was used when expected counts were <5; Chi-squared test was used otherwise.

See Table S1 in appendix for taxonomic classification of bacterial species.

Note: Because of co-isolation, the total number of bacterial isolates exceeds the number of cultures yielding bacterial growth.

3.4.3 Longitudinal trends in serial sputum cultures

Over the course of serial sampling within individuals, significant temporal changes were observed: *P. aeruginosa* prevalence increased markedly in subsequent cultures (from 6.7% to 17.9%; $p < 0.001$), while *H. influenzae* declined (14.5% to 5.3%; $p < 0.001$) (Figure 3.2). Among patients with ≥3 positive cultures, early isolation of *H.*

influenzae often preceded later isolation of *P. aeruginosa* and other species, suggesting microbial succession within the airways. In addition, the prevalence of *M. catarrhalis*, *S. pneumoniae*, and NTM decreased in the following sputum cultures, while that of *S. aureus* and *E. coli* remained unchanged (Figure 3.2).

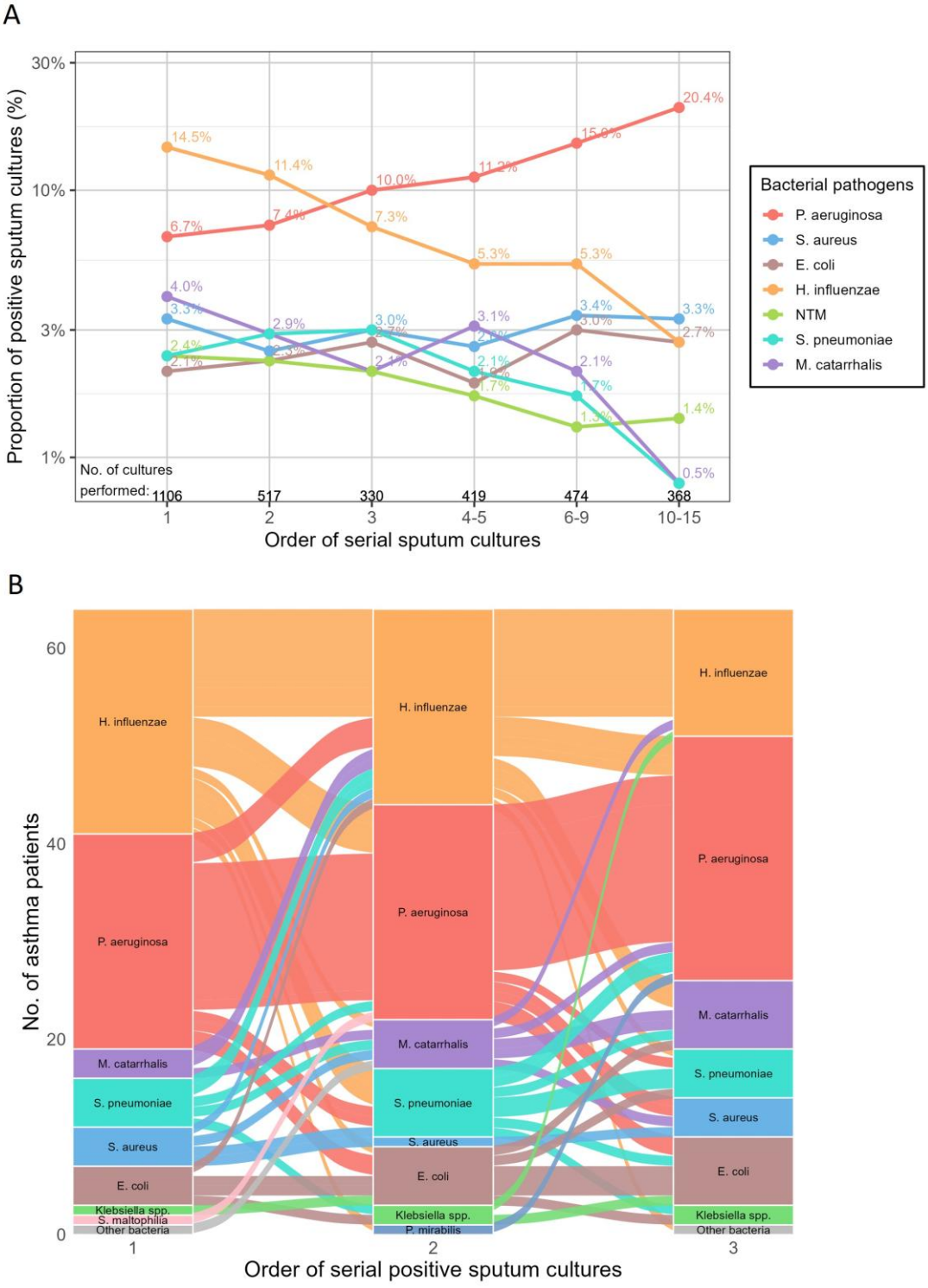


Figure 3.2 Longitudinal trends of bacterial pathogens in serial sputum cultures for patients with asthma.

(A) Proportions of bacterial pathogens identified from serial sputum cultures in patients with asthma (y-axis on log scale).

(B) Sankey plot illustrating progression and replacement of dominant pathogens among patients with ≥3 serial positive cultures (n=63).

3.4.4 Persistent isolation and co-isolation

Persistent isolation (as defined in Methods and Supplement) was most common in patients with initial infections caused by *P. aeruginosa* (18.0%) and *H. influenzae* (14.6%) (Figure 3.3). This was followed by *E. coli* (9.7%) and NTM (6.8%). Patients with these organisms often demonstrated repeated positive cultures across sequential hospital admissions. Persistent isolation of other organisms was less common, including *S. pneumoniae* (4.4%), *S. aureus* (4.3%), and *M. catarrhalis* (2.1%) (Figure 3.3).

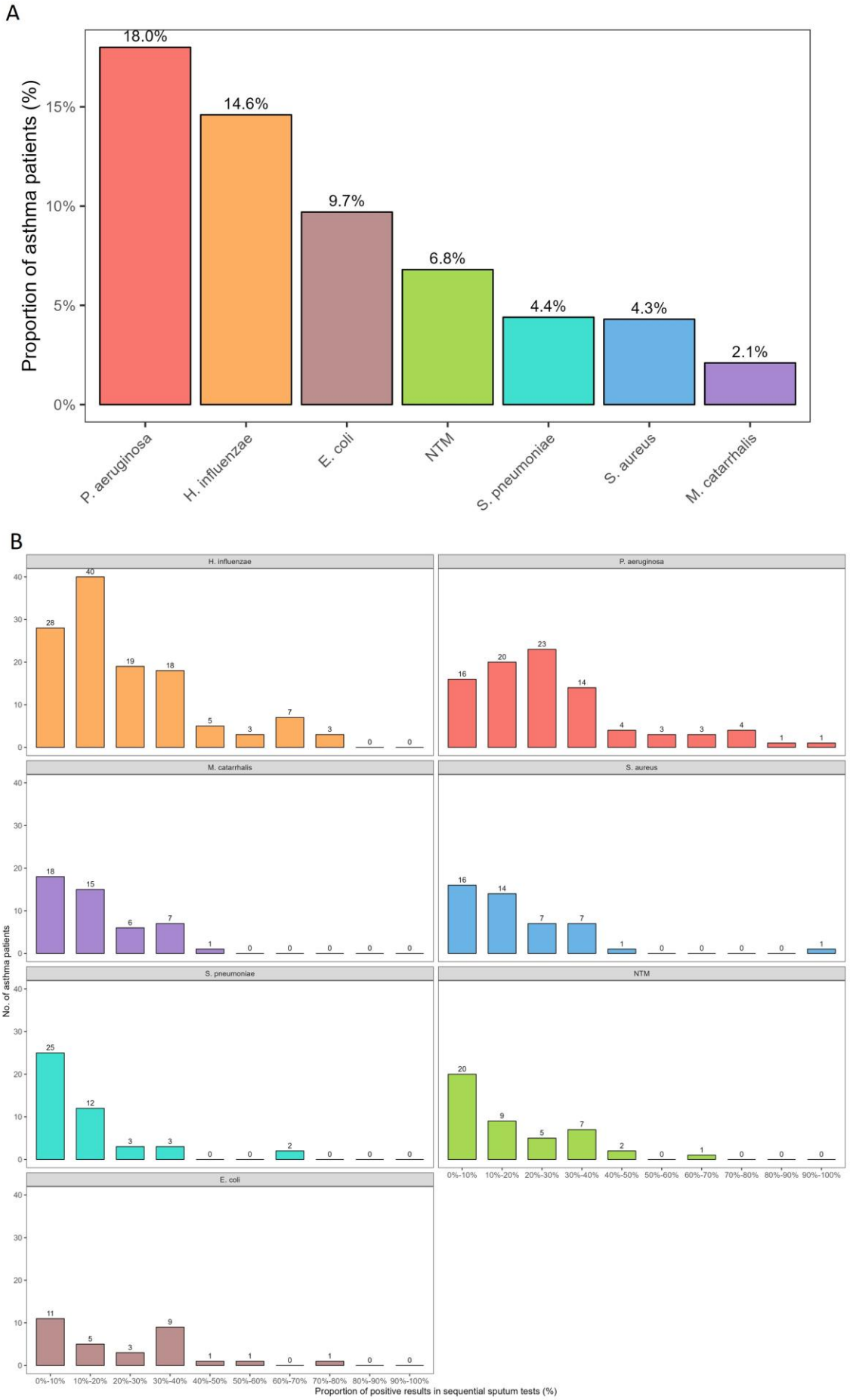

A
Proportion of asthma patients (%)
18.0%
14.6%
9.7%
6.8%
4.4%
4.3%
2.1%
P. aeruginosa
H. influenzae
E. coli
NTM
S. pneumoniae
S. aureus
M. catarrhalis
B
H. influenzae
P. aeruginosa
M. catarrhalis
S. aureus
S. pneumoniae
NTM
E. coli
No. of asthma patients
0%-10%
10%-20%
20%-30%
30%-40%
40%-50%
50%-60%
60%-70%
70%-80%
80%-90%
90%-100%
Proportion of positive results in sequential sputum tests (%)

Figure 3.3 Persistent isolation of bacterial pathogens in patients with asthma.

(A) Proportion of asthma patients with persistent isolation of the initial potentially pathogenic bacteria.

(B) Distribution of the proportion of positive sputum cultures among asthma patients in sequential sputum tests.

Among 1,366 positive sputum cultures, multiple species were isolated in 218 (16.0%). Most bacteria are more likely to co-isolate, including *P. aeruginosa* with *S. aureus* (28 (2.0%)) or with NTM (14 (1.0%)), and *H. influenzae* with *M. catarrhalis* (18 (1.3%)) or with *S. pneumoniae* (13 (1.0%)) or with NTM (9 (0.7%)), which were significantly higher than expected if their co-isolation were independent ($p < 0.001$) (Table 3.3 and Figure 3.4). Moreover, *S. aureus* was more commonly co-isolated with *H. influenzae* ($p = 0.01$), *M. catarrhalis* ($p = 0.02$), *S. pneumoniae* ($p = 0.043$), and *E. coli* ($p = 0.008$). *S. pneumoniae* were more commonly co-isolated with *M. catarrhalis* ($p < 0.001$) and NTM ($p = 0.003$). In contrast, despite being the most frequently isolated PPBs overall, *H. influenzae* and *P. aeruginosa* were rarely isolated from the same sample, occurring in only 3 (0.2%) cultures, which were significantly lower than expected ($p < 0.001$) (Table 3.3 and Figure 3.4).

Table 3.3 Co-isolation frequencies between bacterial pathogens within individual sputum samples.

This table shows the observed number and proportion of co-isolations for each bacterial pair identified in positive sputum cultures (n = 1,366). The expected co-isolation number and proportion under the assumption that their co-isolation was independent. P-values were obtained using exact binomial tests to assess whether observed co-isolation rates deviated significantly from expected rates.

Bacteria A	Bacteria B	Observed (n, %)	Expected (n, %)	p-value
P. aeruginosa	*S. aureus*	28 (2.0%)	6 (0.4%)	<0.001
P. aeruginosa	NTM	14 (1.0%)	4 (0.3%)	<0.001
H. influenzae	*M. catarrhalis*	18 (1.3%)	2 (0.2%)	<0.001
H. influenzae	*S. pneumoniae*	13 (1.0%)	2 (0.1%)	<0.001
H. influenzae	NTM	9 (0.7%)	2 (0.2%)	<0.001
H. influenzae	*S. aureus*	8 (0.6%)	3 (0.2%)	0.01
E. coli	*S. aureus*	4 (0.3%)	1 (0.1%)	0.008
S. pneumoniae	*M. catarrhalis*	5 (0.4%)	1 (0.0%)	<0.001
S. pneumoniae	NTM	4 (0.3%)	1 (0.0%)	0.003
S. pneumoniae	*S. aureus*	3 (0.2%)	1 (0.1%)	0.043
M. catarrhalis	*S. aureus*	4 (0.3%)	1 (0.1%)	0.02
H. influenzae	*P. aeruginosa*	3 (0.2%)	14 (1.0%)	0.001
S. pneumoniae	*P. aeruginosa*	4 (0.3%)	4 (0.3%)	0.8
E. coli	NTM	2 (0.1%)	1 (0.0%)	0.1
E. coli	*P. aeruginosa*	2 (0.1%)	4 (0.3%)	0.6
NTM	*S. aureus*	2 (0.1%)	1 (0.1%)	0.2
E. coli	*H. influenzae*	1 (0.1%)	2 (0.1%)	1
M. catarrhalis	NTM	1 (0.1%)	1 (0.1%)	0.5
M. catarrhalis	*P. aeruginosa*	1 (0.1%)	4 (0.3%)	0.1
E. coli	*M. catarrhalis*	0 (0.0%)	1 (0.0%)	1
E. coli	*S. pneumoniae*	0 (0.0%)	0 (0.0%)	1

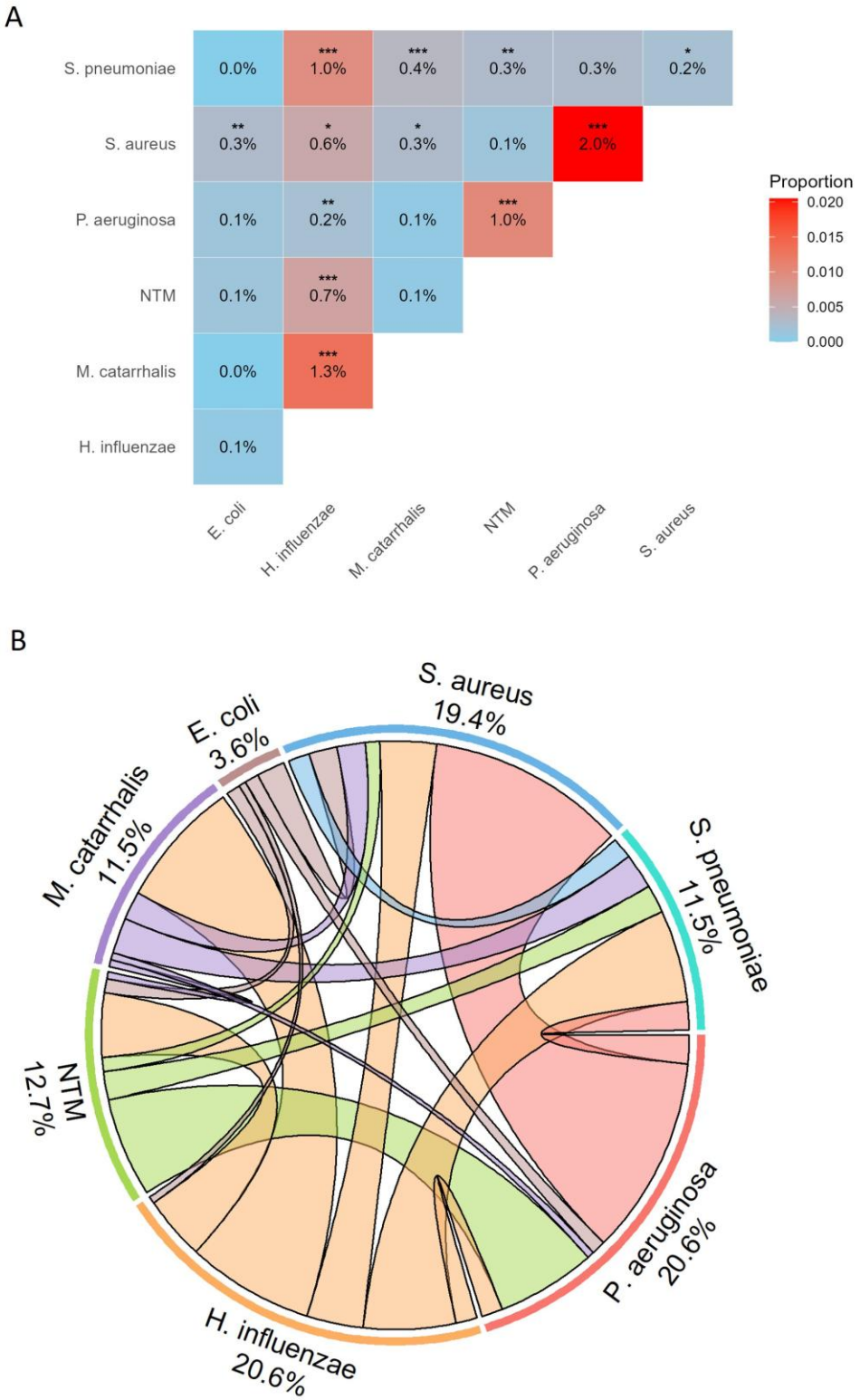


Figure 3.4 Co-isolation of bacterial pathogens in patients with asthma.

(A) Heatmap showing the frequency of co-isolation between bacterial pathogens within individual sputum samples. Statistically significant deviations from expected co-isolation rates under the assumption of independence were determined using exact binomial tests (see Table

S3.3 in appendix for full statistical details).
(B) Chord diagram illustrating the co-isolation of bacterial species identified in sputum cultures. Each segment along the circumference represents a specific bacterium, with the size of each segment corresponding to the relative frequency of that pathogen. Connecting ribbons indicate co-isolation between bacterial species, with the width of each ribbon representing the proportion of co-isolation involving the two connected pathogens.

3.4.5 Bacteria and inflammatory biomarkers

Systemic inflammatory responses varied by pathogen (Figure 3.5). Four inflammatory biomarkers (CRP, blood neutrophils, blood leukocytes and blood eosinophils) in the PPB isolated patients were examined. A high CRP value was found in 60% of patients with positive sputum cultures. The distribution of CRP level varied substantially by pathogen, with *S. pneumoniae* (93%), *E. coli* (86%), and *H. influenzae* (69%) exhibiting the highest proportions of high CRP value, suggesting active infection rather than simple colonisation (Figure 3.5).

Peripheral blood neutrophilia (>7.5 $\times 10^9$/L) was identified in 42% of individuals with positive sputum cultures, with *H. influenzae* (62%) and *S. aureus* (57%) associated with the highest proportions of individuals with high neutrophil counts. Leukocytosis (>10 $\times 10^9$/L) followed a different pattern, with the highest rates observed with *E. coli* (76%) and *H. influenzae* (66%), and the lowest rates with NTM (39%). In contrast, eosinophilia (>0.05 $\times$ 10^9/L) was common in some bacterial infections, particularly those involving NTM (84%) and *E. coli* (75%), compared with non-infectious cases (36%) (Figure 3.5). Although causality cannot be inferred, this finding is consistent with the hypothesis that NTM more readily colonise small airways affected by tenacious sputum plugs, which characterise eosinophilic airways inflammation.

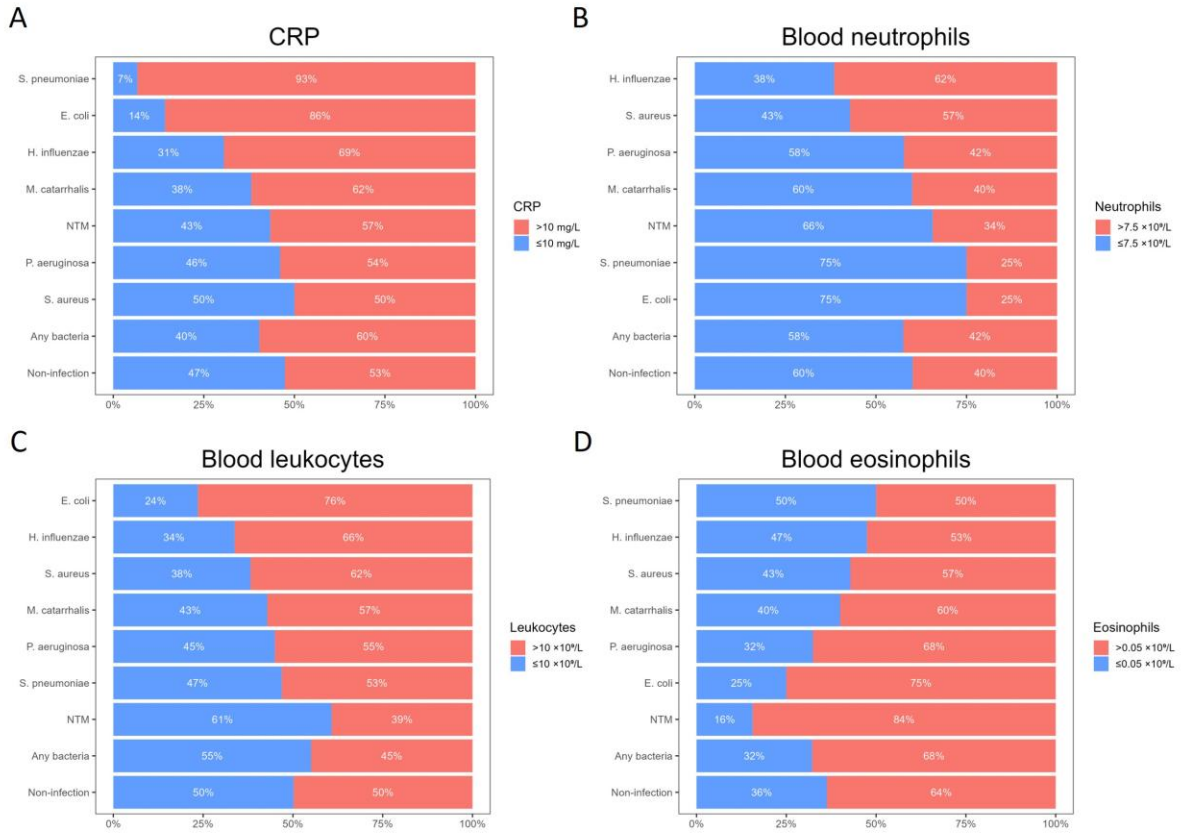


Figure 3.5 The distribution of inflammatory biomarkers by bacterial pathogens in patients with asthma.

(A) The distribution of CRP by bacterial pathogens in patients with asthma.

(B) The distribution of blood neutrophils by bacterial pathogens in patients with asthma.

(C) The distribution of blood leukocytes by bacterial pathogens in patients with asthma.

(D) The distribution of blood eosinophils by bacterial pathogens in patients with asthma.

3.4.6 Distribution of antibiotic resistance

AST revealed substantial species-specific resistance patterns (Figure 3.6). Overall, nearly half of all bacterial isolates (51.9%) exhibited detectable resistance, including 22.0% were multi-drug resistant, 12.8% resistant to two antibiotics, and 17.1% resistant to a single antibiotic. Resistance patterns varied markedly between bacterial species. *E. coli* isolates demonstrated the highest burden of multi-drug resistance (47.4%), followed by *S. aureus* (35.1%) and *P. aeruginosa* (25.4%). In contrast, *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae* exhibited lower levels of resistance, with about 40% of isolates showing resistance (38.2%, 37.1%, and 33.4%, respectively). Notably, NTM were predominantly susceptible, with 95.5% of isolates showing no resistance and only 3.0% being MDR. (Figure 3.6).

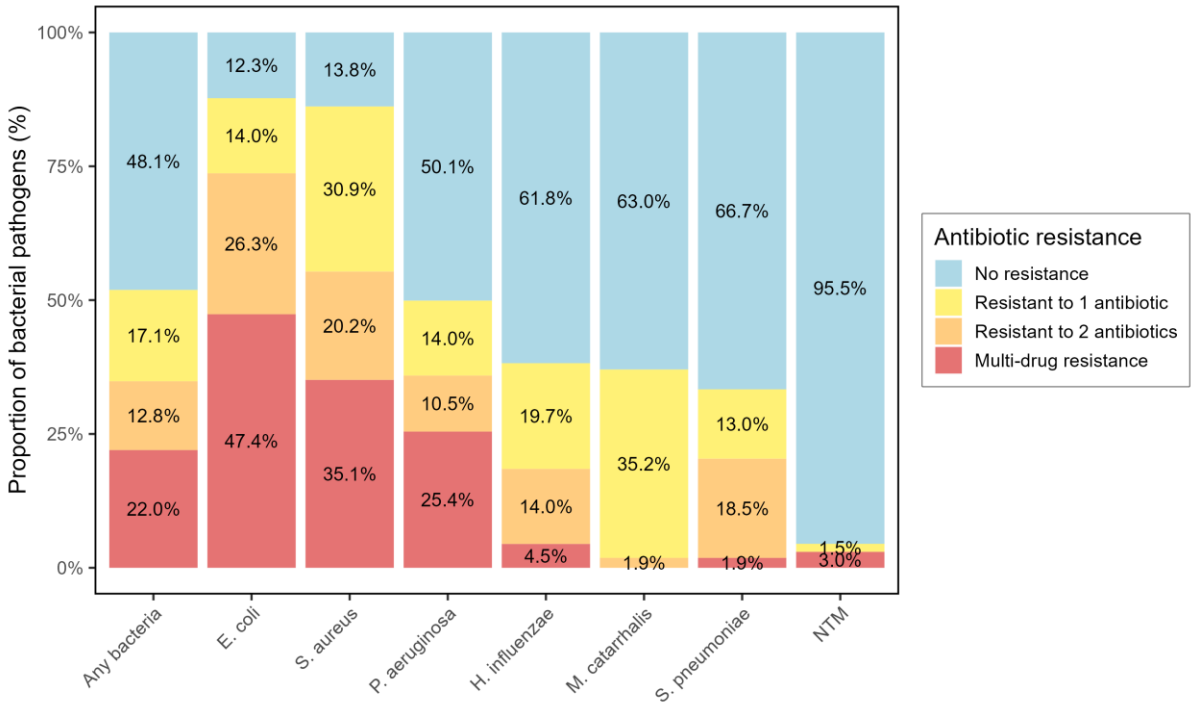


Figure 3.6 Distribution of antibiotic resistance among different bacterial pathogens in antimicrobial susceptibility tests.

Table 3.3 summarises the antibiotic resistance profiles of the major bacterial pathogens isolated from sputum cultures in patients with asthma. See Table S3.2 in appendix for the classification of specific antibiotics. In initial cultures, narrow-spectrum penicillins (i.e., amoxicillin), broad-spectrum penicillins (i.e., β-lactamase inhibitor-containing), and macrolides (i.e., azithromycin) have the highest resistance rates (43.5%, 18.2%, and 17.4%, respectively). It is also worth noticing that in the following sputum cultures, cephalosporins (i.e., ceftriaxone), monobactams (i.e., aztreonam), quinolones (i.e., ciprofloxacin, levofloxacin), and carbapenems (i.e., imipenem, meropenem) have relatively high resistance rates (16.2%, 22.0%, 30.1%, and 19.6%).

In initial sputum cultures, *H. influenzae* showed notable resistance to narrow-spectrum penicillins (30.1%), macrolides (23.3%), and broad-spectrum penicillins (21.1%). Nearly all *M. catarrhalis* isolates were resistant to narrow-spectrum penicillins (90.9%) but remained susceptible to other antibiotics. *S. aureus* commonly exhibited resistance to narrow-spectrum penicillins (50.0%), macrolides (30.8%), and quinolones (16.7%) in initial sputum culture. *S. pneumoniae* displayed resistance to tetracyclines (20.0%) and macrolides (13.3%). *E. coli* exhibits high resistance to many types of antibiotics, such as narrow-spectrum penicillins (100.0%), broad-spectrum penicillins (50.0%), cephalosporins (26.3%), and quinolones (14.3%). In subsequent cultures, *P. aeruginosa* exhibited higher resistance to quinolones (40.2% vs. 8.5% in initial cultures; $p < 0.001$), cephalosporins (22.3% vs. 5.3%; $p = 0.02$), and carbapenems (24.4% vs. 0.0%; $p < 0.001$) (Table 3.3).

Table 3.3 Antibiotic resistance profiles of bacterial pathogens isolated from

sputum cultures in patients with asthma.

	Resistance in initial AST (%) [95% CI]	Resistance in following AST (%) [95% CI]	p-value[†]
Any bacteria	n=419	n=948	
Narrow-spectrum penicillins	43.5 [36.3, 50.8]	46.0 [42.1, 50.0]	0.59
Broad-spectrum penicillins	18.2 [13.2, 24.2]	19.2 [16.8, 21.8]	0.82
Macrolides	17.4 [11.0, 25.6]	24.0 [19.7, 28.7]	0.18
Sulfonamides	10.8 [3.0, 25.4]	10.6 [6.5, 16.0]	1.00
Cephalosporins	9.1 [5.5, 14.1]	16.2 [14.0, 18.7]	0.01
Monobactams	8.2 [2.3, 19.6]	22.0 [18.6, 25.7]	0.04
Quinolones	7.5 [4.0, 12.4]	30.1 [27.4, 32.8]	<0.001
Tetracyclines	6.8 [3.4, 11.8]	8.2 [5.8, 11.1]	0.70
Other antibiotics	5.3 [1.1, 14.6]	9.1 [6.3, 12.8]	0.48
Polypeptides	4.9 [1.6, 11.1]	2.4 [1.4, 3.7]	0.25
Aminoglycosides	3.3 [1.3, 6.7]	6.9 [5.8, 8.1]	0.06
Chloramphenicols	2.9 [0.8, 7.4]	0.6 [0.1, 2.0]	0.10
Carbapenems	1.3 [0.0, 7.1]	19.6 [16.7, 22.7]	<0.001
Haemophilus influenzae	n=157	n=151	
Narrow-spectrum penicillins	30.1 [21.0, 40.5]	31.8 [25.6, 38.5]	0.88
Macrolides	23.3 [11.8, 38.6]	29.6 [20.0, 40.8]	0.59
Broad-spectrum penicillins	21.1 [13.4, 30.6]	18.7 [13.7, 24.6]	0.74
Cephalosporins	9.5 [4.4, 17.2]	3.3 [1.3, 6.6]	0.05
Tetracyclines	6.3 [2.4, 13.2]	1.4 [0.3, 4.0]	0.05
Chloramphenicols	4.2 [1.2, 10.4]	0.9 [0.1, 3.4]	0.14
Quinolones	2.0 [0.1, 10.6]	4.6 [1.7, 9.7]	0.71
Pseudomonas aeruginosa	n=73	n=491	
Quinolones	8.5 [2.4, 20.4]	40.2 [36.3, 44.1]	<0.001
Monobactams	8.1 [1.7, 21.9]	23.0 [19.4, 26.9]	0.06
Cephalosporins	5.3 [0.6, 17.7]	22.3 [18.7, 26.1]	0.02
Aminoglycosides	0.8 [0.0, 4.3]	6.7 [5.6, 8.1]	0.01
Carbapenems	0.0 [0.0, 9.3]	24.4 [20.7, 28.3]	0.001
Polypeptides	0.0 [0.0, 11.6]	1.3 [0.4, 3.0]	1.00
Moraxella catarrhalis	n=44	n=50	
Narrow-spectrum penicillins	90.9 [58.7, 99.8]	86.2 [68.3, 96.1]	1.00
Broad-spectrum penicillins	3.7 [0.1, 19.0]	0.0 [0.0, 5.4]	0.64
Cephalosporins	0.0 [0.0, 28.5]	0.0 [0.0, 13.2]	NA
Chloramphenicols	0.0 [0.0, 12.8]	0.0 [0.0, 5.4]	NA
Macrolides	0.0 [0.0, 13.2]	1.5 [0.0, 8.3]	1.00
Tetracyclines	0.0 [0.0, 12.8]	0.0 [0.0, 5.4]	NA
Staphylococcus aureus	n=36	n=90	
Narrow-spectrum penicillins	50.0 [33.4, 66.6]	52.4 [45.4, 59.3]	0.71
Macrolides	30.8 [14.3, 51.8]	34.4 [26.3, 43.1]	0.90
Quinolones	16.7 [5.6, 34.7]	27.8 [21.4, 34.9]	0.29
Other antibiotics	7.7 [0.9, 25.1]	8.9 [5.5, 13.6]	1.00
Tetracyclines	4.8 [0.1, 23.8]	15.5 [9.1, 24]	0.34
Polypeptides	2.3 [0.1, 12.3]	0.8 [0.1, 2.8]	0.91
Aminoglycosides	0.0 [0.0, 13.2]	0.8 [0.0, 4.4]	1.00
Streptococcus pneumoniae	n=27	n=53	
Tetracyclines	20.0 [4.3, 48.1]	22.2 [12.7, 34.5]	1.00
Macrolides	13.3 [1.7, 40.5]	23.4 [13.8, 35.7]	0.61
Other antibiotics	0.0 [0.0, 21.8]	0.0 [0.0, 5.2]	NA
Narrow-spectrum penicillins	0.0 [0.0, 30.8]	0.0 [0.0, 5.1]	NA
Polypeptides	0.0 [0.0, 20.6]	0.0 [0.0, 4.7]	NA
Escherichia coli	n=22	n=55	
Narrow-spectrum penicillins	100.0 [69.2, 100.0]	88.9 [78.4, 95.4]	0.60
Broad-spectrum penicillins	50.0 [26.0, 74.0]	44.0 [33.2, 55.3]	0.84
Cephalosporins	26.3 [9.1, 51.2]	23.3 [15.5, 32.7]	1.00
Quinolones	14.3 [1.8, 42.8]	35.2 [24.2, 47.5]	0.22
Aminoglycosides	10.5 [1.3, 33.1]	20.0 [12.3, 29.8]	0.52
Carbapenems	0.0 [0.0, 26.5]	6.1 [1.7, 14.8]	0.87

† Chi-squared test for p-value.

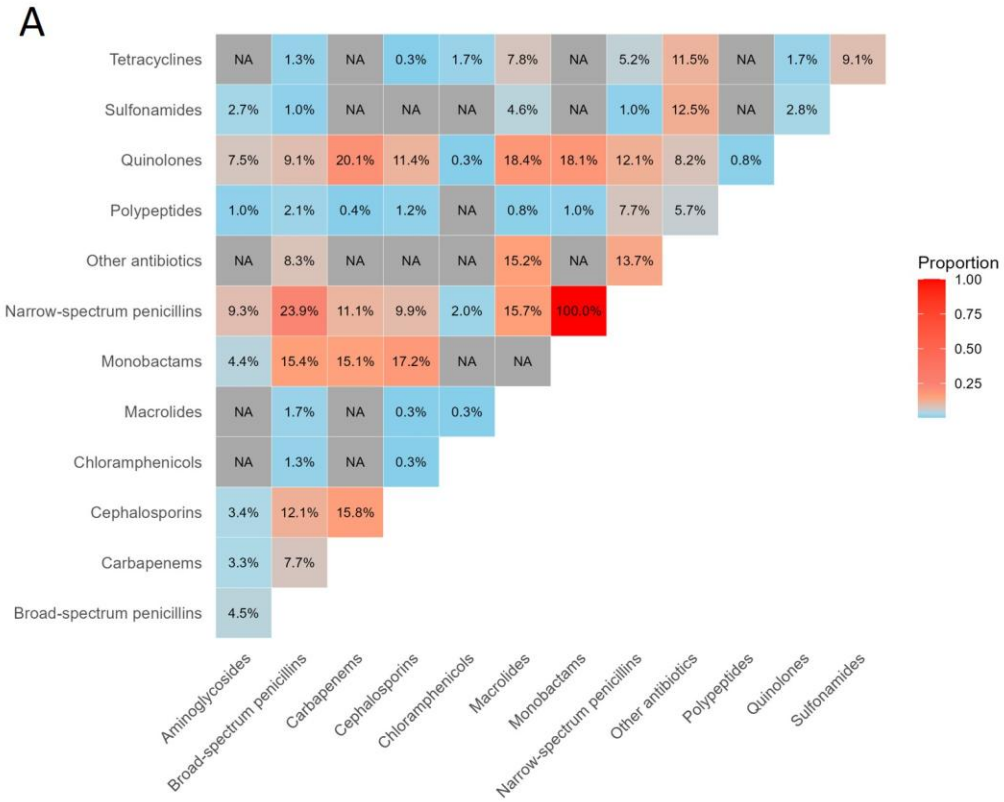
Abbreviations: AST: antimicrobial susceptibility testing.

See Table S2 in appendix for classification of antibiotics.

Note: owing to co-isolation, 393 initial positive cultures yielded 453 PPB, of which 419 (92.5%) had any AST; and the 973 follow-up positive cultures yielded 1,172 PPB, of which 948 (80.1%) had any AST.

Widespread co-resistance across multiple antibiotic classes, particularly β-lactams (penicillins, cephalosporins, monobactams, and carbapenems), macrolides, and quinolones (Figure 3.7 and Figure 3.8). Co-resistance was most prevalent between narrow-spectrum penicillins and broad-spectrum penicillins (23.9%), monobactams (15.7%) and macrolides (15.7%). Strong associations were also observed between quinolones and multiple classes, including carbapenems (20.1%), macrolides (18.4%), and monobactams (18.1%). In contrast, co-resistance to less frequently used antibiotics, such as chloramphenicol and polypeptides, was rare. The co-resistance was not obvious in *H. influenzae*, where only narrow-spectrum penicillins and carbapenems have high co-resistance (100.0%). The co-resistance was more frequently observed among *P. aeruginosa, S. aureus, and S. pneumoniae* isolates. Notably, for *P. aeruginosa,* the co-resistance between quinolones, monobactams, cephalosporins and carbapenems was generally higher than 10%. In *S. aureus,* broad-spectrum penicillins, monobactams, and polypeptides have a 100% co-resistance rate. (Figures 3.7 and Figure 3.8).

A


Tetracyclines NA 1.3% NA 0.3% 1.7% 7.8% NA 5.2% 11.5% NA 1.7% 9.1%
Sulfonamides 2.7% 1.0% NA NA NA 4.6% NA 1.0% 12.5% NA 2.8%
Quinolones 7.5% 9.1% 20.1% 11.4% 0.3% 18.4% 18.1% 12.1% 8.2% 0.8%
Polypeptides 1.0% 2.1% 0.4% 1.2% NA 0.8% 1.0% 7.7% 5.7%
Other antibiotics NA 8.3% NA NA NA 15.2% NA 13.7%
Narrow-spectrum penicillins 9.3% 23.9% 11.1% 9.9% 2.0% 15.7% 100.0%
Monobactams 4.4% 15.4% 15.1% 17.2% NA NA
Macrolides NA 1.7% NA 0.3% 0.3%
Chloramphenicols NA 1.3% NA 0.3%
Cephalosporins 3.4% 12.1% 15.8%
Carbapenems 3.3% 7.7%
Broad-spectrum penicillins 4.5%
Aminoglycosides
Broad-spectrum penicillins
Carbapenems
Cephalosporins
Chloramphenicols
Macrolides
Monobactams
Narrow-spectrum penicillins
Other antibiotics
Polypeptides
Quinolones
Sulfonamides
Proportion
1.00
0.75
0.50
0.25

B

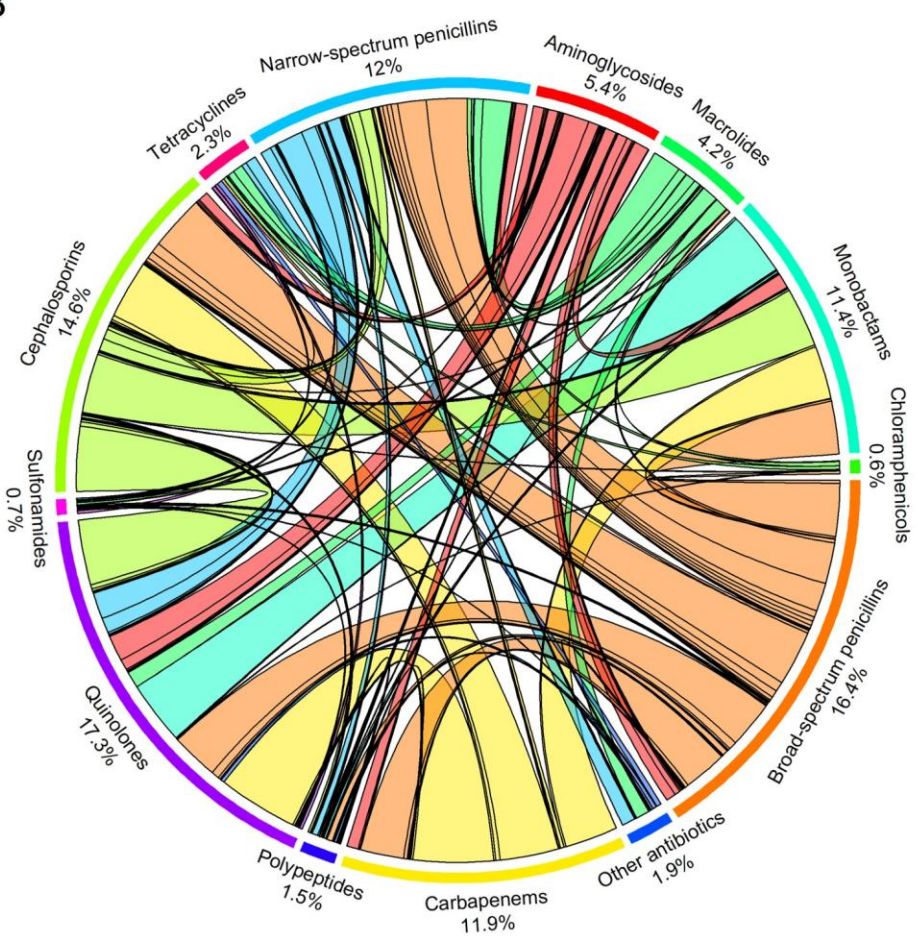

Narrow-spectrum penicillins 12%
Aminoglycosides 5.4%
Macrolides 4.2%
Monobactams 11.4%
Chloramphenicols 0.6%
Broad-spectrum penicillins 16.4%
Other antibiotics 1.9%
Carbapenems 11.9%
Polypeptides 1.5%
Quinolones 17.3%
Sulfonamides 0.7%
Cephalosporins 14.6%
Tetracyclines 2.3%

Figure 3.7 Patterns of co-resistance among antibiotic classes isolates from patients with asthma.

(A) Heatmap showing pairwise co-resistance proportions between antibiotic classes. Each cell represents the proportion of isolates resistant to both antibiotic classes.

(B) Chord diagram illustrating co-resistance relationships among antibiotic classes. The size of each arc corresponds to the overall frequency of resistance to each class, while the connecting ribbons represent co-resistance between pairs, with ribbon width proportional to the co-resistance rate.

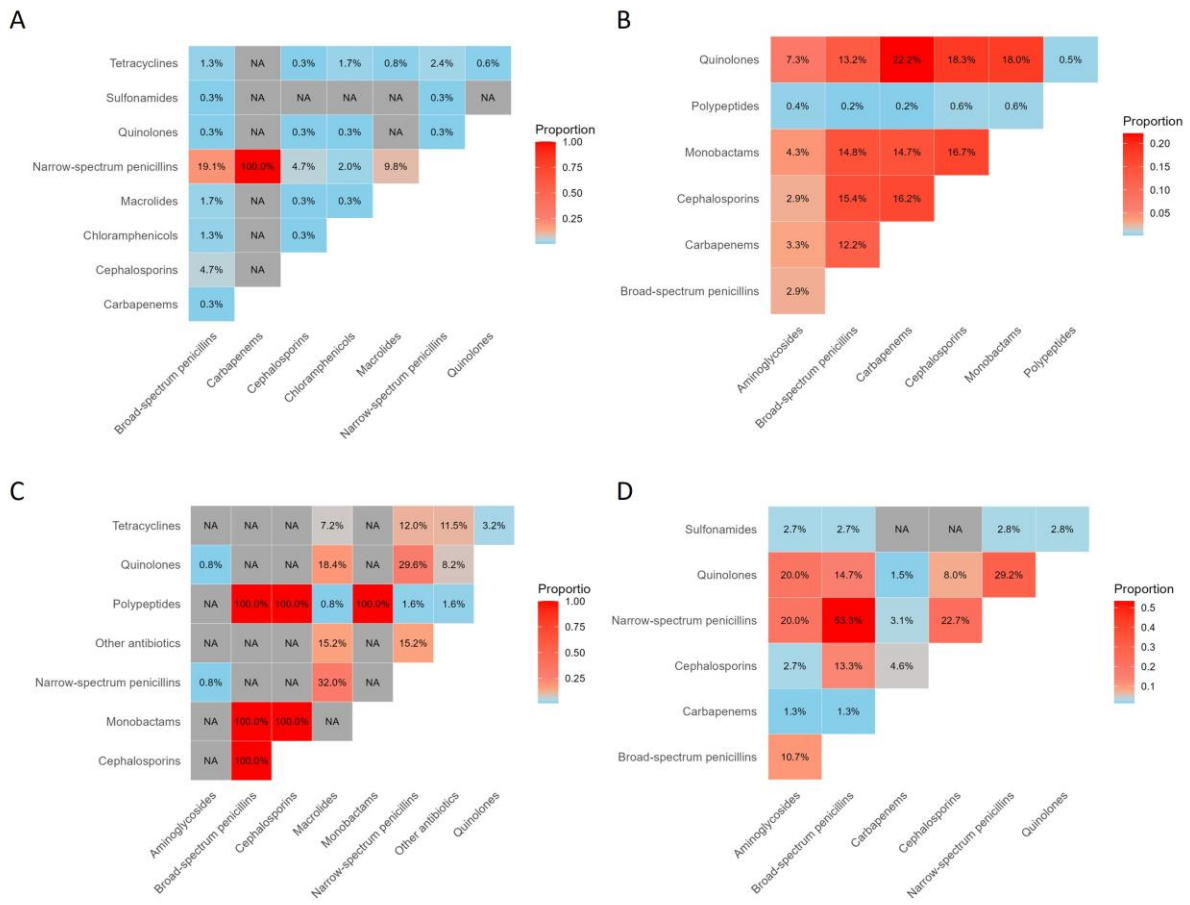


Figure 3.8 Patterns of co-resistance among antibiotic classes in bacterial pathogen isolates from patients with asthma.

(A) Co-resistance proportions between antibiotic classes in *H. influenzae*.

(B) Co-resistance proportions between antibiotic classes in *P. aeruginosa*.

(C) Co-resistance proportions between antibiotic classes in *S. aureus*.

(D) Co-resistance proportions between antibiotic classes in *S. pneumoniae*.

3.4.7 Antibiotic prescription in hospital

From 2016 to 2024, antibiotic prescribing remained common among asthma patients with sputum cultures in hospitals (Figure 3.9). These data reflect prescriptions occurring during the admission for which the primary admission diagnosis was 'asthma', though in some cases, there may have been other indications for these specific prescriptions, which were not necessarily given to treat acute asthma. Penicillins, cephalosporins, tetracyclines, aminoglycosides, and macrolides were the most frequently used antibiotics. The use of cephalosporins and chloramphenicol has markedly increased, while the use of aminoglycosides has shown a rapid decrease. Prescriptions of other antibiotic classes remained at low but stable levels (Figure 3.9).

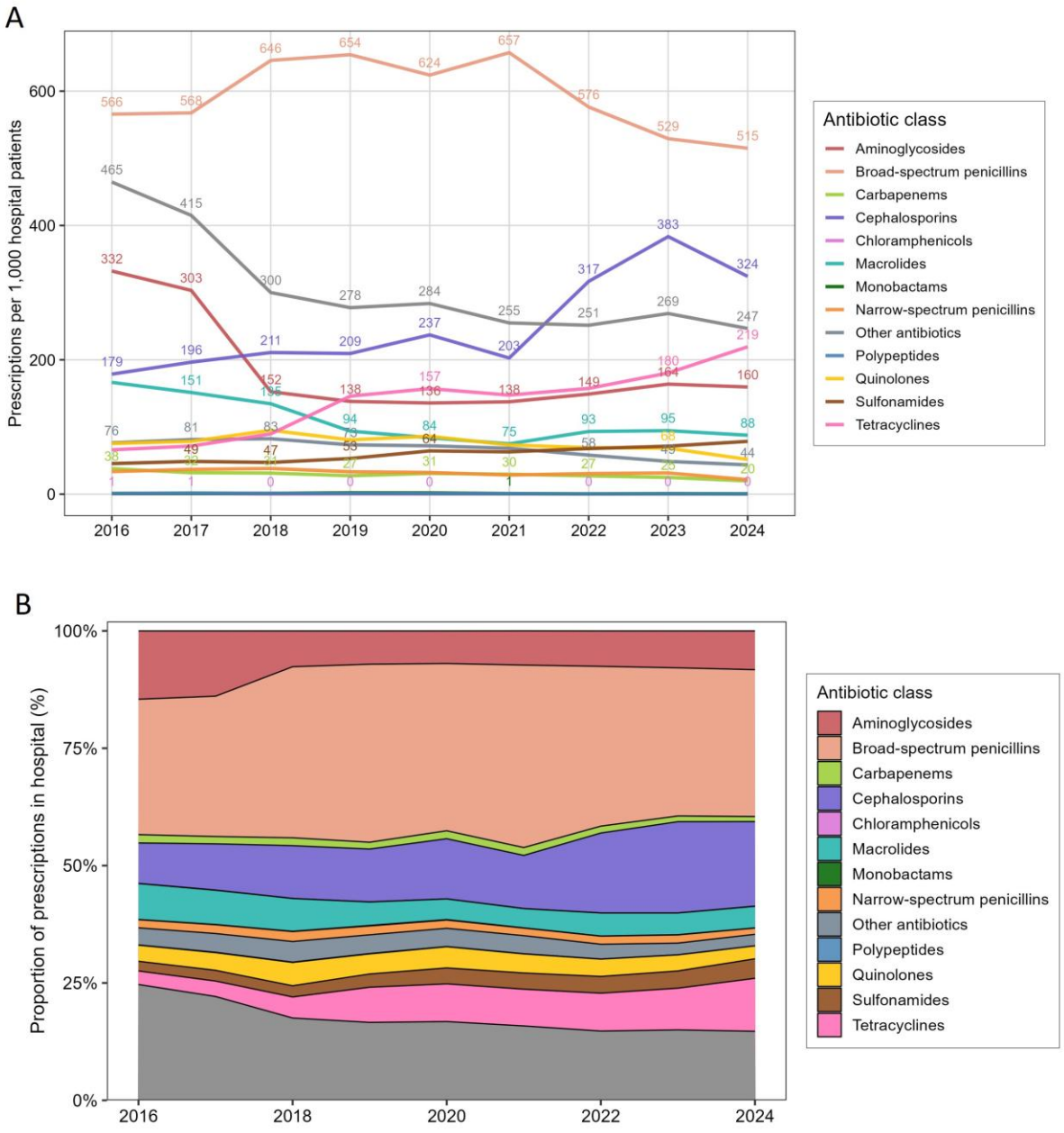


Figure 3.9 Annual trends in hospital antibiotic prescriptions in asthma patients with sputum cultures (2016-2024).

(A) Annual trends in the number of hospital antibiotic prescriptions in asthma patients with sputum cultures.

(B) Annual trends in the proportion of hospital antibiotic prescriptions in asthma patients with sputum cultures.

Figure 3.10 shows the relationship between the proportion of hospital antibiotic prescriptions and the proportion of resistance in positive sputum cultures from asthma

patients between 2016 and 2024. Broad-spectrum penicillins represented the largest share of prescriptions (33.8%), with a resistance rate of 18.0%. In contrast, narrow-spectrum penicillins accounted for only 1.8% of hospital prescriptions yet showed the highest resistance burden (41.0%). Macrolides also demonstrated disproportionate resistance, with 15.7% of resistant isolates compared to 5.6% of prescriptions. Similarly, quinolones (3.9% prescriptions, 12.9% resistance), sulphonamides (3.0% prescriptions, 8.3% resistance) and tetracyclines (6.6% prescriptions, 8.6% resistance).

Some antibiotics have limited clinical use for asthma patients but display high resistance rates, such as monobactams (0.06% prescriptions, 8.4% resistance) and polypeptides (0.05% prescriptions, 6.2% resistance). In contrast, aminoglycosides (9.2% prescriptions, 1.9% resistance) and carbapenems (1.5% prescriptions, 1.8% resistance) retained low resistance despite their clinical use. Other antibiotic classes, including chloramphenicol, polypeptides, monobactams, and miscellaneous agents, represented only a small proportion of prescribing and resistance (Figure 3.10).

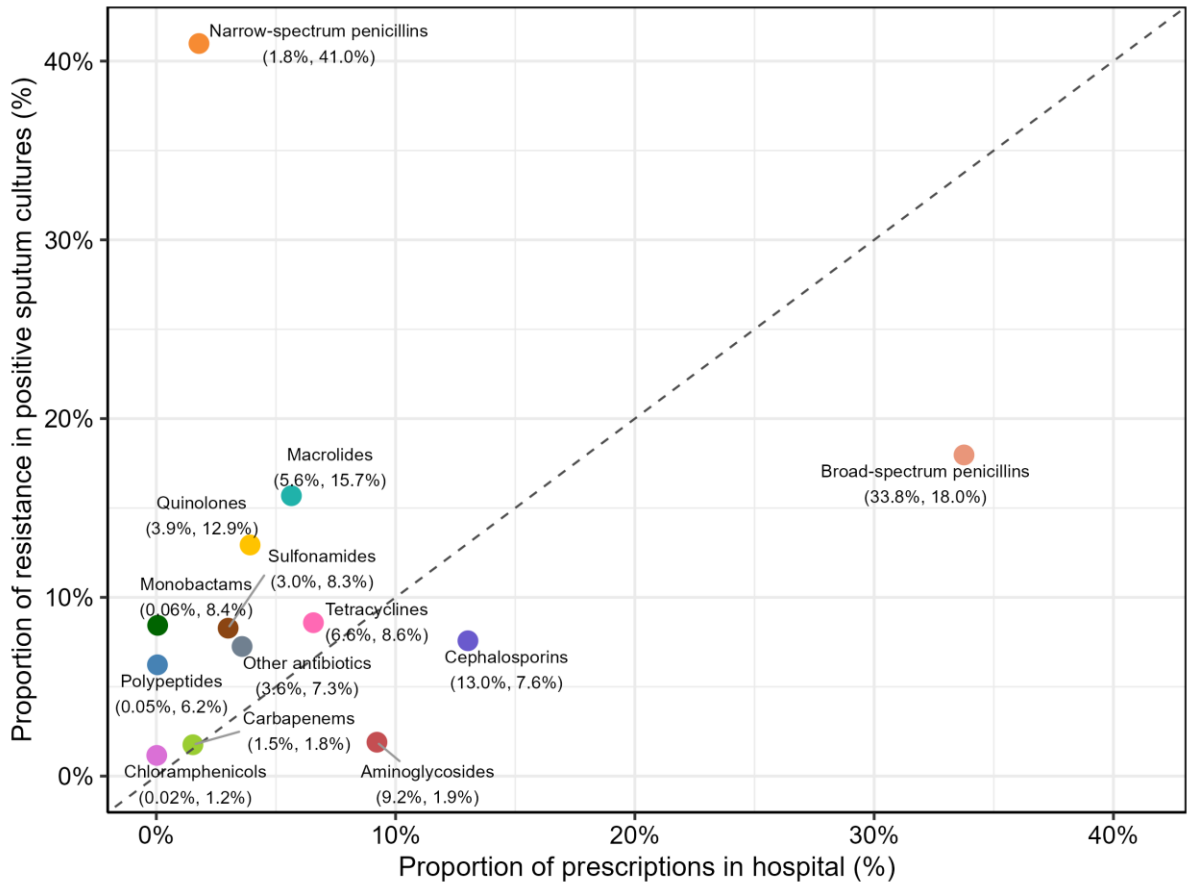


Figure 3.10 Relationship between the proportion of antibiotic prescriptions in the hospital and the proportion of antibiotic resistance in positive sputum cultures (2016-2024).

The proportions of hospital antibiotic prescriptions and the proportions of resistance in positive sputum cultures for different pathogen infections were shown in Figure 3.11. For *H. influenzae*, broad-spectrum penicillins were both highly prescribed (28.6%) and frequently resistant (29.1%). Narrow-spectrum penicillins, despite being prescribed minimally (0.4%), exhibited disproportionately high resistance rates (37.9%), whereas tetracyclines had a relatively low resistance rate (3.6%). In *P. aeruginosa*, resistance was concentrated in quinolones (22.6%), whereas aminoglycosides retained relatively low resistance (1.6%) despite use. M. catarrhalis generally showed a very low resistance rate for different antibiotics. In S. aureus, resistance was very high for

macrolides (20.9%), quinolones (18.6%), and tetracyclines (13.9%), while aminoglycosides and sulphonamides displayed low resistance. For S. pneumoniae, resistance was dominated by tetracyclines (34.5%), macrolides (23.3%), and sulphonamides (14.3%). Cephalosporins were prescribed but showed little resistance. In E. coli, broad-spectrum penicillins were the most commonly prescribed class (28.6%) and accounted for a substantial proportion of resistance (25.0%) (Figure 3.11).

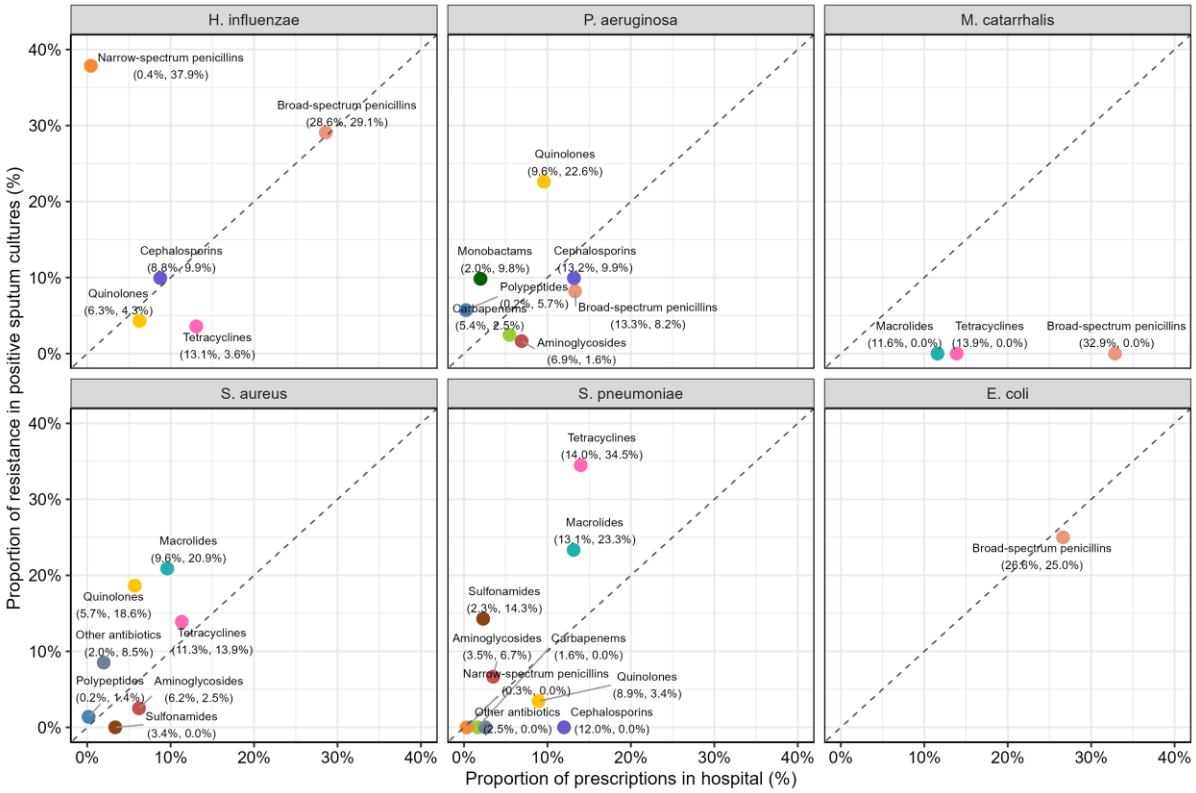


Figure 3.11 Relationship between the proportion of antibiotic prescriptions in the hospital and the proportion of antibiotic resistance in positive sputum cultures for different bacterial pathogens (2016-2024).

3.4.8 Temporal trends in bacteria and resistance

To assess temporal trends, we compared results from 784 sputum cultures obtained between 2005 and 2009 with those from 1,149 sputum cultures obtained between 2020 and 2024. The overall positivity rate for sputum cultures decreased significantly

(43.0% to 24.7%; p-value for trend < 0.001) (Figure 3A). Significant declines were observed between the two periods in the prevalence of *P. aeruginosa* (from 23.1% to 9.6%), *S. aureus* (from 5.4% to 1.2%), and *E. coli* (from 5.9% to 0.3%) (all p-value for trend < 0.001). Across all species combined, resistance to multiple antibiotic classes reached its peak from 2010 to 2014. It decreased significantly afterwards, including for quinolones (from 34.6% to 8.0%), monobactams (from 32.5% to 5.1%), and carbapenems (from 24.2% to 0.7%) (p-value for trend < 0.001 for all; Figure 3B). In contrast, resistance to polypeptides rose from 0.9% to 11.3% over 2010-2024. Species-specific patterns diverged: *H. influenzae* showed increasing resistance across several classes, whereas *P. aeruginosa* exhibited declining resistance rates (Figure 3.12).

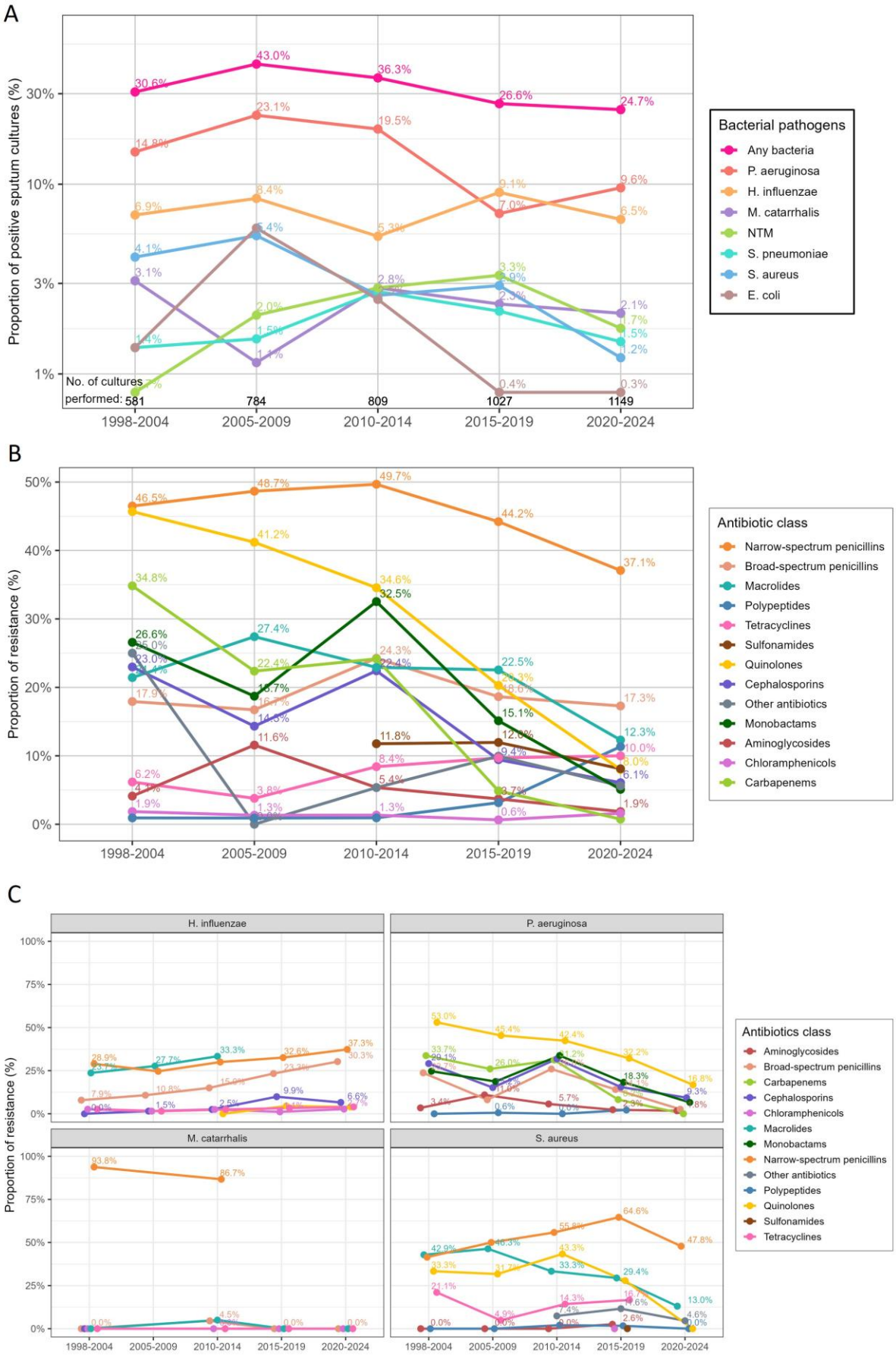

A
Proportion of positive sputum cultures (%)
30%
10%
3%
1%
No. of cultures performed:
581
784
809
1027
1149
1998-2004
2005-2009
2010-2014
2015-2019
2020-2024
Bacterial pathogens
Any bacteria
P. aeruginosa
H. influenzae
M. catarrhalis
NTM
S. pneumoniae
S. aureus
E. coli
B
Proportion of resistance (%)
50%
40%
30%
20%
10%
0%
Antibiotic class
Narrow-spectrum penicillins
Broad-spectrum penicillins
Macrolides
Polypeptides
Tetracyclines
Sulfonamides
Quinolones
Cephalosporins
Other antibiotics
Monobactams
Aminoglycosides
Chloramphenicols
Carbapenems
C
H. influenzae
P. aeruginosa
M. catarrhalis
S. aureus
Proportion of resistance (%)
100%
75%
50%
25%
0%
Antibiotics class
Aminoglycosides
Broad-spectrum penicillins
Carbapenems
Cephalosporins
Chloramphenicols
Macrolides
Monobactams
Narrow-spectrum penicillins
Other antibiotics
Polypeptides
Quinolones
Sulfonamides
Tetracyclines

Figure 3.12 Temporal trends of bacterial pathogen isolations and antibiotic resistance in sputum cultures from patients with asthma (1998-2024).

(A) Temporal trends in the proportion of bacterial pathogens (y-axis on log scale).

(B) Temporal trends in the proportion of antimicrobial resistance across all isolated bacterial pathogens.

(C) Temporal trends in the proportion of antimicrobial resistance in selected bacterial pathogens.

Clear seasonal patterns were observed in this study. For most of the bacterial pathogens, isolation rates were high in winter but low in summer. Particularly, *H. influenzae* peaked in the spring (8.4%) and winter (16.8%) months compared with summer (6.6%) and autumn (5.8%). However, *P. aeruginosa* peaked as a proportion in the summer months (16.8%), but was low in winter (12.7%) and spring (12.4%) months, although its absolute numbers remained stable (Figures 3.13 and Figure 3.14).

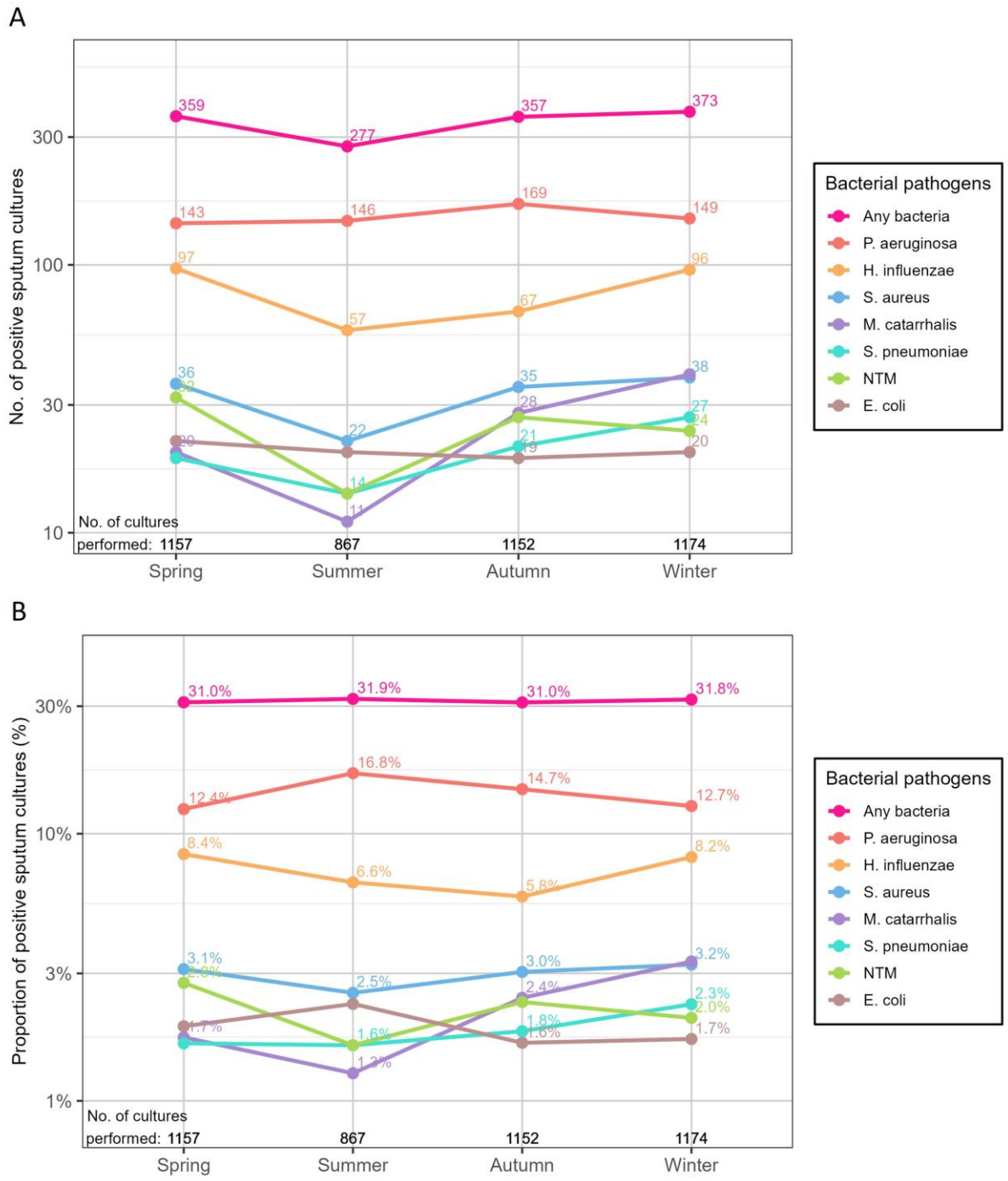


Figure 3.13 Seasonal trends in bacterial pathogen isolations from patients with asthma.

(A) Seasonal trends in the number of bacterial pathogens.

(B) Seasonal trends in the proportion of bacterial pathogens.

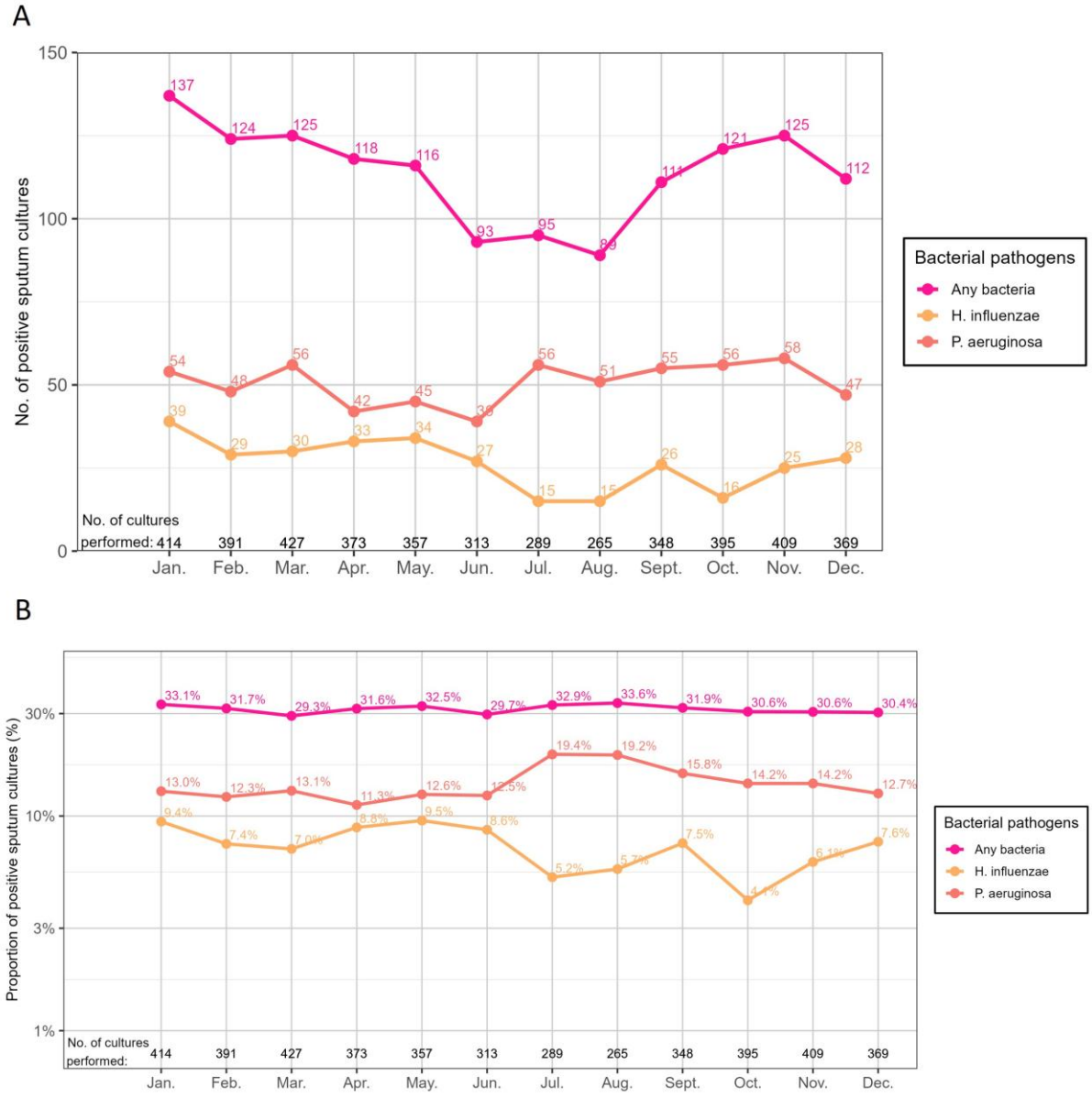


Figure 3.14 Monthly trends in bacterial pathogen isolations from patients with asthma.

(A) Monthly trends in the number of bacterial pathogens.

(B) Monthly trends in the proportion of bacterial pathogens.

3.4.9 Determinants of positive sputum cultures

Logistic regression identified several independent factors associated with positive versus only negative sputum cultures in the 342 patients with complete data who could be included in this analysis (out of a total of 633 patients with a first sputum culture taken after 2016, when hospital prescription records were available) (Table 3.4). Any positive sputum culture was more likely in older patients (adjusted odds ratio aOR (95%CI) per 10 years older: 1.11 [1.01, 1.22], p = 0.03), and those with higher blood neutrophil counts (aOR (95%CI) per 10^9/L higher: 1.05 [1.01, 1.09], p = 0.01), while cardiovascular disease was associated with a lower risk of positive cultures (aOR (95%CI): 0.59 [0.40, 0.86], p = 0.007). For inhaled corticosteroid use, fluticasone propionate was strongly associated with positive sputum cultures (aOR (95%CI): 2.51 [1.57, 4.06], p < 0.001) compared to individuals not using ICS, whereas no significant associations were observed for budesonide or beclomethasone dipropionate. Moreover, high-dose fluticasone propionate (>500 µg/day) was strongly associated with both positive sputum cultures (OR (95%CI): 2.90 [1.78, 4.71], p < 0.001) compared to low-to-medium dose fluticasone propionate (Table 3.5).

Table 3.4 Logistic regression analysis for factors independently associated with positive sputum cultures in patients with asthma.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (10 years older)	1.12 [1.03, 1.21]	0.007	1.11 [1.01, 1.22]	0.03
Sex				
Male	1 (Ref.)		1 (Ref.)	
Female	0.96 [0.71, 1.30]	0.78	0.91 [0.65, 1.26]	0.55
IMD score (5-unit higher)	0.96 [0.90, 1.03]	0.28	0.98 [0.91, 1.05]	0.49
Comorbidities				
Cardiovascular disease	0.65 [0.47, 0.90]	0.01	0.59 [0.40, 0.86]	0.007
Type 2 diabetes	0.98 [0.60, 1.55]	0.94	1.21 [0.71, 2.00]	0.48
Blood tests				
Neutrophils (10^9/L higher)	1.03 [1.00, 1.07]	0.044	1.05 [1.01, 1.09]	0.01
Eosinophils (10^9/L higher)	1.03 [0.77, 1.32]	0.84	0.99 [0.69, 1.33]	0.96
High CRP value	1.21 [0.91, 1.60]	0.19	1.08 [0.80, 1.47]	0.63
ICS usage				
No ICS therapy	1 (Ref.)		1 (Ref.)	
Budesonide	0.70 [0.40, 1.18]	0.17	0.67 [0.36, 1.24]	0.21
Beclomethasone dipropionate	0.87 [0.58, 1.32]	0.50	0.85 [0.54, 1.35]	0.48
Fluticasone propionate	2.62 [1.74, 3.99]	<0.001	2.51 [1.57, 4.06]	<0.001
Prior antibiotic use in past year	1.06 [0.55, 2.21]	0.87	0.88 [0.43, 1.93]	0.74
Calendar year (per year later)	0.98 [0.93, 1.03]	0.40	1.03 [0.97, 1.08]	0.37

Abbreviations: CI: confidence interval; CRP: C-reactive protein; ICS: inhaled corticosteroid; IMD: Index of Multiple Deprivation score (lower values indicate less deprivation); OR: odds ratio; Ref: reference.

A high CRP value indicates a CRP level greater than 10 mg/L.

Table 3.5 Logistic regression analysis for dose of inhaled corticosteroid associated with positive sputum cultures in patients with asthma.

ICS dose (high *vs.* low-to-medium)	Univariate analysis	
	OR (95% CI)	p-value
Beclomethasone dipropionate	1.01 [0.75, 1.38]	0.95
Budesonide	0.89 [0.48, 1.67]	0.71
Fluticasone propionate	2.90 [1.78, 4.71]	<0.001

High-dose thresholds were defined according to guideline recommendations: >400 μg/day for *beclomethasone dipropionate*, >800 μg/day for *budesonide*, and >500 μg/day for *fluticasone propionate*.

3.5 Discussion

In this large-scale retrospective study spanning 27 years, this study provided a comprehensive overview of the bacterial landscape, resistance patterns, and factors associated with culture positivity in patients with asthma in a region covering ~1% of the UK. My findings highlighted substantial heterogeneity in pathogen prevalence, temporal dynamics, and AMR, revealing significant clinical and epidemiological trends in this population, and a novel association with fluticasone usage.

3.5.1 Patterns of bacterial pathogens in asthma

PPB were identified in nearly one-third of patients with asthma who provided sputum cultures, most commonly *H. influenzae, P. aeruginosa*, *M. catarrhalis* and *S. aureus,* which aligns with previous studies (Durack et al., 2020; Green et al., 2014; Jabeen et al., 2022; Jabeen, Sanderson, Tinè, et al., 2024). It has previously been shown through a metagenomic approach that three of these organisms, *H. influenzae*, *M. catarrhalis* and *S. pneumoniae*, are commensals in the upper airway but are pathogenic in the lower airways, and are frequently detected in severe asthma and associated with airways inflammation, including type-1 and type-17 responses, airway neutrophilia, and elevated airway proteases (Vitiello et al., 2024). Thus, persistent lower airways infection is associated with heightened airways inflammation and an increased risk of exacerbations. These patients may not respond adequately to standard anti-inflammatory ICS therapy and may benefit from targeted antimicrobial strategies such as culture-guided antibiotics or long-term macrolide therapy (Jabeen, Sanderson, Tinè,

et al., 2024), which provides significant additional clinical benefit, even in those with severe eosinophilic asthma treated with monoclonal antibodies (Gabriel Lavoie et al., 2025).

The co-isolation of multiple PPB in a subset of sputum cultures suggests polymicrobial colonisation may play a role in the pathophysiology of asthma, particularly among patients with more severe or recurrent disease. Common patterns included *P. aeruginosa* with *S. aureus* or NTM, and of *H. influenzae* with *M. catarrhalis* or *S. pneumoniae.* The correlation analysis cannot determine whether these patterns arise from direct causal interactions with other bacteria or from shared microbial niches within an inflamed airway, but these microbial combinations may exhibit synergistic interactions which could facilitate bacterial survival and may enhance antibiotic tolerance, as has been reported in other polymicrobial conditions such as COPD and skin wounds (DeLeon et al., 2014; Malvisi et al., 2021).

The longitudinal analyses suggest that *H. influenzae* predominates in early airway infections but shows reduced prevalence in subsequent samples, with a progressive emergence of *P. aeruginosa*. This pattern supports the concept of microbial succession within the airway microbiome, consistent with observations in other chronic airway diseases such as COPD and cystic fibrosis (Foundation, 2022; McDonnell et al., 2015). In asthma, early colonisation by *H. influenzae* is likely promoted by respiratory viral infections, particularly rhinovirus, which compromise epithelial barrier

integrity and enhance bacterial adhesion (Gulraiz et al., 2015). *H. influenzae* further upregulates ICAM-1 (Sajjan et al., 2006), a cell surface receptor used by major group rhinoviruses, facilitating viral entry and inflammation, and drives sustained neutrophilic inflammation that creates a permissive environment for more opportunistic pathogens such as *P. aeruginosa* (Brown et al., 2022; T. S. Hinks et al., 2016; Jabeen, Sanderson, Tinè, et al., 2024). Paradoxically, once *P. aeruginosa* is established, the co-isolation rate of *H. influenzae* and *P. aeruginosa* is significantly lower than expected, suggesting competitive exclusion between these species, further supporting a stepwise model of airway colonisation (Rogers et al., 2015). The eventual replacement of *H. influenzae* by the more antibiotic-tolerant *P. aeruginosa* may also partly reflect selective pressure under antimicrobial exposure, as previously reported in bronchiectasis patients receiving long-term macrolide therapy (Rogers et al., 2014).

3.5.2 Antibiotic resistance and asthma management

This study identified distinct, species-specific AMR profiles among sputum pathogens in asthma, with *S. aureus* and *E. coli* showing particularly high rates of resistance and multi-drug resistance. These organisms are known for their persistence in inflamed airways and their association with recurrent exacerbations (Wan et al., 2025). Multi-drug resistance was most frequently involved β-lactams, quinolones, and monobactams, likely driven by shared mechanisms such as plasmid-mediated gene transfer, efflux pumps, and biofilm formation (Guitor & Wright, 2018).

High-volume prescribing of broad-spectrum antibiotics in hospitals, such as penicillins and fluoroquinolones, is typically associated with high rates of resistance. This likely reflects a combination of overuse and suboptimal empirical prescribing practices, consistent with global concerns regarding antimicrobial stewardship. Macrolides have demonstrated efficacy in asthma management, likely mediated through their activity against persistent *H. influenzae* and their antiviral and anti-inflammatory effects (Jabeen, Sanderson, Tinè, et al., 2024). However, the high resistance rates observed in *S. aureus* and *S. pneumoniae* raise concerns regarding the long-term efficacy of these treatments for these pathogens. These findings underscore the importance of implementing effective resistance-guided antibiotic stewardship in asthma care. Future clinical guidelines should incorporate local and up-to-date resistance surveillance data when making recommendations for empirical antibiotic use during asthma exacerbations.

3.5.3 Interpretation of temporal trends in pathogens and resistance

The overall declining trend in both the prevalence of bacterial pathogens and antimicrobial resistance rates over the past 15 years may be attributable to national antimicrobial stewardship efforts, particularly NHS-led initiatives aimed at reducing inappropriate antibiotic use, which may have contributed to reductions in both sputum culture positivity and resistance, especially among opportunistic or healthcare-associated pathogens (D. Ashiru-Oredope et al., 2023). Interestingly, *H. influenzae*

showed a contrasting pattern, with stable prevalence but increasing resistance over time. Its ability to evade host defences through pseudo-biofilm formation, intracellular persistence, and antigenic variation (Garmendia et al., 2014), combined with repeated antibiotic exposure in patients with frequent exacerbations, may contribute to this trend within individuals.

These findings underscore the need for targeted surveillance and nuanced empirical therapy decisions. Reviewing limited data on the benefit of antibiotics in the management of acute asthma exacerbations, and evidence that inappropriate antibiotic use may lead to extended hospital stays and increased healthcare costs (Stefan et al., 2019), a Cochrane collaboration recommended antibiotics only be used in asthma exacerbations where there are clear clinical signs, symptoms, or laboratory evidence indicative of a bacterial infection (Normansell et al., 2018).

Seasonal variations were observed for most pathogens, particularly *H. influenzae,* which typically peaks in winter, following peaks in the prevalence of respiratory viruses, as has been observed in COPD (Wilkinson et al., 2017). In contrast, *P. aeruginosa* peaked in relative terms in late summer, a finding which has also been reported in otitis media (Perencevich et al., 2008; Villedieu et al., 2018). As *P. aeruginosa* thrives in warm, moist conditions and is commonly found in water and soil (Moradali et al., 2017), this may reflect environmental factors, including increased exposure during summer activities, such as swimming (Mena & Gerba, 2009). However, the absolute

numbers of isolates were less variable over the year, so the summer peak may, to some extent, be an artefact of a seasonal relative reduction in *H. influenzae* isolates.

3.5.4 Determinants of positive sputum cultures

Several factors were associated with positive sputum cultures in patients with asthma. Consistent with earlier studies, older age was associated with an increased risk of positive sputum cultures, likely reflecting cumulative steroid exposure, age-related immunosenescence, and progressive damage to airway mucosal defences (Green et al., 2014; Simpson et al., 2016). Elevated peripheral blood neutrophil counts were also associated with positive cultures, particularly with *H. influenzae*, suggesting that airway infection can cause a degree of systemic neutrophilic inflammation. In contrast, given that most participants are likely to have received ICS since early disease onset, the inverse association between infection and cardiovascular disease (CVD) may reflect a protective effect of ICS on systemic inflammation and cardiovascular risk, as has been described in COPD (Gadhvi et al., 2023). Alternatively, statins and angiotensin-converting enzyme inhibitors (ACEIs), two widely prescribed cardiovascular drugs, have been reported to exert protective effects against bacterial infections and might contribute to this effect (Cao et al., 2021; Nassaji et al., 2015).

The differential impact of ICS formulations on infection risk is particularly noteworthy. Fluticasone propionate, especially high-dose, was strongly associated with an increased likelihood of positive sputum cultures compared to individuals not using ICS.

In contrast, no such association was observed for budesonide or beclomethasone dipropionate. This finding was consistent with those in COPD populations (55, 56), although less well understood in asthma, where fluticasone's high glucocorticoid potency and prolonged airway retention may particularly suppress epithelial antimicrobial defences and facilitate bacterial colonisation (T. S. Hinks et al., 2016). These findings support the growing recognition that not all ICS compounds have equivalent effects on host immunity and susceptibility to infection, and highlight the need for formulation-specific risk assessments in both clinical practice and research.

3.5.5 Study limitations

This study has several limitations. First, sputum cultures, although widely used to assess lower airway infections, may not fully capture the diversity of airway microbiota, particularly for certain difficult-to-culture organisms, such as *Mycoplasma pneumoniae* or *Tropheryma whipplei*. Alternative diagnostic approaches, including protected sterile endobronchial brushings or bronchoalveolar lavage, and applying metagenomic next-generation sequencing, could offer more comprehensive and accurate microbial profiles (Jabeen, Sanderson, Tinè, et al., 2024). Moreover, this study was conducted among patients admitted to the hospital with a primary diagnosis of asthma, potentially constituting the more severe cases. Furthermore, sputum sampling in routine clinical practice is typically performed in patients with suspected infection, introducing potential selection bias. PPB isolation may reflect both carriage and infection by the organism, although culture positivity was found to be associated with systemic

inflammation. Additionally, this study was conducted in patients in a single region (Oxfordshire), which may limit the generalisability of the findings to other settings or populations. Diabetes and CVD were the only comorbidities considered, identified through secondary diagnosis codes. Their prevalence was almost certainly under-ascertained, implying that actual effects are likely underestimated. Finally, given the observational nature of this study, findings reflect association rather than causation. Further interventional studies or mechanistic investigations are necessary to establish causality and to assess therapeutic implications.

3.5.6 Clinical implications

This study provides important clinical insights into the role of bacterial infection and antimicrobial resistance in asthma. The observation that one-third of sputum cultures yielded potentially pathogenic bacteria highlights the value of routine microbiological assessment in selected patients, particularly those with frequent exacerbations, neutrophilic inflammation, or older age. The strong association between inhaled fluticasone use and positive bacterial cultures suggests that the choice of corticosteroid may influence airway microbial ecology, with implications for tailoring anti-inflammatory therapy. The strong inverse association between infection and CV disease warrants further research into the causal mechanism, be it a protective effect of ICS on the development of coronary plaques or an antimicrobial effect of statins, aspirin or other cardiovascular medications. The high prevalence of antimicrobial resistance, including widespread multi-drug resistance in *S. aureus* and *E. coli* and

substantial β-lactam resistance in *H. influenzae* and *M. catarrhalis*, underscores the importance of culture-guided antibiotic prescribing and antimicrobial stewardship in asthma care. Longitudinal trends showing declining *H. influenzae* but rising *P. aeruginosa* isolation suggest microbial succession in the airways, which may influence long-term disease progression and treatment strategies. These findings support the use of sputum culture in personalised asthma management, guiding both corticosteroid selection and targeted antibiotic therapy.

3.6 Conclusions

In this 27-year retrospective study, nearly one-third of asthma patients undergoing sputum testing had positive cultures for potentially pathogenic bacteria, most frequently *H. influenzae* and *P. aeruginosa*. Over the past decade, overall pathogen prevalence and antimicrobial resistance rates declined, although *P. aeruginosa*, *S. aureus*, and *E. coli* continued to exhibit the highest resistance levels. We observed intra-individual microbial succession, with early *H. influenzae* isolation often followed by *P. aeruginosa*. Positive cultures were associated with older age, neutrophilic inflammation, and inhaled fluticasone use. These findings highlight the need for infection surveillance, resistance-guided antibiotic stewardship, and judicious selection of inhaled corticosteroid formulations in the management of asthma.

Chapter 4 Sex differences in asthma: a large-scale multi-omics analysis

Overview

Adult women exhibit a higher prevalence and greater severity of asthma compared with men, yet the underlying molecular and physiological mechanisms remain poorly understood.

Clinical and multi-omics data (transcriptomic, proteomic, metabolomic, microbiomic) were analysed from 568 adults in the U-BIOPRED asthma cohort. Differential expression analysis, functional enrichment analysis, and WGCNA were conducted. Key findings were validated using the RASP-UK cohort.

In mild to moderate asthma, clinical characteristics were largely comparable, although women exhibited a higher prevalence of non-T2 phenotypes and a history of respiratory infections. In severe asthma, men exhibited more pronounced airflow limitation, reflected by lower FEV_1 and greater residual volume (RV), whereas women experienced a higher symptom burden, more frequent exacerbations and higher prevalence of comorbidities in general. Among women with severe asthma, bronchial biopsy transcriptomic analysis revealed enhanced airway remodelling and impaired mitochondrial and ciliary function, while blood transcriptomic profiling indicated stronger innate and adaptive immune responses with enhanced T-cell activation.

Proteomic analysis showed heightened type 2 immune activity in women with severe asthma, evidenced by increased protein levels of *GZMB*, *TSLP*, and *CCL11*. Sputum microbiome profiling further revealed a higher relative abundance of potentially pathogenic genera in women with severe asthma, with a higher prevalence of *Haemophilus*, *Moraxella*, and *Pseudomonas* species. Circulating androgen levels were inversely associated with asthma severity in both sexes.

To conclude, women with severe asthma experienced more inflammation, remodelling, and pathogenic airway bacteria, while men exhibited greater lung function loss.

4.1 Introduction

4.1.1 Sex differences in asthma burden

Asthma burden varies by sex throughout the lifespan. In childhood, asthma is more prevalent in boys, while this trend reverses after puberty, with adult women showing higher prevalence (10.0% vs. 5.7%) and asthma-related mortality (16.0 vs. 10.2 per million) compared to men (Pate et al., 2021).

Women with asthma tend to experience poorer symptom control than men, reporting more asthma-related symptoms and being more likely to be affected by specific manifestations, such as coughing or environmental triggers (McCallister et al., 2013). In cross-sectional analyses, women more frequently present with late-onset asthma, obesity, gastroesophageal reflux disease (GORD), and lower levels of T2 biomarkers, whereas previous smoking and nasal polyposis are more commonly observed in males (Senna et al., 2021). Large observational studies have similarly shown that women are more likely to be obese, have a greater symptom burden, and exhibit lower levels of type 2 inflammatory biomarkers and lower spirometric indices (Eastwood et al., 2023; L. Loewenthal et al., 2024; Tessa et al., 2024).

Several factors have been investigated that contribute to sex differences, including variations in pulmonary physiology, hormonal regulation, immune response variability, and genetic influences (N. U. Chowdhury et al., 2021).

4.1.2 The role of sex hormones in asthma

Sex hormones are increasingly recognised as critical modulators of asthma pathophysiology, modulating airway structure and immune function. Epidemiological studies show sex-specific patterns in asthma: boys are more affected in childhood, but after puberty, women have higher prevalence and greater severity (Pate et al., 2021). This shift suggests that sex hormones influence asthma risk and severity across puberty, menstrual cycles, pregnancy, and menopause, implicating oestrogens, progesterone, and androgens in disease modulation (McCleary et al., 2018; Yung et al., 2018).

Oestrogen can promote type 2 inflammation by enhancing Th2 cell differentiation, with increased production of IL-4, IL-5, and IL-13, while progesterone has been associated both with airway smooth muscle relaxation and enhanced mucus secretion (Radzikowska & Golebski, 2023). These hormonal influences may partly explain symptom fluctuations across the menstrual cycle, pregnancy, menopause, and during hormone replacement therapy (HRT) (McCleary et al., 2018). Androgens, by contrast, appear to exert predominantly protective effects. Circulating levels of dehydroepiandrosterone sulphate (DHEA-S), testosterone, and related androgen metabolites have been inversely associated with asthma severity and exacerbation frequency (DeBoer et al., 2018; Zein et al., 2021). Experimental studies suggest that androgens suppress airways inflammation by inhibiting type 2 cytokine production, promoting regulatory T-cell activity, and modulating innate immune pathways (Ejima

et al., 2022; Laffont et al., 2017). These findings suggest that sex hormones are not only biomarkers but also potential mediators of sex differences in asthma, offering a rationale for therapeutic strategies targeting hormonal pathways.

4.1.3 Sex differences in pulmonary physiology

Pulmonary physiology exhibits marked sex differences that influence asthma pathophysiology across the life course. Women generally have smaller lungs, narrower airways, and lower maximal expiratory flows than men, even after adjustment for body size (LoMauro & Aliverti, 2021). In contrast, men typically demonstrate larger airway calibres, higher lung volumes, and are predisposed to greater airflow limitation with ageing (Dominelli & Molgat-Seon, 2022).

Sex hormones play a significant role in shaping pulmonary physiology and airway responsiveness (LoMauro & Aliverti, 2021). Estrogens exert protective effects by suppressing cholinergic airway reactivity and reducing constriction. These effects are partly mediated via upregulation of epithelial acetylcholinesterase and modulation of muscarinic receptor activity (Dimitropoulou et al., 2005). Estrogen receptor-α (ERα) has been identified as a key regulator of respiratory rhythm and airway responsiveness, with disruption leading to enhanced bronchoconstriction and altered breathing patterns (Carey et al., 2007). Conversely, testosterone has been linked to airway smooth muscle relaxation (Carbajal-García et al., 2024). In human asthma, increased bronchial airway androgen receptor expression together with higher circulating

androgen levels are associated with improved lung function, fewer clinical symptoms, and reduced FeNO (Zein et al., 2021).

4.1.4 Sex differences in asthma immunology

Women typically exhibit stronger innate and adaptive immune responses than men, a phenomenon reported across infectious, autoimmune, and allergic diseases (Dunn et al., 2024). This heightened immune reactivity is thought to provide advantages in pathogen defence but can also predispose women to excessive inflammation and immune-mediated tissue damage (Klein & Flanagan, 2016).

In asthma, several immune pathways have been implicated in sex-specific differences. Women exhibit stronger type 2 immune responses and eosinophilic inflammation, together with enhanced activation of innate lymphoid cells (ILC2s) (Dunn et al., 2024; Roved et al., 2017). Transcriptomic and proteomic analyses of severe asthma have also identified exaggerated T-cell activation and upregulation of interferon-stimulated genes in women, indicating immune hyperactivity extending beyond classical T2 pathways (Peng et al., 2025). Conversely, men with severe asthma may display relatively higher neutrophilic and non-type 2 inflammatory phenotypes, which are associated with faster lung function decline (Hsiao et al., 2019; Ray & Kolls, 2017).

4.1.5 Sex-related genetic differences in asthma

Previous genomic studies have identified sex-specific genetic variants associated with

asthma, including immune-regulatory loci such as *IRF1*, *CD52*, *ORMDL3*, *TSLP*, *IL4RA* and *IL1RL1* (El-Husseini et al., 2020; Yi Han et al., 2020; Mersha et al., 2015). Many asthma-related genes are located on the sex chromosomes, such as *TLR7*, *IL2RG*, *CD40LG*, and *IRAK1*, which exhibit different expression patterns in males and females (Odimba et al., 2023). In general, women show higher expression of X-chromosome genes due to incomplete X inactivation, which affects 15-25% of X-linked genes (Klein & Flanagan, 2016; Schurz et al., 2019). Gautam *et al.* found men showed dysregulation of *FBXL7*, *ITPR3*, *RAD51B*, and *ALOX15* and enrichment of the hypoxia-inducible factor-1 (HIF-1) pathway, whereas women demonstrated enrichment of IL-17 and chemokine signalling pathways (Gautam et al., 2019). Epigenetic analyses have also revealed sex-specific DNA methylation patterns at key CpG sites such as cg20891917 (Patel et al., 2021). In sputum microbiome profiling, *Streptococcus salivarius* was significantly more abundant in women than in men (Chen et al., 2020). However, comprehensive investigations integrating multiple omics layers are still lacking (Peng et al., 2025).

4.1.6 Research gaps and rationale

Several important gaps remain in understanding sex differences in asthma. Although epidemiological studies consistently report a higher prevalence and greater severity of asthma in adult women, the molecular and physiological mechanisms underlying these differences are poorly defined. Existing research is constrained by modest sample sizes, single-centre cohorts, and a predominant focus on clinical or hormonal

factors in isolation, which limits understanding of the multi-layered biological basis of sex-specific asthma pathobiology. Addressing these gaps through large-scale, multi-omics analyses holds promise for revealing the complex interplay between sex, immunity, airway remodelling, and microbial ecology, with the potential to provide new insights into disease heterogeneity and guiding potential personalised treatment strategies.

4.2 Objectives

Previous studies have lacked comprehensive investigations into sex differences in the characteristics and mechanisms of asthma. The primary objective of this study was to address this gap by exploring sex-specific molecular and physiological mechanisms of asthma through the integration of clinical, transcriptomic, proteomic, metabolomic, microbiomic, and radiomic data from a large adult asthma cohort, with validation performed in an external cohort. Specifically, this study aimed to:

1. Assess how clinical presentation, lung function, and inflammatory features of asthma differ between men and women with clinical data analysis.
2. Uncover sex-specific molecular pathways and gene networks in airway tissues linked to asthma severity with bronchial biopsy transcriptomics analysis.
3. Determine circulating protein signatures that reflect sex-related immune and inflammatory responses in asthma with blood proteomics analysis.
4. Investigate hormonal and metabolic contributions to sex-related differences in asthma severity and treatment response with urine metabolomics analysis.
5. Characterise sex differences in airway microbiome composition, particularly the abundance of pathogenic bacteria with sputum metagenomics analysis.
6. Validate key findings with an external cohort.

These objectives aimed to provide a comprehensive and multi-omics characterisation of sex differences in asthma, thereby advancing understanding of the biological

mechanisms of sex differences in asthma pathophysiology.

4.3 Methods

4.3.1 Study population

This study utilised cross-sectional data from the U-BIOPRED (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes) adult cohort, a multi-centre, pan-European asthma study (ClinicalTrials.gov: NCT01982162) conducted at 16 clinical centres across 11 European countries (D. E. Shaw et al., 2015). Before enrolment, participants with severe asthma were required to have been under specialist respiratory care for ≥6 months, during which their asthma management was optimised and adherence ensured (Bel et al., 2011).

A total of 568 non-smoking adults (aged ≥18 years) were included, comprising 101 healthy controls, 88 with mild-to-moderate asthma, and 379 with severe asthma (Figure 4.1). Asthma diagnosis and severity classification were agreed upon at a U-BIOPRED consensus meeting (Bel et al., 2011). The definition of mild/moderate asthma and severe asthma was illustrated in Chapter 1.1.2.

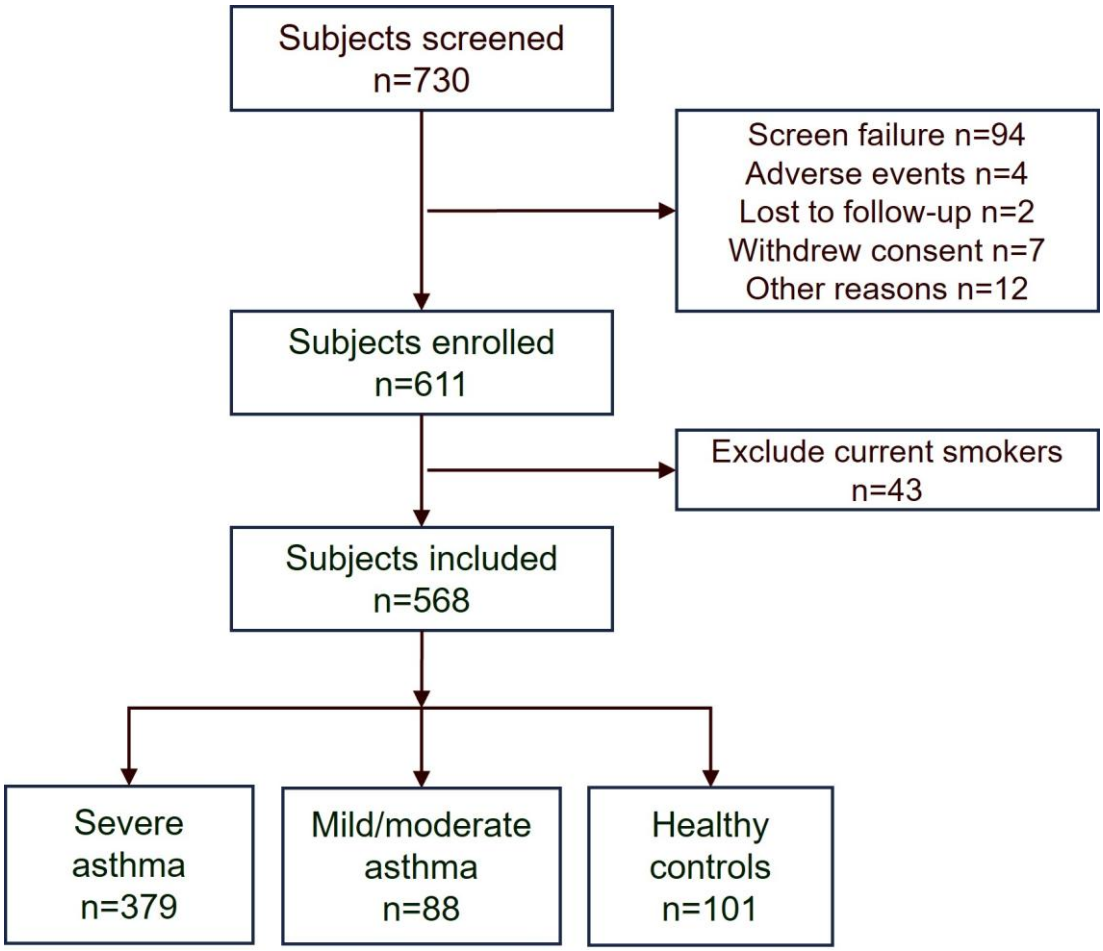


Figure 4.1 Cohort flow diagram.

The figure was adapted from Shaw *et al* (D. E. Shaw et al., 2015).

Biological samples were anonymised and stored centrally. Clinical and omics data were accessed via the tranSMART knowledge platform (Athey et al., 2013). Participants were stratified by sex to examine differences in asthma characteristics and mechanisms. Clinical data, histology data, and transcriptomics data from the RASP-UK bronchoscopy study (Khalfaoui et al., 2022b) were used to validate key findings. The detailed introduction of RASP-UK was provided in Chapter 2.3.1.

4.3.2 Clinical data collection

At the initial screening visit, participants were evaluated for eligibility. Within 28 days, they attended a baseline visit, where core and optional assessments were performed. Demographic, clinical, lung function (spirometry and body plethysmography), and physiological data were collected as previously described (Dominick E Shaw et al., 2015; Susan J Wilson et al., 2021). Cell counts were obtained from blood, induced sputum, and biopsies, together with biomarkers including serum IgE, skin prick test results, serum periostin, FeNO, C-reactive protein (CRP), and 30 additional blood biomarkers, as previously described (Kuo, Pavlidis, Loza, Baribaud, Rowe, Pandis, Sousa, et al., 2017). Type 2 phenotypes were classified according to the RASP criteria (Heaney et al., 2021). Two-millimetre sections embedded in glycol methacrylate were stained with monoclonal antibodies against CD3, CD4, CD8, neutrophil elastase, and EG2. Cell counts were performed in a blinded manner and reported as the number of positive cells per square millimetre. All procedures were undertaken in accordance with standardised operating protocols (D. E. Shaw et al., 2015).

4.3.3 Omics data collection

Multi-omics data included transcriptomics, proteomics, metabolomics, microbiomics, and radiomics. Data collection and processing procedures for each omics layer have been described previously. Specifically, transcriptomic data were obtained from bronchial biopsies (Kuo, Pavlidis, Loza, Baribaud, Rowe, Pandis, Hoda, et al., 2017) and blood (J. Bigler et al., 2017). proteomic analyses from serum and plasma (Khezia

Asamoah et al., 2024); metabolomics from urine samples (Reinke et al., 2022); microbiomics profiling from sputum samples (Abdel-Aziz et al., 2021); and quantitative CT (qCT) parameters derived from high-resolution CT (HRCT) scans (S. J. Wilson et al., 2021). The sample sizes for each omics analysis are shown in Table 4.1.

Table 4.1 Sample size in each omics dataset in this study.

	Healthy controls		Mild/moderate asthma		Severe asthma	
	Female	Male	Female	Male	Female	Male
Total	39	62	44	44	237	142
Bronchial biopsy transcriptomics	10	16	16	12	23	23
Blood transcriptomics	34	53	37	40	182	114
Blood proteomics	35	57	38	39	203	124
Urine metabolomics	38	62	43	44	236	140
Sputum microbiomics	7	16	11	13	52	36
Radiomics	6	8	4	5	38	29

4.3.4 Bioinformatic and computational analysis

Raw count data were initially pre-processed and quality-filtered using the *edgeR* package to remove lowly expressed genes and stabilise variance across samples. Differential expression analysis was performed using *DESeq2*, with Benjamini-Hochberg FDR correction (FDR < 0.05 considered significant). Models were adjusted for relevant covariates, including age, body mass index (BMI), smoking status, and oral corticosteroid (OCS) use to minimise potential confounding. Functional annotation of differentially expressed genes was performed using GO enrichment analysis with the clusterProfiler package (Yu et al., 2012). To explore gene co-expression architecture, weighted gene co-expression network analysis (WGCNA) (Langfelder &

Horvath, 2008b) was employed to cluster genes into modules, which were subsequently interrogated to identify modules associated with sex and asthma phenotypes. Sources of transcriptional variability across individuals were assessed using the variancePartition package (Hoffman & Schadt, 2016), enabling quantification of the relative contributions of demographic, clinical, and biological factors. In addition, transcription factor (TF) activity was inferred by mapping expression data to the *DoRothEA* regulon database (Garcia-Alonso et al., 2019) to identify upstream regulatory mechanisms potentially contributing to sex-specific transcriptional signatures.

4.3.5 Statistical analysis

Categorical variables were compared using Chi-square or Fisher's exact tests, as appropriate, and presented as frequencies and percentages. Continuous variables were summarised as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on data distribution, and compared using ANOVA, the Mann–Whitney U test, or the Kruskal–Wallis test. Linear regression models adjusted for age, body mass index (BMI), smoking status, and oral corticosteroid (OCS) use were applied to assess sex differences in clinical outcomes. Spearman's rank correlation coefficients were calculated to assess associations between demographic variables and biomarkers. All analyses were performed in R (version 4.4.0), with p-values < 0.05 considered statistically significant.

4.3.6 Ethics and consent

All participants provided written informed consent. The U-BIOPRED study was approved by the ethics committees in each participating country and was registered on ClinicalTrials.gov (NCT01982162).

4.4 Results

4.4.1 Sex differences in clinical characteristics

The sex differences in clinical characteristics were assessed. In the severe asthma group, women were significantly younger (50.4 vs. 54.6 years, p = 0.004) and had a higher BMI (30.0 vs. 28.5 kg/m², p = 0.026). Men with severe asthma exhibited lower spirometry values, including lower pre-bronchodilator FEV_1% predicted (62.7 vs. 70.2, p = 0.010), post-bronchodilator FVC% predicted (84.8 vs. 88.8, p = 0.043), and pre-bronchodilator FEV_1/FVC ratio (61.3 vs. 66.7, p < 0.001). Moreover, male patients with severe asthma have a higher residual volume (RV) (p < 0.001), higher specific airway conductance (sGaw) (p < 0.001), and lower total lung capacity (TLC) (p = 0.020). Men with severe asthma had a significantly higher proportion of ex-smokers (p = 0.030) and more pack-years than women (p = 0.018) (Table 4.2).

Table 4.2 Sex differences in demographic characteristics and pulmonary function of patients with asthma and healthy controls.

	Healthy controls			Mild/moderate asthma			Severe asthma		
	Female	Male	p-value	Female	Male	p-value	Female	Male	p-value
Subjects, n	39	62		44	44		237	142	
Age, years	40.3 ± 13.9	38.0 ± 13.2	0.399	41.5 ± 14.4	41.8 ± 16.7	0.924	50.4 ± 13.9	54.6 ± 12.9	0.004
Age at diagnosis, years	NA	NA	NA	16 [5, 34]	13 [6, 28]	0.956	23 [9, 40]	30 [8, 44]	0.139
Asthma duration, years	NA	NA	NA	23 [14, 29]	18 [9, 28]	0.316	23 [12, 37]	24 [11, 38]	0.734
Race, Caucasian	36/39 (92.3%)	58/62 (13.5%)	1.000	41/44 (93.2%)	41/44 (93.2%)	1.000	213/237 (89.9%)	129/142 (90.8%)	0.897
BMI, $kg \cdot m^{-2}$	23.8 ± 3.4	26.3 ± 3.4	0.001	25.2 ± 4.7	26.3 ± 4.2	0.255	30.0 ± 6.9	28.5 ± 5.3	0.026
Pre-bronchodilator FEV_1, % pred	98.4 [92.7, 107.2]	104.3 [95.7, 111.2]	0.180	92.3 [81.8, 101.8]	91.6 [73.1, 100.6]	0.387	70.2 [52.4, 86.4]	62.7 [48.3, 77.3]	0.010
Post-bronchodilator FEV_1, % pred	NA	NA	NA	101.9 [89.7, 109.4]	101.0 [81.9, 108.6]	0.482	81.4 [63.3, 92.8]	73.1 [55.5, 86.3]	0.001
Pre-bronchodilator FVC, % pred	108.1 [99.7, 120.1]	108.7 [100.8, 114.5]	0.594	108.3 [97.3, 113.2]	104.2 [86.8, 118.5]	0.325	88.8 [75.9, 103.2]	84.8 [73.5, 97.1]	0.043
Post-bronchodilator FVC, % pred	NA	NA	NA	106.0 [99.0, 115.7]	108.3 [94.2, 123.7]	0.860	97.8 [85.5, 111.3]	91.9 [80.2, 102.1]	0.001
Pre-bronchodilator FEV_1/FVC, %	79.8 [74.9, 83.3]	80.1 [75.3, 82.8]	0.810	75.5 [70.1, 78.8]	71.3 [63.7, 77.4]	0.094	66.7 [56.2, 75.9]	61.3 [49.2, 68.6]	<0.001
Pre-bronchodilator $FEF_{25-75\%}$, % pred	70.9 [62.4, 95.4]	85.80 [73.1, 104.7]	0.034	60.9 [43.1, 70.1]	59.7 [44.2, 69.4]	0.932	32.1 [19.1, 52.7]	29.6 [17.6, 45.9]	0.370
Pre-bronchodilator PEF, %pred	108.7 [92.5, 115.5]	100.4 [91.4, 115.1]	0.385	93.1 [78.8, 108.3]	91.6 [78.5, 102.7]	0.494	71.9 [57.6, 88.8]	72.1 [53.5, 91.8]	0.738
Airflow limitation, %	0/39 (0.0%)	0/62 (0.0%)	NA	5/44 (11.4%)	10/44 (22.7%)	0.257	113/237 (47.7%)	79/142 (55.6%)	0.164
RV, L	1.7 [1.4, 2,0]	1.7 [1.4, 2.3]	0.957	1.9 [1.4, 2.2]	2.2 [1.8, 2.7]	0.016	2.4 [1.9, 2.9]	2.8 [2.4, 3.6]	<0.001
TLC, L	5.4 [4.8, 6.1]	7.4 [6.8, 8.0]	<0.001	5.4 [5.0, 5.9]	7.4 [6.7, 7.9]	<0.001	5.4 [4.8, 6.1]	7.3 [6.5, 8.2]	<0.001
RV/TLC	0.31 [0.29, 0.34]	0.23 [0.19, 0.28]	<0.001	0.34 [0.28, 0.39]	0.30 [0.25, 0.35]	0.112	0.44 [0.36, 0.54]	0.40 [0.35, 0.48]	0.020
sGaw, $kPa^{-1} \cdot s^{-1}$	1.5 [1.1, 2.0]	1.7 [1.1, 2.3]	0.438	1.4 [1.1, 2.6]	1.1 [0.7, 1.8]	0.118	0.7 [0.4, 1.1]	0.8 [0.4, 1.1]	0.383
Ex-smokers/non-smokers, n	11/28	9/53	0.154	7/37	6/38	1.000	62/175	53/89	0.030
Smoking history, pack-years	1.0 [0.6, 3.5]	0.7 [0.2, 3.0]	0.447	3.5 [1.4, 4.1]	4.3 [1.5, 4.9]	0.352	5.2 [2.0, 14.8]	13.0 [4.0, 23.0]	0.018

Data are presented as n (%), mean ± SD, or median [interquartile range], unless otherwise stated.

Abbreviations: BMI: body mass index; $FEF_{25-75\%}$: forced expiratory flow at 25% and 75% of the pulmonary volume; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; RV: residual volume; sGaw: specific airway conductance; TLC: total lung capacity.

On quantitative CT, women with severe asthma demonstrated statistically significantly lower lung volumes (4,832 mL vs. 6,416 mL, $p < 0.001$). Moreover, women with asthma exhibited smaller luminal areas (20.1 mm^2 vs. 23.7 mm^2, $p = 0.012$) and wall areas (28.2 mm^2 vs. 34.1 mm^2, $p < 0.001$), indicating a smaller central airway diameter (Table 4.3 and Table 4.4). Men with severe asthma demonstrated significantly more gas trapping than women ($LAA_{-856}E$: 24.2% vs. 17.0%, $p = 0.045$). Furthermore, men with severe asthma also had more radiological evidence of emphysema than women, either by percentage of low attenuation areas (LAA_{-950} 12.6% vs. 8.7%, $p = 0.004$), by inspiratory mean lung density (-840 vs. -824, $p = 0.034$), or by the 15^{th} percentile of the cumulative frequency distribution of CT numbers (-940 vs. -927, $p = 0.009$), compared with women. The airway per cent wall area, a marker of remodelling, did not differ significantly between males and females with severe asthma (Table 4.3 and Table 4.4).

Table 4.3 Sex differences in quantitative CT in patients with asthma and healthy controls.

	Healthy controls			Mild/moderate asthma			Severe asthma		
	Female (n=6)	Male (n=8)	p-value	Female (n=4)	Male (n=5)	p-value	Female (n=38)	Male (n=29)	p-value
Airways dimension indexes									
Lung volume I, mL	4535 ± 561	6693 ± 1292	<0.001	5469 ± 934	7115 ± 651	0.03	4832 ± 963	6416 ± 1242	<0.001
Median lumen area I, mm^2	18.8 ± 2.9	27.1 ± 8.1	0.03	22.2 ± 1	30.8 ± 9.2	0.047	20.1 ± 7.3	23.7 ± 5.4	0.01
Median wall area I, mm^2	26.5 ± 3.8	33.3 ± 6.9	0.04	31.5 ± 1.4	33.6 ± 5.4	0.4	28.2 ± 4.4	34.1 ± 6.9	<0.001
Median total area I, mm^2	45.3 ± 5.6	60.4 ± 14.2	0.02	53.7 ± 1.3	62.8 ± 13.7	0.1	49.1 ± 11.6	57.0 ± 11.6	0.003
Wall area I, %	61.1 ± 4.1	60.8 ± 4.9	0.9	61.8 ± 4.9	60.8 ± 3.1	0.8	64.1 ± 3.3	63.1 ± 3.6	0.2
Air trapping indexes									
LAA_{-856} E, %	3.6 ± 3.4	8.1 ± 5.8	0.1	7.8 ± 10.9	10.5 ± 10.7	0.7	17.0 ± 14.9	24.2 ± 15.8	0.045
Lung volume E/I	0.4 ± 0.1	0.4 ± 0.1	0.9	0.4 ± 0.1	0.4 ± 0.1	0.8	0.6 ± 0.1	0.6 ± 0.1	0.9
MLD E/I	0.8 ± 0.1	0.8 ± 0.1	0.8	0.7 ± 0.1	0.7 ± 0.1	0.9	0.8 ± 0.1	0.8 ± 0.1	0.5
$LAA_{-856/-950}$ E-I, %	-1.2 ± 3.2	-2.6 ± 6.3	0.6	2.3 ± 10.7	-1.3 ± 10.5	0.6	8.1 ± 12.4	11.7 ± 12.7	0.2
LAA_{-856} E-I, %	-54.8 ± 16.7	-58.4 ± 13.1	0.7	-56.6 ± 13.7	-60.7 ± 11.1	0.6	-38.5 ± 15.3	-39.9 ± 15.1	0.7
Emphysema indexes									
LAA_{-950} I, %	4.8 ± 3.4	10.6 ± 4.9	0.02	5.5 ± 1.5	11.8 ± 5.3	0.01	8.7 ± 5.6	12.6 ± 6.2	0.004
MLD I, HU	-831 ± 31	-846 ± 24	0.3	-840 ± 21	-857 ± 15	0.2	-824 ± 35	-840 ± 35	0.03
Percentile 15 I, HU	-914 ± 19	-934 ± 134	0.1	-921 ± 11	-940 ± 20	0.1	-927 ± 23	-940 ± 20	0.01

Abbreviations: E: expiration; HU: Hounsfield units; I: inspiration; LAA: low attenuation areas; MLD: mean lung density.

$LAA_{-856/-950}$ E-I, %=LAA_{-856} E − LAA_{-950} I; LAA_{-856} E-I, %=LAA_{-856} E − LAA_{-856} I.

Table 4.4 Sex-stratified comparison of quantitative CT between healthy controls and severe asthma patients.

	Female				Male			
	Healthy controls (n=6)	Severe asthma (n=38)	Mean differences	p-value	Healthy controls (n=8)	Severe asthma (n=29)	Mean differences	p-value
Airways dimension indexes								
Lung volume I, mL	4535 ± 561	4832 ± 963	297	0.3	6693 ± 1292	6416 ± 1242	-278	0.6
Median lumen area I, mm^2	18.8 ± 2.9	20.1 ± 7.3	1.2	0.5	27.1 ± 8.1	23.7 ± 5.4	-3.4	0.3
Median wall area I, mm^2	26.5 ± 3.8	28.2 ± 4.4	1.7	0.3	33.3 ± 6.9	34.1 ± 6.9	0.8	0.8
Median total area I, mm^2	45.3 ± 5.6	49.1 ± 11.6	3.7	0.2	60.4 ± 14.2	57.0 ± 11.6	-3.4	0.6
Wall area I, %	61.1 ± 4.1	64.1 ± 3.3	3.0	0.1	60.8 ± 4.9	63.1 ± 3.6	2.3	0.3
Air trapping indexes								
LAA_{-856} E, %	3.6 ± 3.4	17.0 ± 14.9	13.4	<0.001	8.1 ± 5.8	24.2 ± 15.8	16.1	<0.001
Lung volume E/I	0.4 ± 0.1	0.6 ± 0.1	0.1	0.002	0.4 ± 0.1	0.6 ± 0.1	0.1	0.002
MLD E/I	0.8 ± 0.1	0.8 ± 0.1	0.1	0.1	0.8 ± 0.1	0.8 ± 0.1	0.1	0.002
$LAA_{-856/-950}$ E-I, %	-1.2 ± 3.2	8.1 ± 12.4	9.4	<0.001	-2.6 ± 6.3	11.7 ± 12.7	14.3	<0.001
LAA_{-856} E-I, %	-54.8 ± 16.7	-38.5 ± 15.3	16.4	0.1	-58.4 ± 13.1	-39.9 ± 15.1	18.5	0.003
Emphysema indexes								
LAA-950 I, %	4.8 ± 3.4	8.7 ± 5.6	3.9	0.04	10.6 ± 4.9	12.6 ± 6.2	2.0	0.3
MLD I, HU	-831 ± 31	-824 ± 35	7.2	0.6	-846 ± 24	-840 ± 35	5.8	0.6
Percentile 15 I, HU	-914 ± 19	-927 ± 23	-13.3	0.2	-934 ± 14	-940 ± 20	-5.9	0.3

Abbreviations: E: expiration; HU: Hounsfield units; I: inspiration; LAA: low attenuation areas; MLD: mean lung density.

$LAA_{-856/-950}$ E-I, %=LAA_{-856} E − LAA_{-950} I; LAA_{-856} E-I, %=LAA_{-856} E − LAA_{-856} I.

On biopsy remodelling, we assess epithelial mucin staining, submucosal extracellular matrix proteins, lamina reticularis thickness, or airway smooth muscle (ASM) volume fraction (Table 4.5 and Table 4.6). In females, no significant differences were observed between healthy controls and patients with severe asthma. In males, severe asthma was associated with higher submucosal elastin deposition compared with healthy controls (20.9% [15.6–28.9] vs. 9.2% [6.8–13.6], p = 0.003; Table 4.5 and Table 4.6).

Table 4.5 Sex differences in biopsy remodelling in patients with asthma and healthy controls.

	Healthy controls			Mild/moderate asthma			Severe asthma		
	Female	Male	p-value	Female	Male	p-value	Female	Male	p-value
Mucin, % epithelial cells positive	11.6 [7.8-17.1] (n=8)	10.2 [3.8-13.6] (n=18)	0.3	13.6 [8.8-17.8] (n=13)	10.5 [3.1-14.6] (n=10)	0.3	10.3 [5.8-13.8] (n=12)	10.9 [6.5-22.2] (n=15)	0.5
Mucin, % epithelial area positive	7.6 [4.6-15.6] (n=8)	5.0 [1.4-6.9] (n=18)	0.1	10.6 [4.5-13.4] (n=13)	6.9 [2.6-12.4] (n=10)	0.8	4.7 [1.6-8.2] (n=12)	8.5 [3.0-14.6] (n=15)	0.2
Elastin, % submucosal area positive	13.2 [8.8-15.2] (n=9)	9.2 [6.8-13.6] (n=24)	0.4	19.0 [11.9-22.8] (n=12)	9.5 [6.4-12.9] (n=12)	0.1	12.8 [7.9-23.2] (n=17)	20.9 [15.6-28.9] (n=17)	0.1
Collagen, % submucosal area positive	21.1 [18.2-24.3] (n=9)	22.8 [17.6-24.9] (n=24)	1	17.0 [15.1-28.5] (n=13)	24.2 [14-29.5] (n=13)	0.7	25.9 [21.7-29.9] (n=17)	18.4 [11.3-28.9] (n=18)	0.2
Lamina reticularis thickness, μm	8.2 [7.8-8.8] (n=9)	9.0 [8.4-9.4] (n=19)	0.048	8.2 [7.6-9.4] (n=15)	9.2 [7.9-9.8] (n=15)	0.4	8.8 [6.7-10.1] (n=18)	8.9 [7.3-10.2] (n=28)	0.6
ASM volume fraction	0.2 [0.1-0.4] (n=13)	0.2 [0.2-0.3] (n=26)	0.8	0.3 [0.3-0.5] (n=18)	0.3 [0.2-0.4] (n=16)	0.3	0.3 [0.2-0.4] (n=26)	0.3 [0.2-0.4] (n=30)	0.9

Abbreviations: ASM: airway smooth muscle.

Table 4.6 Sex-stratified comparison of biopsy remodelling between healthy controls and severe asthma patients.

	Female				Male			
	Healthy controls	Severe asthma	Median differences	p-value	Healthy controls	Severe asthma	Median differences	p-value
Mucin, % epithelial cells positive	11.6 [7.8-17.1] (n=8)	10.3 [5.8-13.8] (n=12)	-1.3	0.5	10.2 [3.8-13.6] (n=18)	10.9 [6.5-22.2] (n=15)	0.7	0.2
Mucin, % epithelial area positive	7.6 [4.6-15.6] (n=8)	4.7 [1.6-8.2] (n=12)	-2.9	0.2	5.0 [1.4-6.9] (n=18)	8.5 [3.0-14.6] (n=15)	3.5	0.1
Elastin, % submucosal area positive	13.2 [8.8-15.2] (n=9)	12.8 [7.9-23.2] (n=17)	-0.4	0.6	9.2 [6.8-13.6] (n=24)	20.9 [15.6-28.9] (n=17)	11.7	0.003
Collagen, % submucosal area positive	21.1 [18.2-24.3] (n=9)	25.9 [21.7-29.9] (n=17)	4.8	0.4	22.8 [17.6-24.9] (n=24)	18.4 [11.3-28.9] (n=18)	-4.4	0.9
Lamina reticularis thickness, μm	8.2 [7.8-8.8] (n=9)	8.8 [6.7-10.1] (n=18)	0.6	0.6	9.0 [8.4-9.4] (n=19)	8.9 [7.3-10.2] (n=28)	-0.1	0.8
ASM volume fraction	0.2 [0.1-0.4] (n=13)	0.3 [0.2-0.4] (n=26)	0.1	0.9	0.2 [0.2-0.3] (n=26)	0.3 [0.2-0.4] (n=30)	0.1	0.3

Abbreviations: ASM: airway smooth muscle.

Among healthy individuals, males have a higher proportion of positive atopy tests ($p = 0.040$), but there are no significant differences among patients with asthma. In the mild to moderate asthma group, a higher proportion of women had a history of pneumonia or bronchitis (31.7% vs. 9.5%, $p = 0.026$) (Table 4.7). In the severe asthma group, women had more comorbidities in general, including allergic rhinitis (57.6% vs. 42.5%, $p = 0.008$), GORD (52.8% vs. 38.6%, $p = 0.011$), and psychiatric disease (12.7% vs. 3.5%, $p = 0.005$), but strikingly had fewer nasal polyps (25.3% vs. 49.6%, $p < 0.001$). Medication use was generally comparable between sexes, although women with severe asthma were more frequently prescribed short-acting β_2-agonists (SABAs) (84.9% vs. 74.1%, $p = 0.015$, Table 4.7).

Table 4.7 Sex differences in clinical characteristics of patients with asthma and healthy controls.

	Healthy controls			Mild/moderate asthma			Severe asthma		
	Female	Male	p-value	Female	Male	p-value	Female	Male	p-value
Atopy test positive	10/37 (27.0%)	27/53 (50.9%)	0.040	32/38 (84.2%)	37/40 (92.5%)	0.429	126/180 (70.0%)	79/110 (71.8%)	0.844
Medical histories									
Pneumonia or bronchitis	0/39 (0.0%)	0/62 (0.0%)	NA	13/41 (31.7%)	4/42 (9.5%)	0.026	147/226 (65.0%)	78/134 (41.8%)	0.237
Emphysema or COPD	0/39 (0.0%)	0/62 (0.0%)	NA	1/44 (2.3%)	2/44 (4.5%)	1.000	13/234 (5.6%)	6/141 (4.3%)	0.754
Comorbidities									
Allergic rhinitis diagnosed	1/38 (2.6%)	4/58 (6.9%)	0.653	22/43 (51.2%)	20/39 (51.3%)	1.000	129/224 (57.6%)	57/134 (42.5%)	0.008
Eczema diagnosed	3/39 (7.7%)	2/62 (3.2%)	0.592	15/43 (34.9%)	10/44 (22.7%)	0.310	79/234 (33.8%)	43/142 (30.3%)	0.559
GORD diagnosed	1/39 (2.6%)	3/62 (4.8%)	0.963	10/44 (22.7%)	6/43 (14.0%)	0.436	121/229 (52.8%)	54/140 (38.6%)	0.011
Hay fever diagnosed	1/38 (2.6%)	9/61 (14.8%)	0.109	25/44 (56.8%)	21/41 (51.2%)	0.764	107/224 (47.8%)	55/140 (39.3%)	0.140
Nasal polyps diagnosed	1/39 (2.6%)	2/61 (3.3%)	1.000	4/44 (9.1%)	3/44 (6.8%)	1.000	59/233 (25.3%)	69/139 (49.6%)	<0.001
Non-allergic rhinitis diagnosed	0/39 (0.0%)	1/61 (1.6%)	1.000	5/42 (11.9%)	3/42 (7.1%)	0.710	32/229 (14.0%)	22/137 (16.1%)	0.695
Psychiatric disease diagnosed	3/39 (7.7%)	2/62 (3.2%)	0.592	7/44 (15.9%)	0/44 (0.0%)	0.018	30/237 (12.7%)	5/142 (3.5%)	0.005
Medications									
ICS	NA	NA	NA	44/44 (100.0%)	43/44 (97.7%)	1.000	229/237 (100.0%)	138/142 (99.3%)	1.000
OCS	NA	NA	NA	0/44 (0.0%)	0/44 (0.0%)	NA	113/237 (47.8%)	63/142 (44.4%)	0.603
OCS dose, prednisolone (equ.) mg	NA	NA	NA	NA	NA	NA	16.4±11.0	15.7±10.7	0.667
SABA	NA	NA	NA	30/44 (68.2%)	37/44 (84.1%)	0.132	197/232 (84.9%)	103/139 (74.1%)	0.015
LABA	NA	NA	NA	2/44 (4.5%)	2/44 (4.5%)	1.000	226/229 (98.7%)	137/139 (98.6%)	1.000
Antibiotic	NA	NA	NA	0/44 (0.0%)	0/44 (0.0%)	NA	53/234 (22.6%)	27/142 (19.0%)	0.481
LAMA	NA	NA	NA	0/44 (0.0%)	0/44 (0.0%)	NA	50/230 (21.7%)	36/141 (25.5%)	0.475
Omalizumab	NA	NA	NA	0/44 (0.0%)	0/44 (0.0%)	NA	36/236 (15.3%)	16/141 (11.3%)	0.363
LTM	NA	NA	NA	0/44 (0.0%)	0/44 (0.0%)	NA	100/232 (43.1%)	63/142 (44.4%)	0.895
Severe events									
ER admission for breathing problems	NA	NA	NA	13/43 (30.2%)	8/44 (18.2%)	0.288	170/236 (72.0%)	91/141 (64.5%)	0.158

	Healthy controls			Mild/moderate asthma			Severe asthma		
	Female	Male	p-value	Female	Male	p-value	Female	Male	p-value
Endotracheal intubation	NA	NA	NA	0/43 (0%)	0/44 (0%)	NA	28/235 (11.9%)	12/139 (8.6%)	0.413
ICU admission	NA	NA	NA	0/43 (0%)	1/43 (2.3%)	1.000	64/234 (27.4%)	28/140 (20.0%)	0.141

Abbreviations: COPD: chronic obstructive pulmonary disease; ER: emergency room; GORD: Gastro-oesophageal reflux disease; ICU: intensive care unit; ICS: Inhaled corticosteroids; LABA: long-acting β-agonist; LAMA: long-acting muscarinic antagonist; LTM: Leukotriene modifier; NA: not applicable; OCS: oral corticosteroids; SABA: short-acting β-agonist.

Sex differences in symptoms and exacerbations in mild/moderate asthma are not significant (Figure 4.2). However, women with severe asthma reported worse symptom control, as reflected by higher ACQ-5 scores (2.38 vs. 2.02, p = 0.032), lower AQLQ scores (4.37 vs. 4.68, p = 0.044) (The meaning of ACQ-5 and AQLQ scores was illustrated in Chapter 1.1). Women with severe asthma also have more frequent overall exacerbations (p = 0.010) and severe exacerbations (p = 0.021) with linear regression (Figure 4.3).

Characteristics	Female	Male	Adjusted β [95% CI]
ACQ-5	0.80 ± 0.57	0.92 ± 0.7	-0.14 (-0.58 to 0.30)
AQLQ			
Total	5.84 ± 0.90	5.85 ± 0.97	-0.05 (-0.49 to 0.40)
Symptoms	5.88 ± 0.92	5.85 ± 0.98	-0.01 (-0.45 to 0.43)
Emotional	6.09 ± 1.21	5.88 ± 1.18	0.14 (-0.30 to 0.58)
Environmental stimuli	5.64 ± 1.09	5.66 ± 1.44	-0.03 (-0.48 to 0.42)
Activity limitation	5.76 ± 0.94	5.89 ± 0.98	-0.18 (-0.63 to 0.27)
HADS			
Total	7.43 ± 5.82	6.31 ± 5.76	0.18 (-0.27 to 0.62)
Anxiety	4.82 ± 3.51	3.53 ± 3.21	0.34 (-0.09 to 0.78)
Depression	2.61 ± 3.04	2.79 ± 3.18	-0.05 (-0.50 to 0.40)
SNOT-20 (total)	18.19 ± 16.95	14.16 ± 10.2	0.32 (-0.13 to 0.77)
ESS (total)	6.00 ± 3.97	5.01 ± 3.52	0.24 (-0.19 to 0.67)
MARS (total)	22.09 ± 3.11	20.56 ± 4.06	0.37 (-0.06 to 0.80)
Exacerbations			
Exacerbations (past year), n	0 [0, 1]	0 [0, 0]	0.15 (-0.25 to 0.55)
Severe exacerbations (past year), n	0 [0, 0]	0 [0, 0]	0.08 (-0.30 to 0.45)

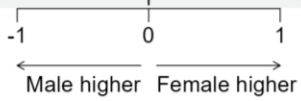


Figure 4.2 Linear regression analysis of sex differences in symptoms and exacerbations of patients with mild/moderate asthma.

Forest plot showing adjusted regression coefficients (β) and 95% confidence intervals (CI) for female versus male patients. Values to the right of the vertical line indicate higher scores in females, while values to the left indicate higher scores in males. *p < 0.05.

Abbreviations: ACQ-5: Asthma Control Questionnaire-5; AQLQ: Asthma Quality of Life Questionnaire; HADS: Hospital Anxiety and Depression Scale; SNOT-20: Sino-Nasal Outcome Test-20; ESS: Epworth Sleepiness Scale; MARS: Medication Adherence Report Scale.

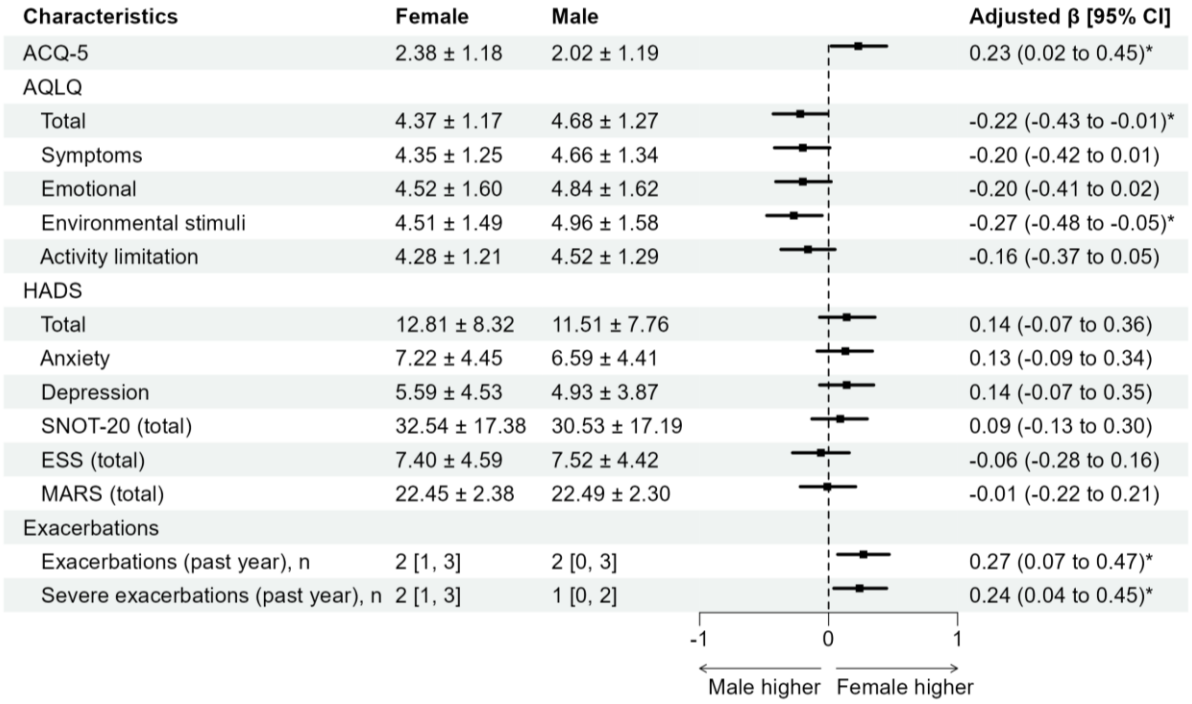


Figure 4.3 Linear regression analysis of sex differences in symptoms and exacerbations of patients with severe asthma.

Forest plot showing adjusted regression coefficients (β) and 95% confidence intervals (CI) for female versus male patients. Values to the right of the vertical line indicate higher scores in females, while values to the left indicate higher scores in males. *p < 0.05.

Abbreviations: ACQ-5: Asthma Control Questionnaire-5; AQLQ: Asthma Quality of Life Questionnaire; HADS: Hospital Anxiety and Depression Scale; SNOT-20: Sino-Nasal Outcome Test-20; ESS: Epworth Sleepiness Scale; MARS: Medication Adherence Report Scale.

4.4.2 Sex differences in inflammatory characteristics

Women with mild to moderate asthma exhibited a significantly different T2 phenotype distribution compared to men: lower T2-high, comparable T2-intermediate, and higher T2-low prevalence ($p = 0.013$). However, no significant sex differences were observed in sputum inflammatory phenotype, allergic phenotype, or early- and late-onset asthma phenotypes (Figure 4.4).

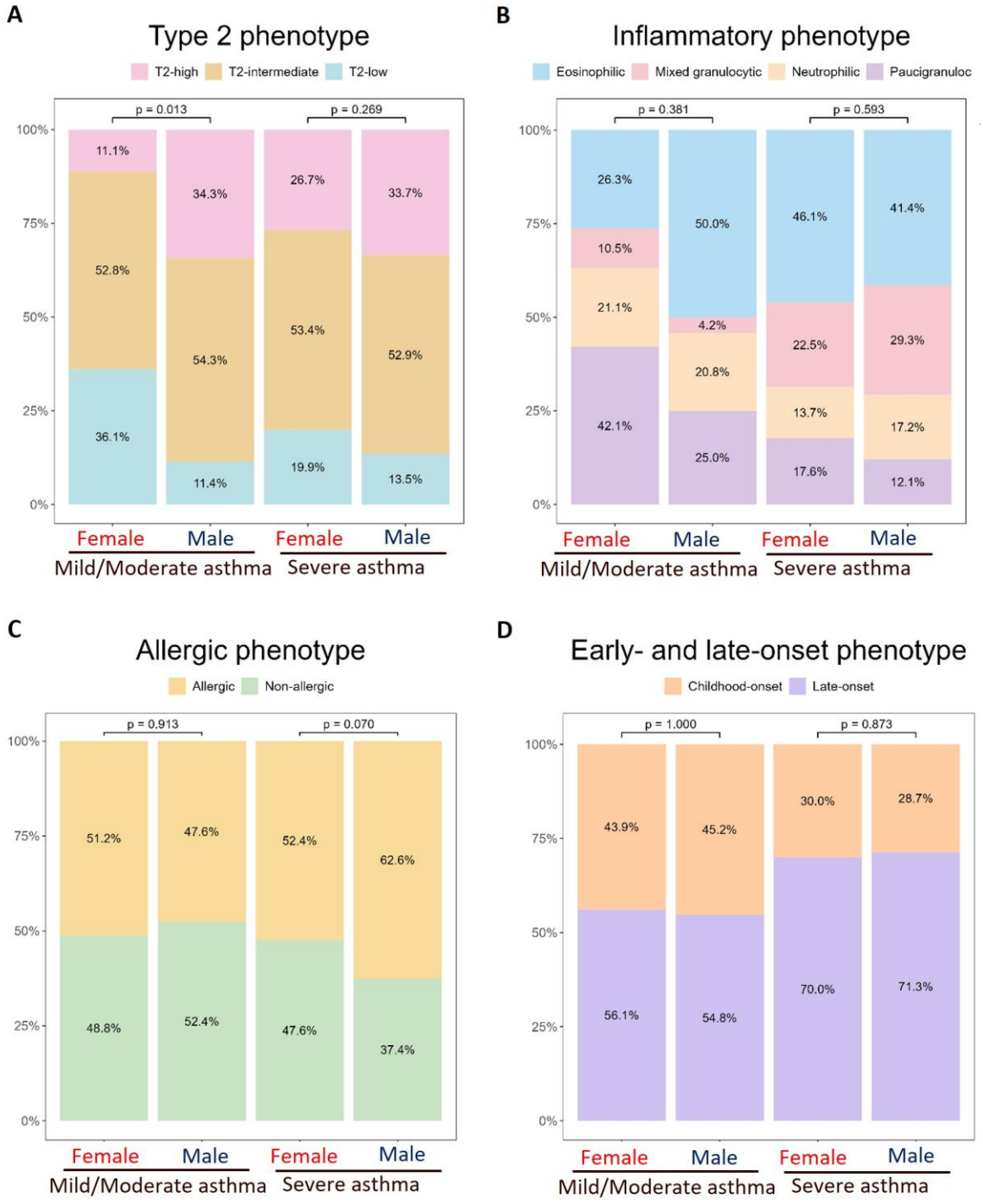


Figure 4.4 Sex differences in asthma phenotype.

Type 2 phenotypes were classified according to the RASP criteria (Heaney et al., 2021). Inflammatory phenotypes were defined based on Simpson *et al.* (Simpson et al., 2006). The Allergic phenotypes were identified using a serum IgE threshold of ≥100 IU/mL. The early- and late-phenotypes were implemented with a cutoff at 12 years of age.

Regarding biomarkers, FeNO levels were higher in men across all asthma severity levels, including mild to moderate and severe asthma ($p < 0.001$ and $p = 0.013$, respectively). C-reactive protein (CRP) levels were numerically elevated in women with mild to moderate asthma; however, differences were not significant after correction for multiple comparisons (Figure 4.5).

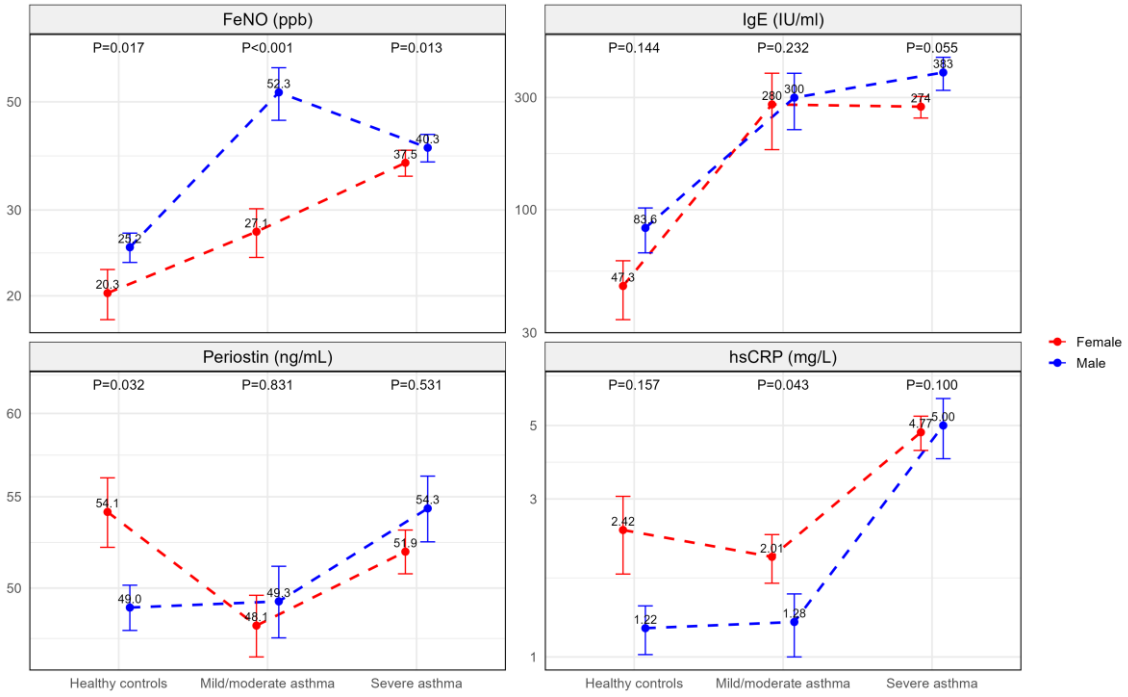


Figure 4.5 Sex differences in type 2 biomarkers of patients with asthma and healthy controls.

Data are presented as mean ± standard error of the mean (SEM), with females in red and males in blue. P-values indicate sex differences within each disease severity group.

Women with mild to moderate asthma had lower peripheral blood eosinophil counts (p = 0.004). Immunohistology of bronchial biopsies showed women with severe asthma had higher $CD3^+$ (p = 0.004), $CD4^+$ (p = 0.002), and $CD8^+$ T cell counts (p = 0.02) in submucosal biopsies (Figure 4.6).

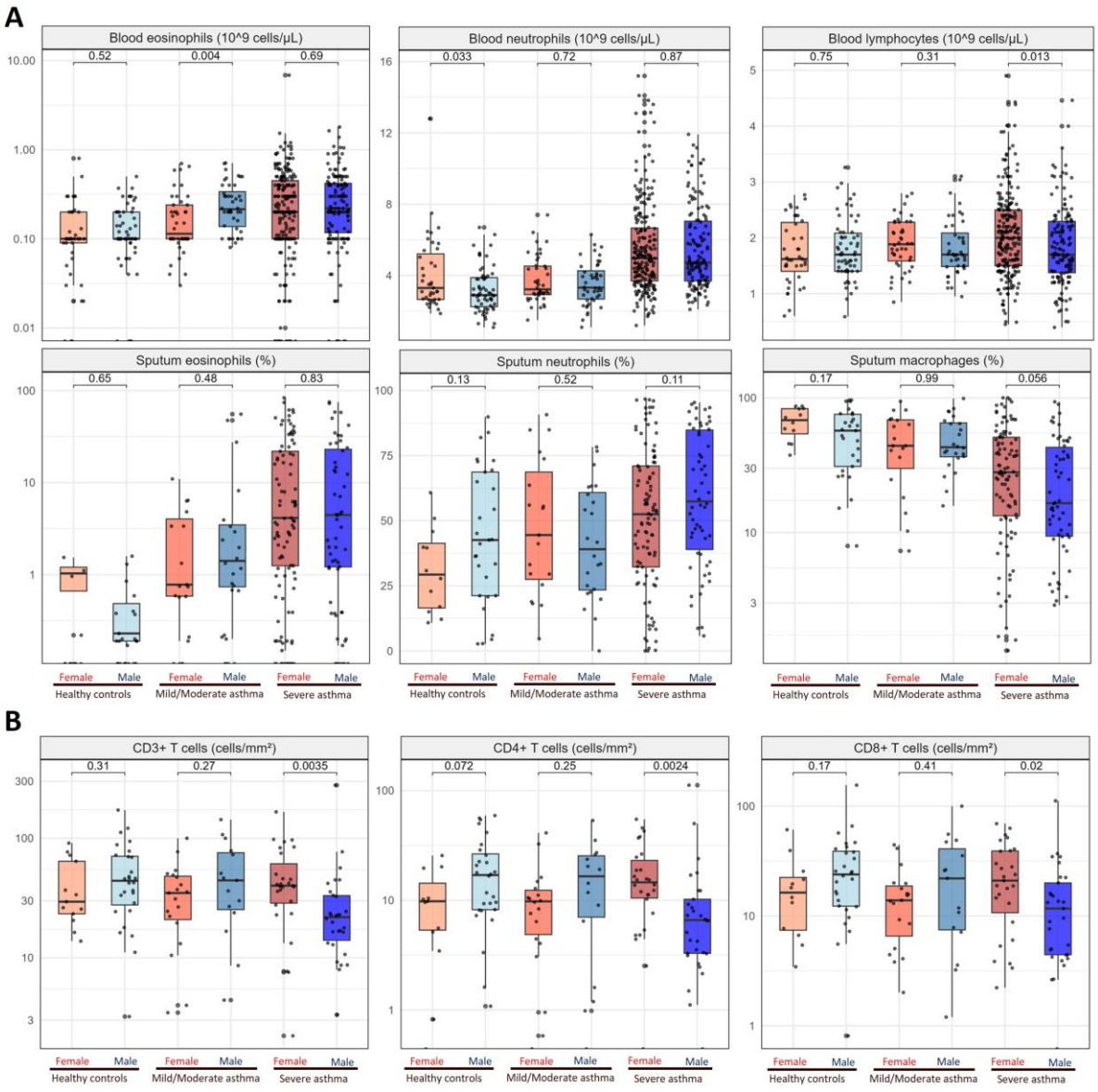


Figure 4.6 Sex differences in immune cells of patients with asthma and healthy controls.

(A) Sex differences in peripheral blood and sputum immune cells in asthma. P-values indicate

sex differences within each disease severity group.

(B) Sex differences in submucosa T cells in asthma. P-values indicate sex differences within each disease severity group.

4.4.3 Sex differences in bronchial biopsy and blood transcriptomics

In bronchial biopsy transcriptomics, approximately 1.6% of autosomal gene expression in severe asthma varied by sex, and 7.6% of genes had sex as an explanatory factor for more than 10% of the variance (Figure 4.7). By analysing this dataset, we derived severe asthma signatures and identified sex as the primary factor accounting for variance in these signatures both on autosomal chromosomes and sex chromosomes (Figure 4.8 and Figure 4.9).

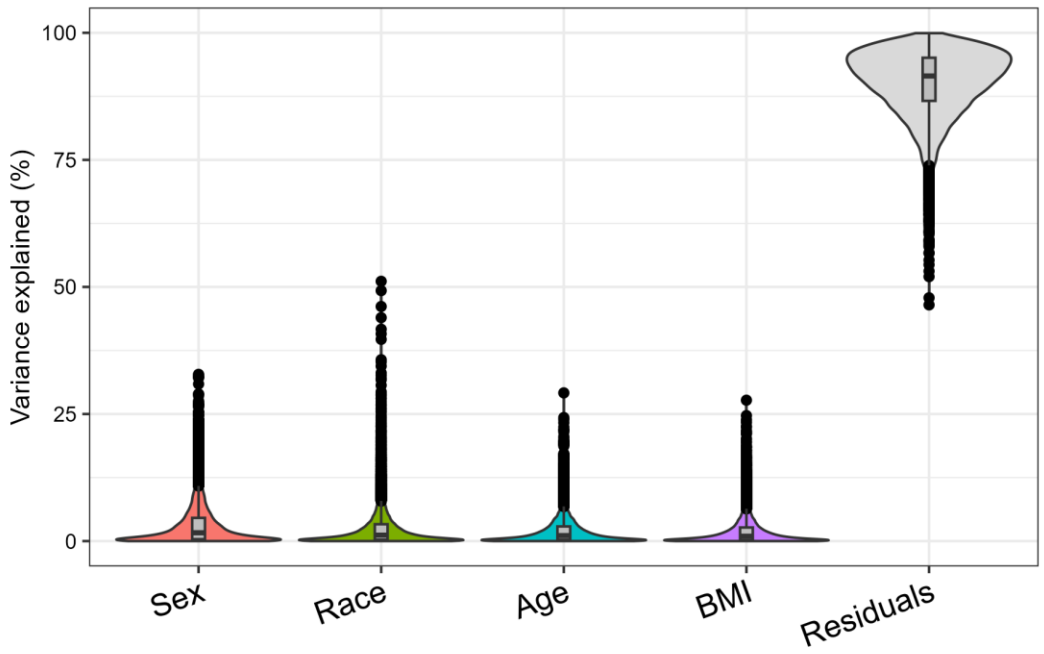


Figure 4.7 Variance partition analysis of the main drivers of expression

variability in autosomal genes of severe asthma with bronchial biopsy transcriptomics.

Violin plots showing the variance fraction of each gene across variables, ranked by the total contribution of variability per variable.

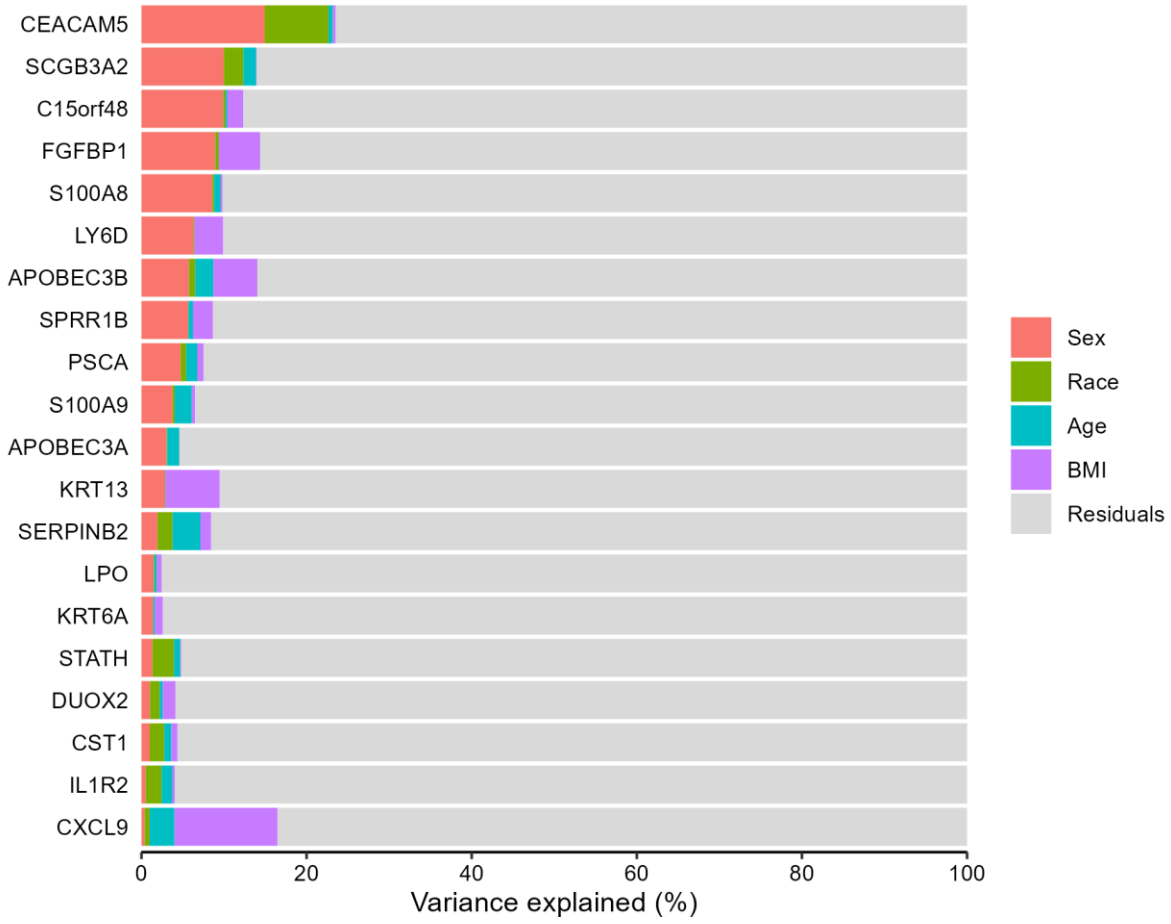


Figure 4.8 Variance explained of severe asthma signatures on autosomal chromosomes in bronchial biopsy transcriptomics.

Bar plot depicting variance fractions for the top 20 most variable genes.

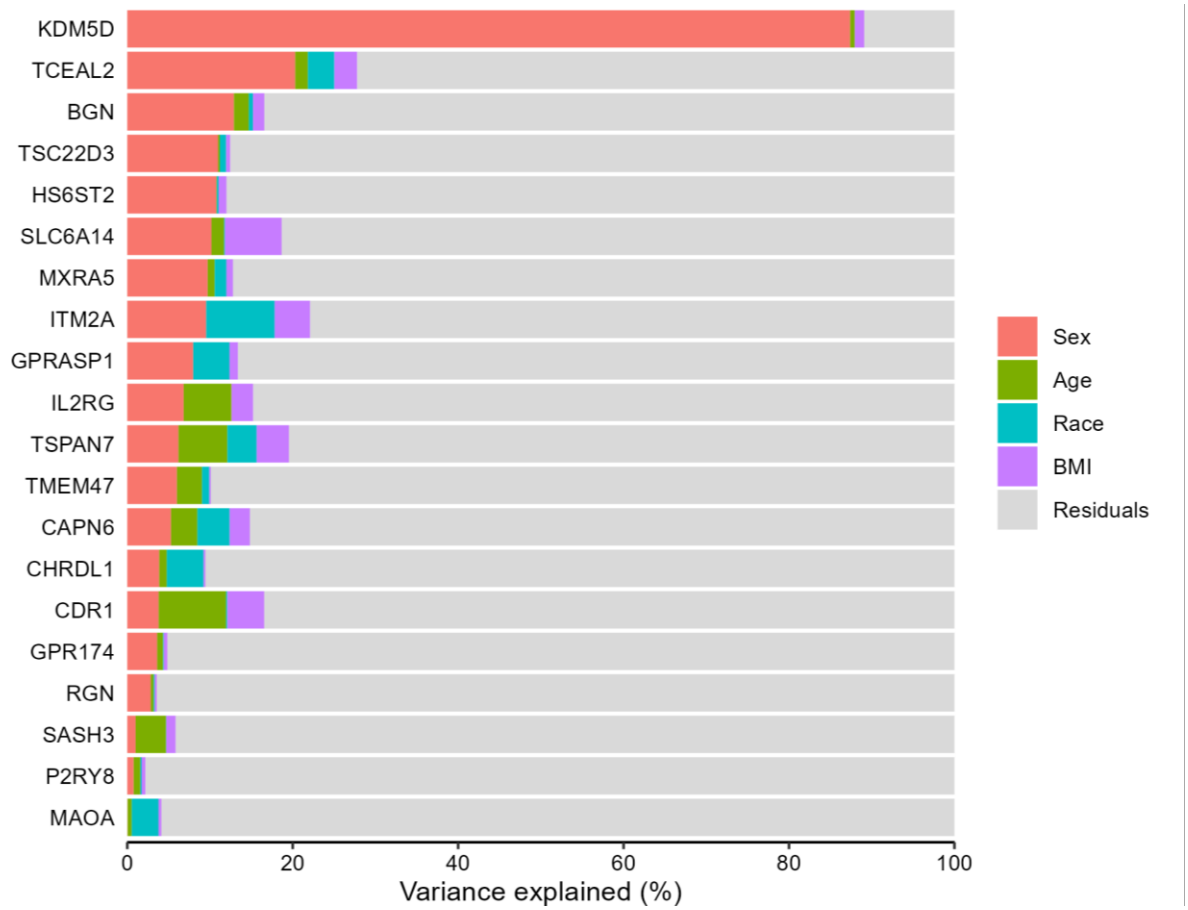


Figure 4.9 Variance explained of severe asthma signatures on sex chromosomes in bronchial biopsy transcriptomics.

Bar plot depicting variance fractions for the top 20 most variable genes.

Transcriptomic differences by sex were assessed in biopsies from healthy controls and asthma patients (Table S4.1 in appendix and Figure 4.10). In healthy controls, a distinct sex-biased transcriptional profile was detected. Female samples showed strong upregulation of X-linked transcripts, including *XIST* and *TSIX*, whereas multiple Y chromosome–linked genes (*RPS4Y1*, *EIF1AY*, *DDX3Y*, *USP9Y*, *KDM5D*, *UTY*) were significantly upregulated in males (Ohno, 2013). In addition, *RPS4X* and *EIF1AX* were elevated in females, while *COL2A1* and *CYTL1* were higher in males (Figure

4.10A). However, many sex-specific DEGs were identified in asthma patients only, some of which were associated with inflammatory processes. In mild-to-moderate asthma (panel B), females demonstrated genes associated with inflammatory and epithelial functions (*ODAM*, *SCGB3A2*, *COX7A1*), while males showed upregulation of immune-related genes (*CXCL9*, *CXCL10*, *PSMB9*, *HLA-DQB1*, *IFI27*) (Figure 4.10B). Genes upregulated in women *versus* men only in severe asthma included *IL7R,* which is part of the TSLP receptor complex*, CYTL1* and *CD34,* molecules involved in eosinophil/mast cell recruitment (Whetstone et al., 2025), the adipokine *RBP4*, and immunoglobulin genes *IGHG1, IGHD* (Table S4.1 in appendix and Figure 4.10). Genes upregulated in men vs. women only in severe asthma included a regulator of IL-33/ILC2 axis, *SPRR3, LCN2,* which encodes the antimicrobial peptide lipocalin 2 (NGAL), and the keratins *KRT4* and *KRT24* (Zhu et al., 2021) (Figure 4.10C).

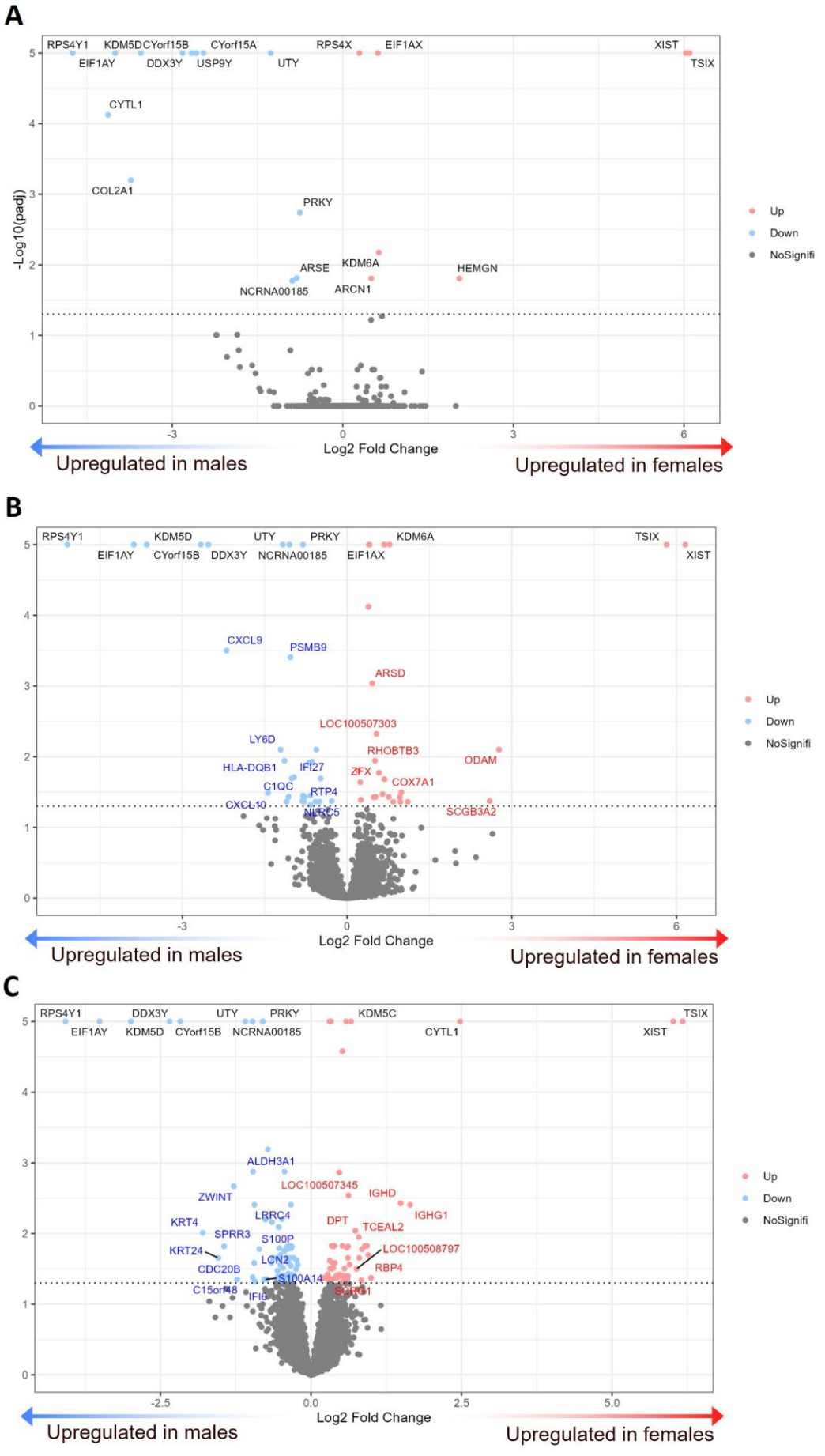

A
RPS4Y1
KDM5D
CYorf15B
CYorf15A
RPS4X
EIF1AX
XIST
EIF1AY
DDX3Y
USP9Y
UTY
TSIX
CYTL1
COL2A1
PRKY
ARSE
KDM6A
HEMGN
NCRNA00185
ARCN1
-Log10(padj)
Log2 Fold Change
Upregulated in males
Upregulated in females
Up
Down
NoSignifi
B
RPS4Y1
KDM5D
UTY
PRKY
KDM6A
TSIX
EIF1AY
CYorf15B
DDX3Y
NCRNA00185
EIF1AX
XIST
CXCL9
PSMB9
ARSD
LOC100507303
LY6D
RHOBTB3
ODAM
HLA-DQB1
IFI27
ZFX
COX7A1
C1QC
RTP4
CXCL10
NLRC5
SCGB3A2
Log2 Fold Change
Upregulated in males
Upregulated in females
Up
Down
NoSignifi
C
RPS4Y1
DDX3Y
UTY
PRKY
KDM5C
TSIX
EIF1AY
KDM5D
CYorf15B
NCRNA00185
CYTL1
XIST
ALDH3A1
LOC100507345
IGHD
ZWINT
IGHG1
LRRC4
DPT
KRT4
TCEAL2
SPRR3
S100P
LOC100508797
KRT24
LCN2
RBP4
CDC20B
S100A14
C15orf48
IFI6
SCGB1
Log2 Fold Change
Upregulated in males
Upregulated in females
Up
Down
NoSignifi

Figure 4.10 Sex differences in differentially expressed genes (DEGs) in patients with asthma and healthy controls with bronchial biopsies transcriptomics.

(A) Volcano plot representing the DEGs between male healthy controls and female healthy controls (Minimum adjusted p-value is 1×10^{-5}). The top 20 upregulated and top 20 downregulated genes are labelled.

(B) Volcano plot representing the DEGs between male mild to moderate asthma and female mild to moderate asthma (Minimum adjusted p-value is 1×10^{-5}). The top 20 upregulated and top 20 downregulated genes are labelled.

(C) Volcano plot representing the DEGs between male severe asthma and female severe asthma (Minimum adjusted p-value is 1×10^{-5}). The top 20 upregulated and top 20 downregulated genes are labelled.

GO enrichment analysis of bronchial biopsy transcriptomes revealed sex-specific differences in both mild-to-moderate and severe asthma (Figure 4.11). In mild-to-moderate asthma (Figure 4.11A), females displayed enrichment in cytoplasmic translation, Golgi vesicle transport, and signalling pathways responsive to peptide hormones and BMP stimulation, while males exhibited strong enrichment in immune- and inflammation-related pathways, including antigen processing and presentation, leukocyte-mediated immunity, cytokine production, and response to viral processes.. In severe asthma (Figure 4.11B), females showed significant enrichment of pathways related to extracellular structure and tissue remodelling, including extracellular matrix and structure organisation, mesenchyme development, cell-substrate adhesion, and regulation of chemotaxis, as well as growth and morphogenesis-related processes. In contrast, males demonstrated enrichment in pathways linked to cell cycle regulation, energy metabolism, and protein turnover, such as mitotic nuclear division, electron

transport chain, proteasome-mediated protein catabolic process, and mitochondrial translation. These results suggest that in asthma, females tend to activate pathways associated with tissue remodeling and cellular signaling, whereas males display stronger transcriptional signatures of energy metabolism.

A


cytoplasmic translation
Golgi vesicle transport
transmembrane receptor protein serine/threonine kinase signaling pathway
response to peptide hormone
response to BMP
cellular response to BMP stimulus
astrocyte differentiation
gland development
positive regulation of epithelial cell proliferation
antigen processing and presentation
leukocyte mediated immunity
immune response-regulating signaling pathway
response to virus
positive regulation of leukocyte cell-cell adhesion
regulation of response to biotic stimulus
positive regulation of cytokine production
viral process
Up in female
Up in male
GeneRatio
0.02
0.04
0.06
0.08
p.adjust
0.015
0.010
0.005

B

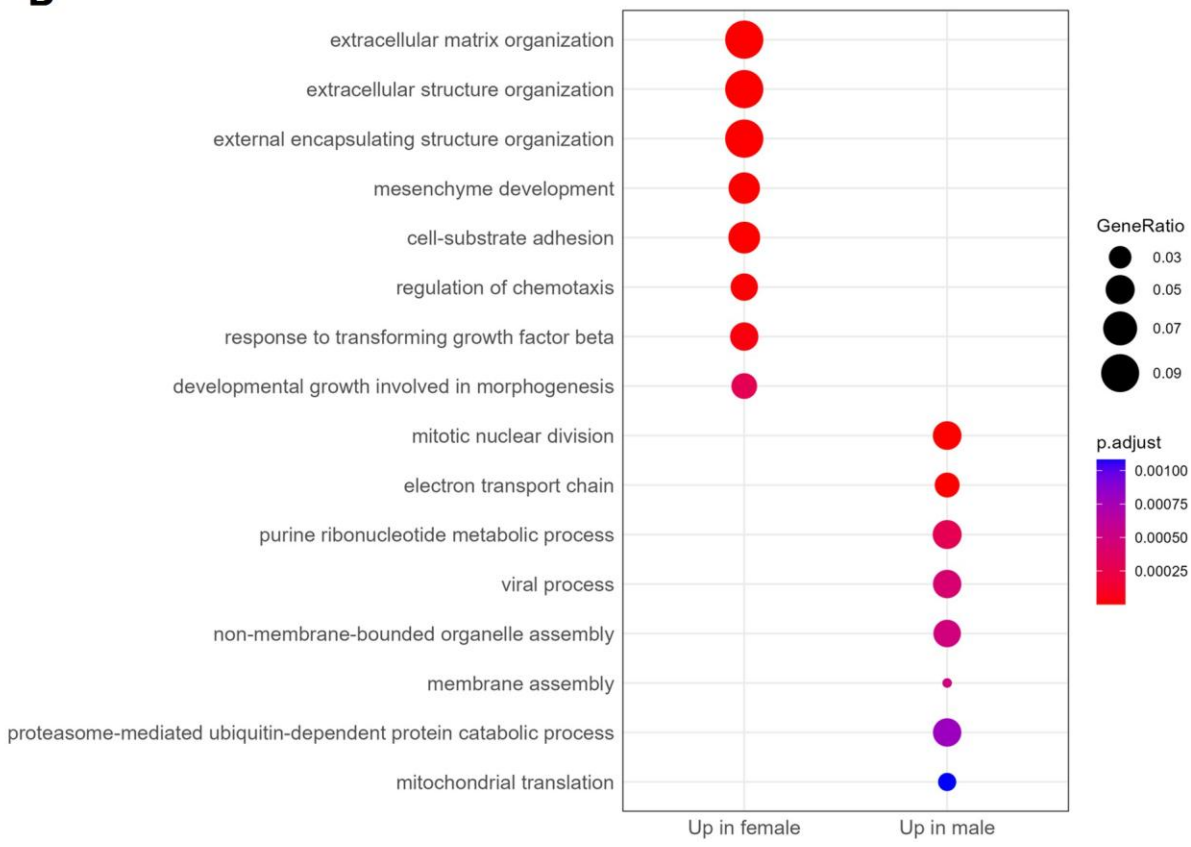

extracellular matrix organization
extracellular structure organization
external encapsulating structure organization
mesenchyme development
cell-substrate adhesion
regulation of chemotaxis
response to transforming growth factor beta
developmental growth involved in morphogenesis
mitotic nuclear division
electron transport chain
purine ribonucleotide metabolic process
viral process
non-membrane-bounded organelle assembly
membrane assembly
proteasome-mediated ubiquitin-dependent protein catabolic process
mitochondrial translation
Up in female
Up in male
GeneRatio
0.03
0.05
0.07
0.09
p.adjust
0.00100
0.00075
0.00050
0.00025

Figure 4.11 Sex differences in GO enrichment pathways in patients with asthma in bronchial biopsies transcriptomics.

(A) GO enrichment pathways in male mild to moderate asthma and female mild to moderate asthma.

(B) GO enrichment pathways in male severe asthma and female severe asthma.

WGCNA identified several distinct gene modules (Figure 4.12A), which were further examined for associations with clinical traits. Correlation analysis revealed that the mitochondrial function module was significantly negatively associated with sex ($r = -0.32$, $p < 0.001$) and severity ($r = -0.29$, $p = 0.012$). In contrast, the extracellular matrix module showed positive correlations with sex ($r = 0.31$, $p < 0.001$) and severity ($r = 0.25$, $p = 0.03$), while the cilium and microtubule module was negatively correlated with sex ($r = -0.19$, $p = 0.01$) (Figure 4.12B). Functional enrichment analysis highlighted that the cilium and microtubule module was enriched in processes related to cilium assembly, cilium organisation, and microtubule-based transport. The mitochondrial metabolism module was enriched in ATP synthesis, electron transport, and mitochondrial translation, whereas the extracellular matrix module was strongly associated with the organisation of extracellular structure and cell adhesion (Figure 4.12C). These results highlight that female patients with severe asthma were linked to a module related to extracellular matrix regulation and showed negative correlations with a module involved in mitochondrial and cilium function.

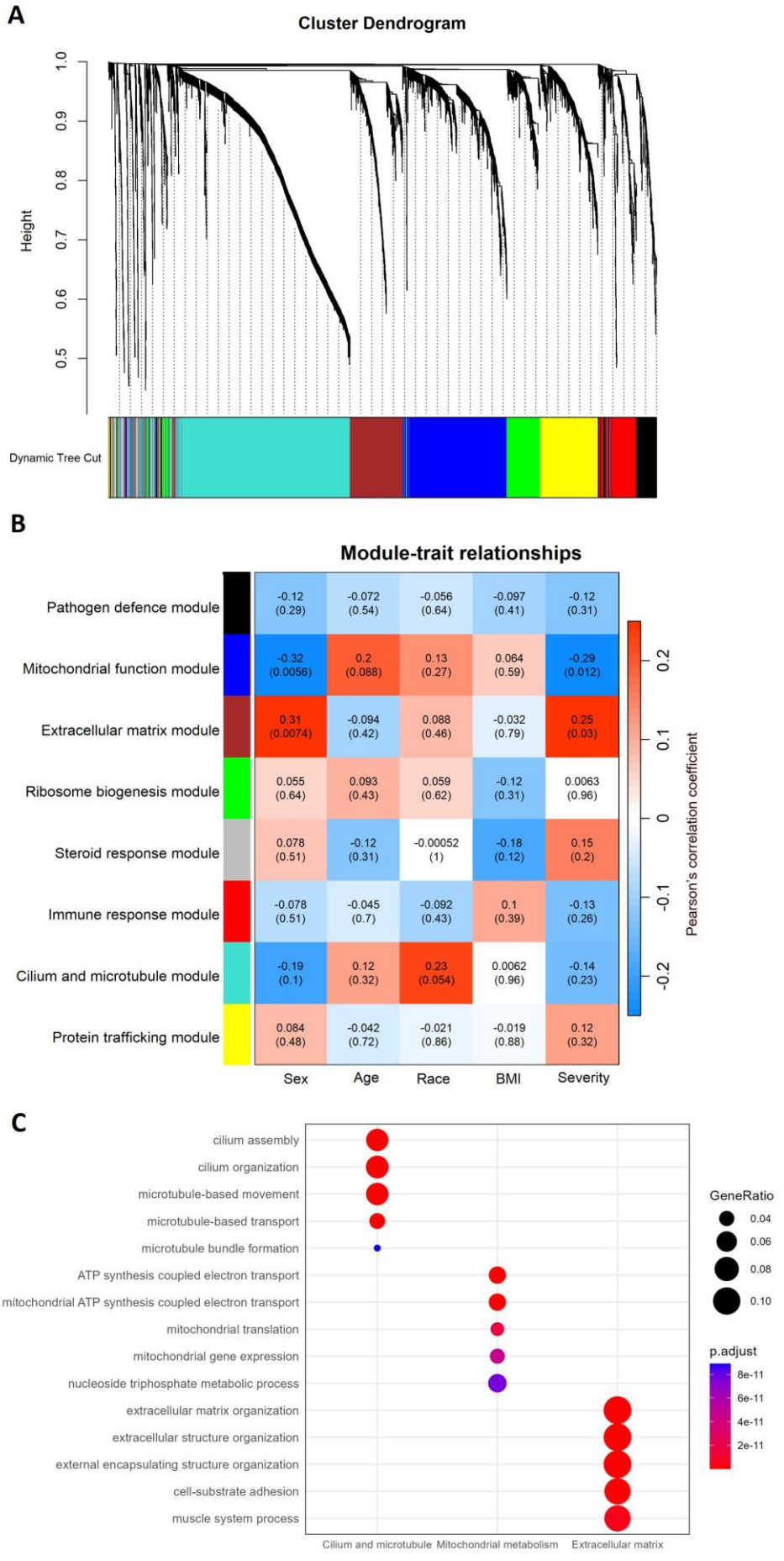

A
Cluster Dendrogram
Height
1.0
0.9
0.8
0.7
0.6
0.5
Dynamic Tree Cut
B
Module-trait relationships
Pathogen defence module
Mitochondrial function module
Extracellular matrix module
Ribosome biogenesis module
Steroid response module
Immune response module
Cilium and microtubule module
Protein trafficking module
-0.12 (0.29)
-0.072 (0.54)
-0.056 (0.64)
-0.097 (0.41)
-0.12 (0.31)
-0.32 (0.0056)
0.2 (0.088)
0.13 (0.27)
0.064 (0.59)
-0.29 (0.012)
0.31 (0.0074)
-0.094 (0.42)
0.088 (0.46)
-0.032 (0.79)
0.25 (0.03)
0.055 (0.64)
0.093 (0.43)
0.059 (0.62)
-0.12 (0.31)
0.0063 (0.96)
0.078 (0.51)
-0.12 (0.31)
-0.00052 (1)
-0.18 (0.12)
0.15 (0.2)
-0.078 (0.51)
-0.045 (0.7)
-0.092 (0.43)
0.1 (0.39)
-0.13 (0.26)
-0.19 (0.1)
0.12 (0.32)
0.23 (0.054)
0.0062 (0.96)
-0.14 (0.23)
0.084 (0.48)
-0.042 (0.72)
-0.021 (0.86)
-0.019 (0.88)
0.12 (0.32)
Sex
Age
Race
BMI
Severity
Pearson's correlation coefficient
0.2
0.1
0
-0.1
-0.2
C
cilium assembly
cilium organization
microtubule-based movement
microtubule-based transport
microtubule bundle formation
ATP synthesis coupled electron transport
mitochondrial ATP synthesis coupled electron transport
mitochondrial translation
mitochondrial gene expression
nucleoside triphosphate metabolic process
extracellular matrix organization
extracellular structure organization
external encapsulating structure organization
cell-substrate adhesion
muscle system process
Cilium and microtubule
Mitochondrial metabolism
Extracellular matrix
GeneRatio
0.04
0.06
0.08
0.10
p.adjust
8e-11
6e-11
4e-11
2e-11

Figure 4.12 Weighted gene co-expression network analysis (WGCNA) analysis reveals distinct functional modules associated with severe asthma clinical traits in bronchial biopsies transcriptomics.

(A) Cluster dendrogram of gene co-expression modules generated using hierarchical clustering based on topological overlap. Modules were identified via dynamic tree cutting, and each module is represented by a distinct colour bar beneath the dendrogram.

(B) Heatmap showing Pearson correlations between module genes to mild to moderate and severe asthma clinical traits in WGCNA analysis. Modules were named according to their top significantly enriched GO biological process term. Each cell displays the correlation coefficient (top) and the corresponding p-value (bottom, in parentheses). The colour gradient represents the strength and direction of the correlation (red: positive; blue: negative). Sex is coded as female *vs.* male (female = 1, male = 0).

(C) GO enrichment analysis of selected WGCNA modules.

Analysis of transcription factor activity revealed pronounced sex- and severity-specific patterns (Figure 4.13). In mild to moderate asthma, females showed higher expression of lymphocyte- and differentiation-associated regulators, including *EBF1*, *TCF7*, *TEAD1*, *MEF2A*, and *TBX21*, whereas males exhibited elevated levels of interferon signalling–related factors, such as *STAT1* and *STAT2*, together with myeloid-associated regulators (*RFX5*, *LYL1*). Transcription factor enrichment in severe asthma showed enhanced expression of two type 1 immune response master regulators, *STAT1* and *TBX21* (T-bet) and higher expression of adaptive immune–associated transcription factors (e.g., *NR2C2*, *TBX21*, *FOXP1*) in women, but male severe asthma displayed strong upregulation of epithelial and metabolic regulators (*ZNF263*, *ZEB2*, *KLF5*, *GRHL2*, *E2F4*, *NFE2L2*), while expression of canonical immune-related

TFs was markedly suppressed.

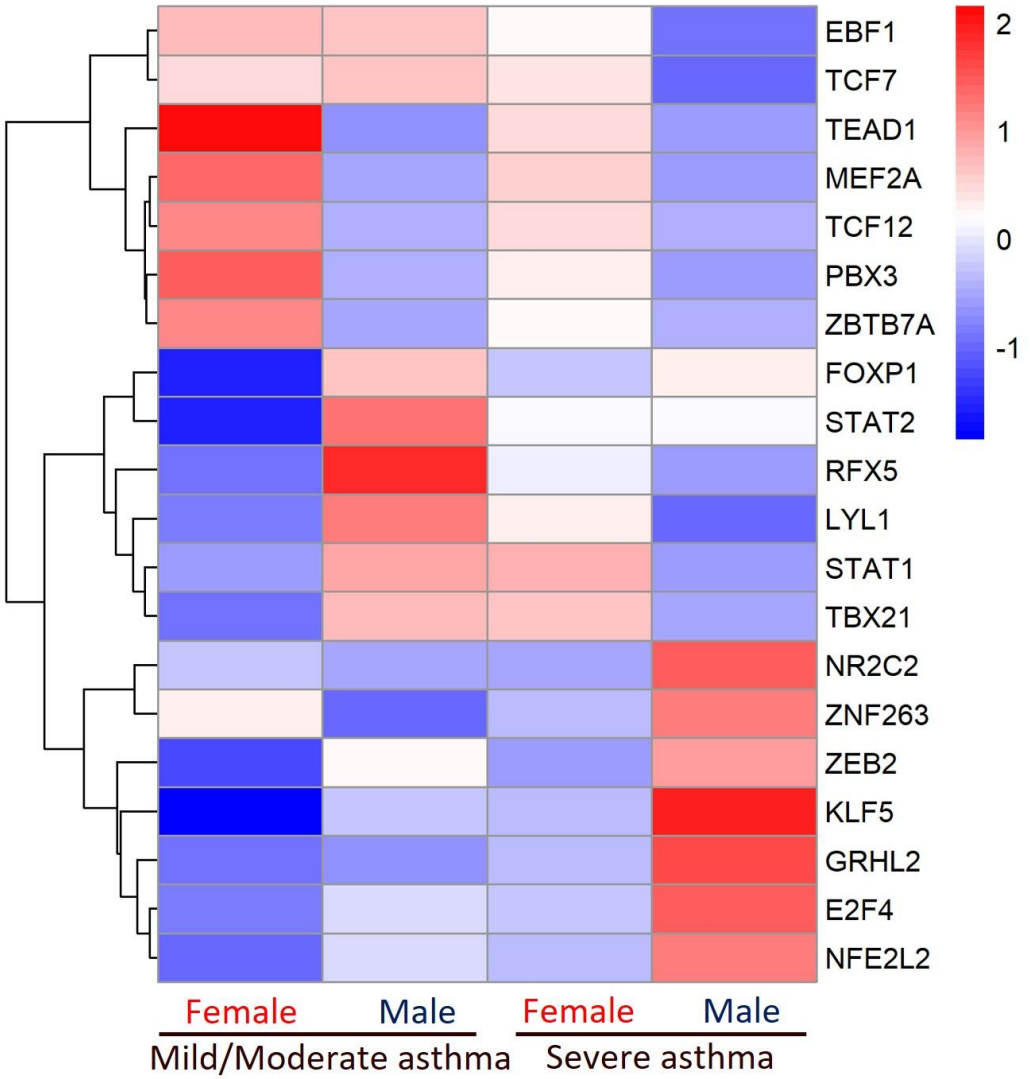
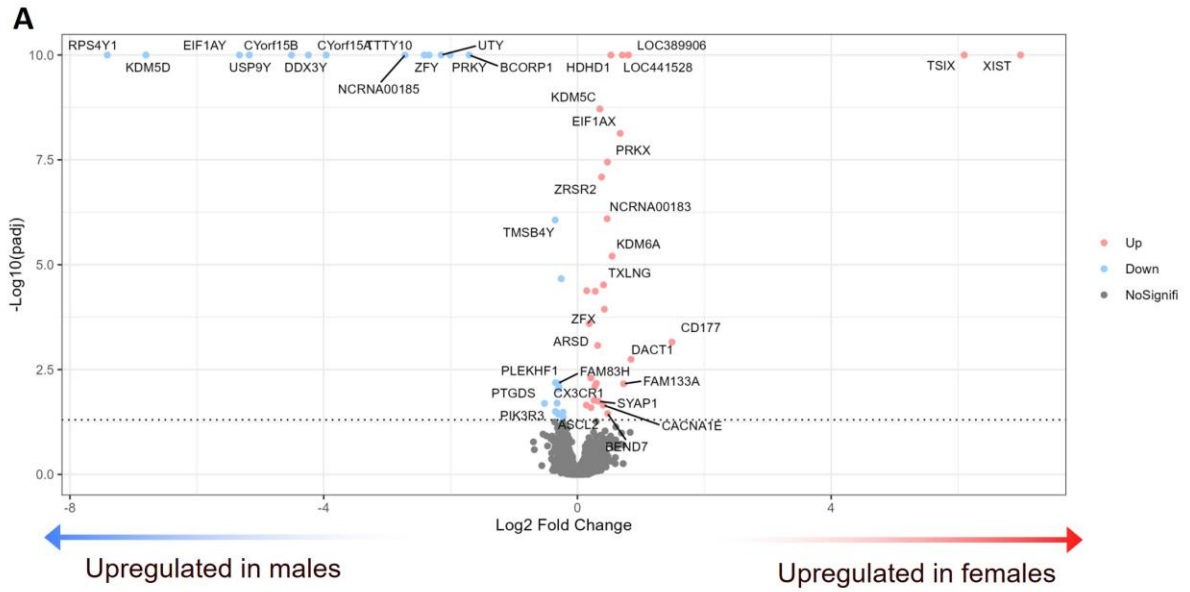


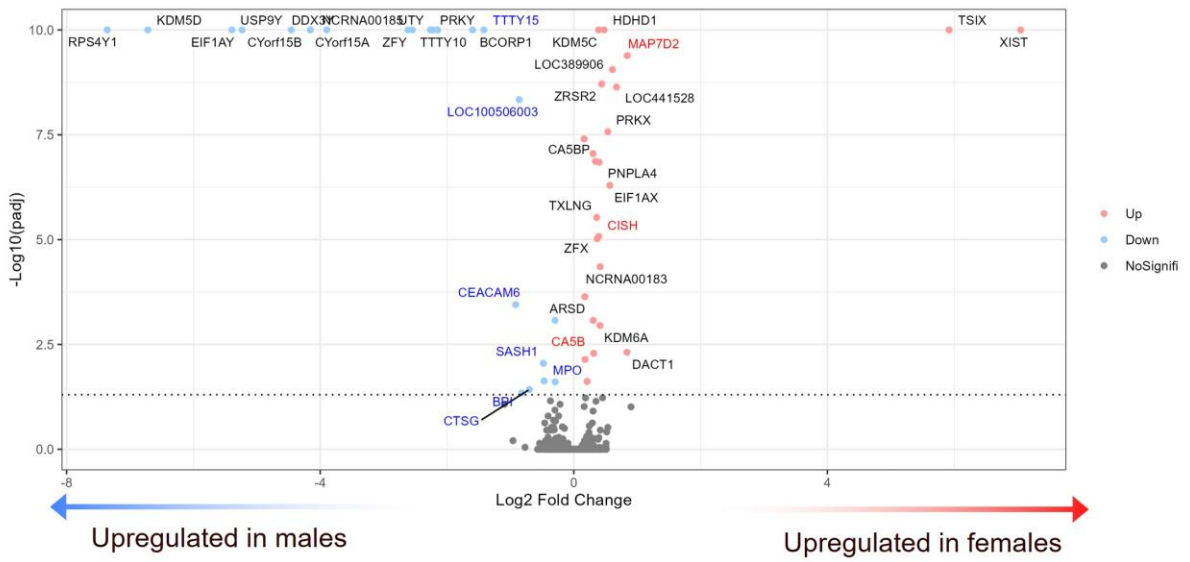
Figure 4.13 Sex differences in transcription factor (TF) expression patterns in asthma with bronchial biopsies transcriptomics.

The heatmap displays inferred TF activity scores calculated from target gene expression, using the DoRothEA regulon database.

Gene expression differences by sex were examined across healthy controls and asthma subgroups (Table S4.2 in appendix Figure 4.14). In healthy controls, a clear sex-biased transcriptional pattern was observed. Genes located on sex chromosomes

were among the most significantly differentially expressed, with *XIST* and *TSIX* strongly upregulated in females, whereas multiple Y-linked genes (*RPS4Y1*, *EIF1AY*, *DDX3Y*, *USP9Y*, *KDM5D*, *UTY*) were upregulated in males (Ohno, 2013) (Figure 4,14A). In mild to moderate asthma, genes higher in males were dominated by neutrophil/antimicrobial genes (*CEACAM6*, *CTSG*, *BPI*, and *MPO*) (Li et al., 2022) (Figure 4.14B). In severe asthma, females upregulated multiple antiviral/IFN-related or immune-regulatory genes (*ICOS*, *CCR6*, *IL7R*, and *GATA3*) while genes higher in males encompassed a broad innate/neutrophil programme, including *ELANE*, *MPO*, *DEFA4*, *BPI* and *LTF* (Ray & Kolls, 2017) (Figure 4.14C).


A
RPS4Y1
KDM5D
EIF1AY
USP9Y
CYorf15B
DDX3Y
CYorf15A
TTTY10
NCRNA00185
UTY
ZFY
PRKY
BCORP1
HDHD1
LOC389906
LOC441528
TSIX
XIST
KDM5C
EIF1AX
PRKX
ZRSR2
NCRNA00183
TMSB4Y
KDM6A
TXLNG
ZFX
CD177
ARSD
DACT1
PLEKHF1
FAM83H
FAM133A
PTGDS
CX3CR1
SYAP1
PIK3R3
ASCL2
CACNA1E
BEND7
-Log10(padj)
Log2 Fold Change
Up
Down
NoSignifi
Upregulated in males
Upregulated in females


B
KDM5D
RPS4Y1
USP9Y
EIF1AY
DDX3Y
CYorf15B
NCRNA00185
CYorf15A
UTY
ZFY
PRKY
TTTY15
TTTY10
BCORP1
HDHD1
KDM5C
MAP7D2
LOC389906
ZRSR2
LOC441528
LOC100506003
PRKX
CA5BP
PNPLA4
EIF1AX
TXLNG
CISH
ZFX
NCRNA00183
CEACAM6
ARSD
CA5B
KDM6A
SASH1
DACT1
MPO
BPI
CTSG
TSIX
XIST
-Log10(padj)
Log2 Fold Change
Up
Down
NoSignifi
Upregulated in males
Upregulated in females

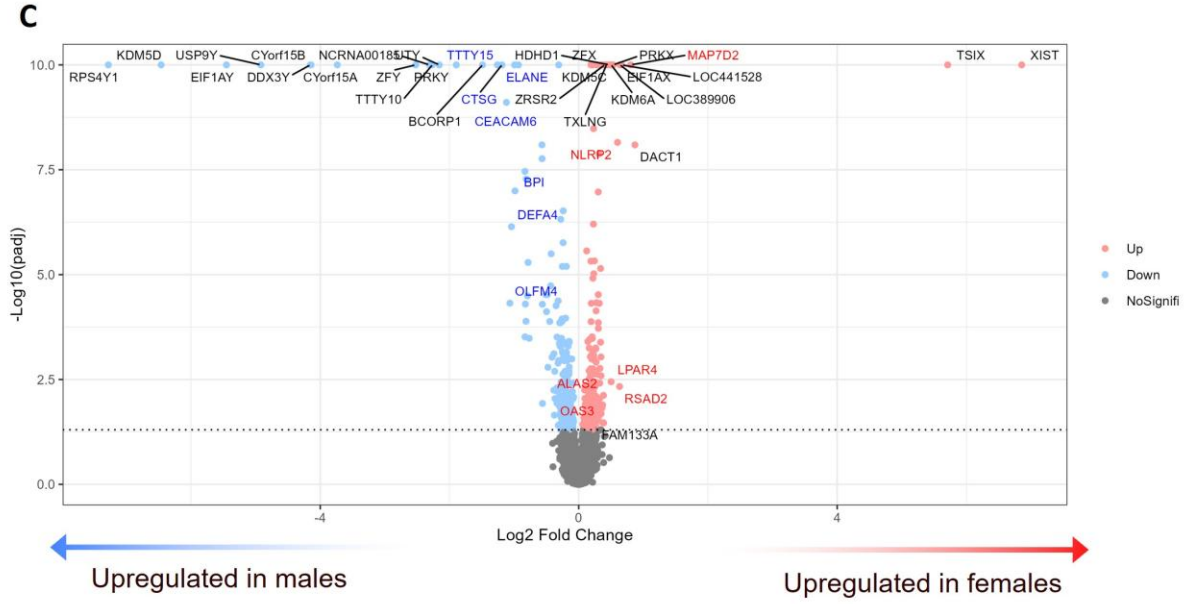

C
KDM5D
RPS4Y1
USP9Y
EIF1AY
CYorf15B
DDX3Y
NCRNA00185
CYorf15A
UTY
ZFY
TTTY15
PRKY
TTTY10
HDHD1
ELANE
CTSG
BCORP1
ZFX
KDM5C
ZRSR2
CEACAM6
PRKX
MAP7D2
EIF1AX
KDM6A
LOC441528
LOC389906
TXLNG
NLRP2
DACT1
BPI
DEFA4
OLFM4
LPAR4
ALAS2
RSAD2
OAS3
FAM133A
TSIX
XIST
-Log10(padj)
Log2 Fold Change
Up
Down
NoSignifi
Upregulated in males
Upregulated in females

Figure 4.14 Sex differences in differentially expressed genes (DEGs) in patients with asthma and healthy controls with blood transcriptomics.

(A) Volcano plot representing the DEGs between male healthy controls and female healthy controls (Minimum adjusted p-value is 1×10^{-10}). The top 20 upregulated and top 20 downregulated genes are labelled.

(B) Volcano plot representing the DEGs between male mild to moderate asthma and female mild to moderate asthma (Minimum adjusted p-value is 1×10^{-10}). The top 20 upregulated and top 20 downregulated genes are labelled.

(C) Volcano plot representing the DEGs between male severe asthma and female severe asthma (Minimum adjusted p-value is 1×10^{-10}). The top 20 upregulated and top 20 downregulated genes are labelled.

GO enrichment analysis of blood transcriptomes revealed clear sex-specific differences in both mild-to-moderate and severe asthma (Figure 4.15). In mild-to-moderate asthma (Figure 4.15A), females exhibited enrichment of immune-related pathways, including regulation of innate immune response, T cell activation, lymphocyte activation, cytokine production, and leukocyte-mediated immunity, indicating stronger activation of adaptive and innate immune processes. In contrast, males showed significant enrichment in mitochondrial and metabolic pathways, such as ATP synthesis coupled to electron transport, mitochondrial electron transport, respiratory electron transport chain, and purine nucleotide catabolic process, suggesting a greater involvement of energy metabolism and oxidative phosphorylation. In severe asthma (Figure 4.15B), a similar pattern was observed, with females continuing to show enrichment in immune regulatory processes, while males were enriched in mitochondrial activity, protein ubiquitination, and metabolic pathways.

These findings indicate that in blood transcriptomics, females with asthma display stronger immune-related transcriptional signatures, whereas males exhibit a predominance of mitochondrial and metabolic pathways, with this divergence being consistent across both mild-to-moderate and severe disease.

A


regulation of innate immune response
regulation of response to biotic stimulus
regulation of T cell activation
positive regulation of lymphocyte activation
extrinsic apoptotic signaling pathway via death domain receptors
positive regulation of cytokine production
leukocyte mediated immunity
establishment of endothelial barrier
ATP synthesis coupled electron transport
mitochondrial ATP synthesis coupled electron transport
mitochondrial electron transport, cytochrome c to oxygen
protein K48-linked ubiquitination
aerobic electron transport chain
regulation of attachment of spindle microtubules to kinetochore
respiratory electron transport chain
purine nucleotide catabolic process
Up in female
Up in male
p.adjust
0.03
0.02
0.01
GeneRatio
0.02
0.04
0.06

B


regulation of innate immune response
regulation of response to biotic stimulus
regulation of T cell activation
positive regulation of lymphocyte activation
extrinsic apoptotic signaling pathway via death domain receptors
positive regulation of cytokine production
leukocyte mediated immunity
establishment of endothelial barrier
ATP synthesis coupled electron transport
mitochondrial ATP synthesis coupled electron transport
mitochondrial electron transport, cytochrome c to oxygen
protein K48-linked ubiquitination
aerobic electron transport chain
regulation of attachment of spindle microtubules to kinetochore
respiratory electron transport chain
purine nucleotide catabolic process
Up in female
Up in male
p.adjust
0.03
0.02
0.01
GeneRatio
0.02
0.04
0.06

Figure 4.15 Sex differences in GO enrichment pathways in patients with asthma in blood transcriptomics.

(A) GO enrichment pathways in male mild to moderate asthma and female mild to moderate asthma.

(B) GO enrichment pathways in male severe asthma and female severe asthma.

Using WGCNA, I identified several biologically relevant gene modules (Figure 4.16A) and assessed their associations with clinical traits (Figure 4.16B). The innate immune response module showed a positive correlation with sex ($r = 0.27$, $p < 0.001$) and a negative correlation with age ($r = –0.31$, $p < 0.001$); the immune activation module correlated positively with disease severity ($r = 0.18$, $p < 0.001$); and the RNA regulation module was positively associated with severity ($r = 0.26$, $p < 0.001$) but negatively with age ($r = –0.24$, $p < 0.001$). Functional enrichment analysis (Figure 4.16C) revealed that the immune activation module was enriched in lymphocyte differentiation and activation, the innate immune response module in humoral and mucosal immune pathways, and the RNA regulation module in RNA splicing and stability processes. It revealed that sex-associated modules were enriched in pathways related to innate immune responses, suggesting that sex differences in immune regulation may underlie variation in disease outcomes.

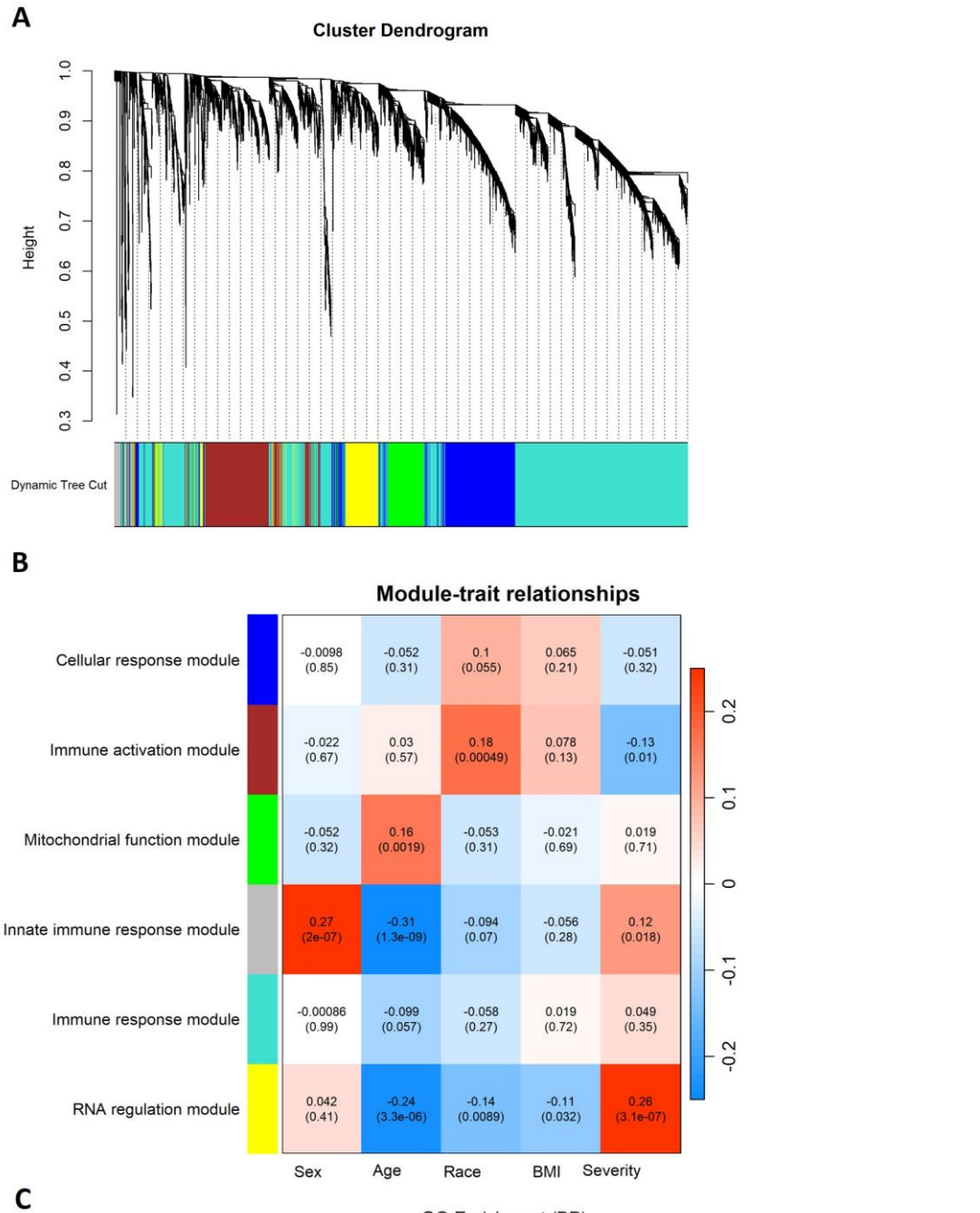

A
Cluster Dendrogram
Height
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
Dynamic Tree Cut
B
Module-trait relationships
Cellular response module
Immune activation module
Mitochondrial function module
Innate immune response module
Immune response module
RNA regulation module
-0.0098 (0.85)
-0.052 (0.31)
0.1 (0.055)
0.065 (0.21)
-0.051 (0.32)
-0.022 (0.67)
0.03 (0.57)
0.18 (0.00049)
0.078 (0.13)
-0.13 (0.01)
-0.052 (0.32)
0.16 (0.0019)
-0.053 (0.31)
-0.021 (0.69)
0.019 (0.71)
0.27 (2e-07)
-0.31 (1.3e-09)
-0.094 (0.07)
-0.056 (0.28)
0.12 (0.018)
-0.00086 (0.99)
-0.099 (0.057)
-0.058 (0.27)
0.019 (0.72)
0.049 (0.35)
0.042 (0.41)
-0.24 (3.3e-06)
-0.14 (0.0089)
-0.11 (0.032)
0.26 (3.1e-07)
Sex
Age
Race
BMI
Severity
0.2
0.1
0
-0.1
-0.2
C
GO Enrichment (BP)
cytoplasmic translation
immune response-activating cell surface receptor signaling pathway
mononuclear cell differentiation
lymphocyte differentiation
B cell activation
positive regulation of lymphocyte activation
production of molecular mediator of immune response
mucosal immune response
organ or tissue specific immune response
antimicrobial humoral response
humoral immune response
RNA splicing
negative regulation of RNA catabolic process
RNA stabilization
regulation of RNA stability
regulation of mRNA metabolic process
Immune activation
Innate immune response
RNA regulation
GeneRatio
0.03
0.06
0.09
0.12
p.adjust
3e-04
2e-04
1e-04

Figure 4.16 Weighted gene co-expression network analysis (WGCNA) analysis reveals distinct functional modules associated with severe asthma clinical traits in blood transcriptomics.

(A) Cluster dendrogram of gene co-expression modules generated using hierarchical clustering based on topological overlap. Modules were identified via dynamic tree cutting, and each module is represented by a distinct colour bar beneath the dendrogram.

(B) Heatmap showing Pearson correlations between module genes to mild to moderate and severe asthma clinical traits in WGCNA analysis. Modules were named according to their top significantly enriched GO biological process term. Each cell displays the correlation coefficient (top) and the corresponding p-value (bottom, in parentheses). The colour gradient represents the strength and direction of the correlation (red: positive; blue: negative). Sex is coded as female *vs.* male (female = 1, male = 0).

(C) GO enrichment analysis of selected WGCNA modules.

Transcription factor profiling revealed distinct sex- and severity-dependent expression patterns (Figure 4.17). In mild to moderate asthma, males exhibited a marked upregulation of *ZNF639*, *SPI1*, *ZNF263*, *STAT1*, and *STAT2*, indicating a preferential activation of interferon and myeloid-associated regulators. In severe asthma, females retained relatively higher expression of a subset of lymphocyte- and differentiation-associated factors (e.g., *IRF4*, *TBX21*, *PAX5*).

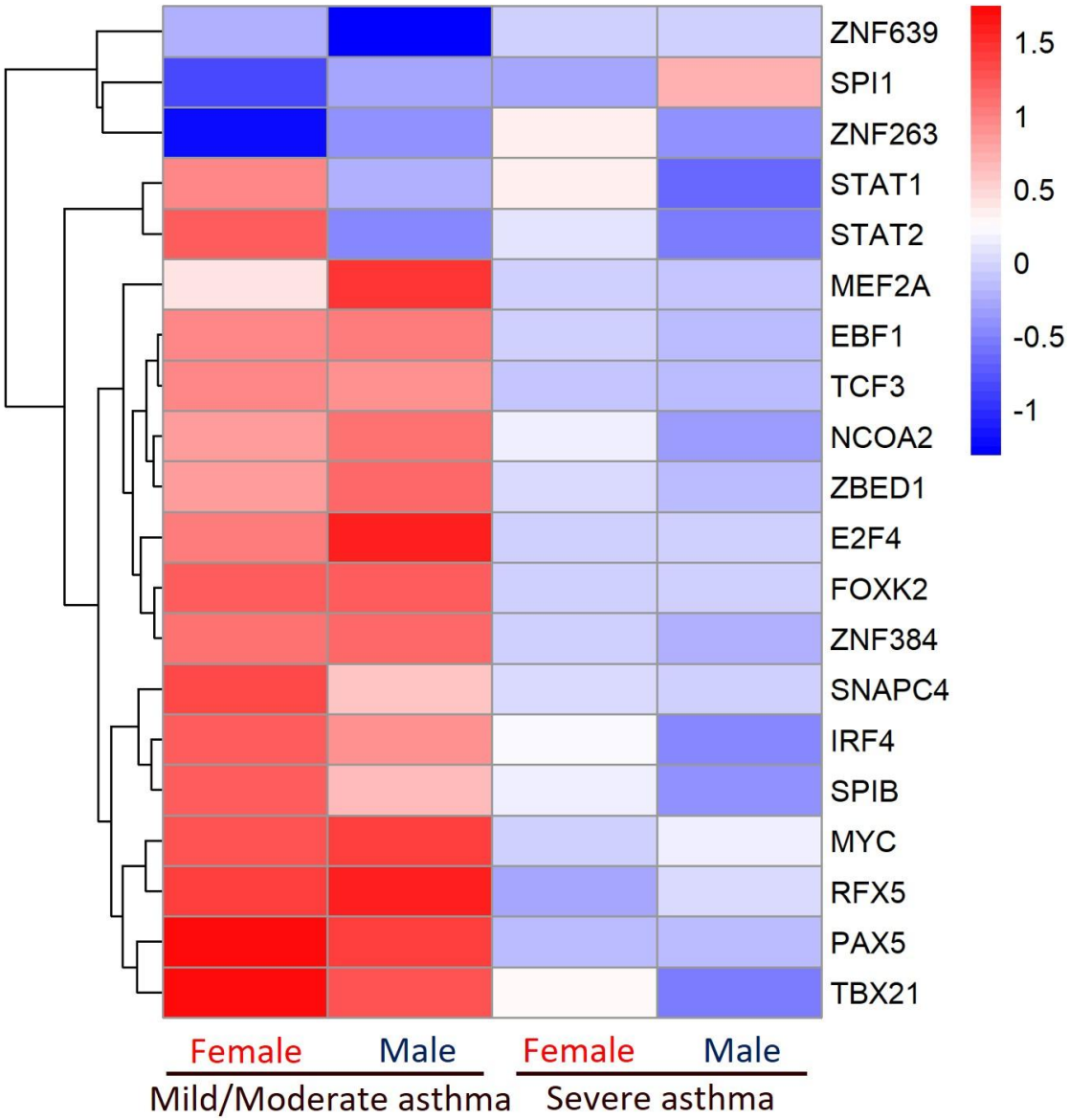


Figure 4.17 Sex differences in transcription factor (TF) expression patterns in asthma with bronchial biopsies transcriptomics.

The heatmap displays inferred TF activity scores calculated from target gene expression, using the DoRothEA regulon database.

4.4.4 Sex differences in blood proteomics

Sex differences in circulating proteins were observed across health and asthma severity groups (Table S4.3 in appendix and Figure 4.18). In healthy controls, only a

small number of proteins showed sex-biased expression. IL6 and MMP10 were significantly downregulated in females, while CERS1 was upregulated in females relative to males. In mild to moderate asthma, more pronounced sex differences emerged. Proteins such as HLA-DQA1, EPX, ACER1, FETUB, B4GALT5, ZNF688, and TNNI3 were significantly upregulated in females compared with males, whereas no proteins were significantly enriched in males. In severe asthma, sex differences in protein expression were even more striking. Females exhibited higher levels of several immune- and inflammation-related proteins, including CCL11 (eotaxin-1), TSLP, GZMB, EPX, and HLA-DQA1, alongside metabolic and structural proteins (LEP, FETUB, TNNI3, PSORS1C1). In contrast, males showed enrichment of proteins involved in lipid metabolism, such as UGT8, SGMS1, SPHK1, and SMPD3 (Table S4.3 in appendix and Figure 4.18).

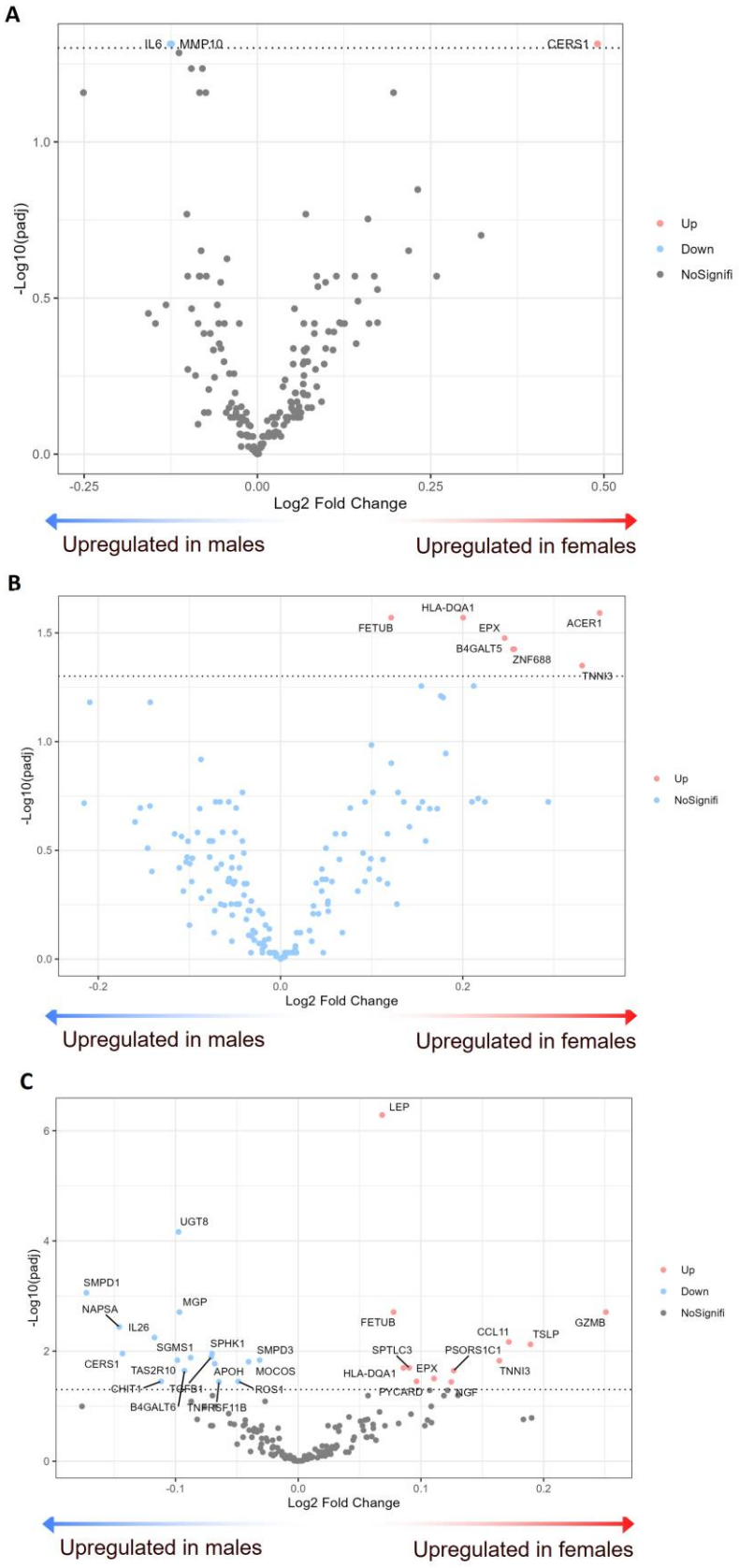


Figure 4.18 Sex differences in differentially expressed proteins (DEPs) in asthma

with blood proteomics.

(A) Volcano plot representing the DEPs between male healthy controls and female healthy controls.

(B) Volcano plot representing the DEPs between male mild to moderate asthma and female mild to moderate asthma.

(C) Volcano plot representing the DEPs between male severe asthma and female severe asthma.

GO enrichment analysis of differentially expressed proteins revealed distinct sex-specific biological processes across asthma severities (Figure 4.19). In mild to moderate asthma (Figure 4.19A), females showed enrichment of immune activation pathways, including positive regulation of phagocytosis, leukocyte migration, leukocyte cell–cell adhesion, defence response to Gram-negative bacterium, regulation of inflammatory response, and cytokine-mediated signalling pathway. Males, by contrast, exhibited enrichment in processes associated with ceramide biosynthesis, negative regulation of immune and external stimulus responses, positive regulation of microRNA transcription, and regulation of memory T cell differentiation, suggesting a greater contribution of lipid metabolic regulation and immune suppression. In severe asthma (Figure 4.19B), females remained enriched for immune activation processes, with upregulated pathways linked to leukocyte migration, cytokine-mediated signalling, positive regulation of leukocyte and lymphocyte activation, mononuclear cell proliferation, and interleukin-17 production, indicating sustained immune cell activation and pro-inflammatory responses. Conversely, males demonstrated enrichment of ceramide and sphingolipid metabolic processes, response to xenobiotic stimulus, and

regulation of smooth muscle cell apoptotic processes, highlighting a shift towards lipid metabolism and tissue remodelling pathways.


A
positive regulation of phagocytosis
leukocyte migration
positive regulation of leukocyte cell-cell adhesion
leukocyte cell-cell adhesion
defense response to Gram-negative bacterium
production of molecular mediator of immune response
regulation of inflammatory response
positive regulation of MAPK cascade
cytokine-mediated signaling pathway
regulation of leukocyte cell-cell adhesion
ceramide biosynthetic process
negative regulation of response to external stimulus
negative regulation of immune system process
positive regulation of miRNA transcription
regulation of memory T cell differentiation
Up in female
Up in male
GeneRatio
0.10
0.15
0.20
0.25
0.30
p.adjust
0.003
0.002
0.001

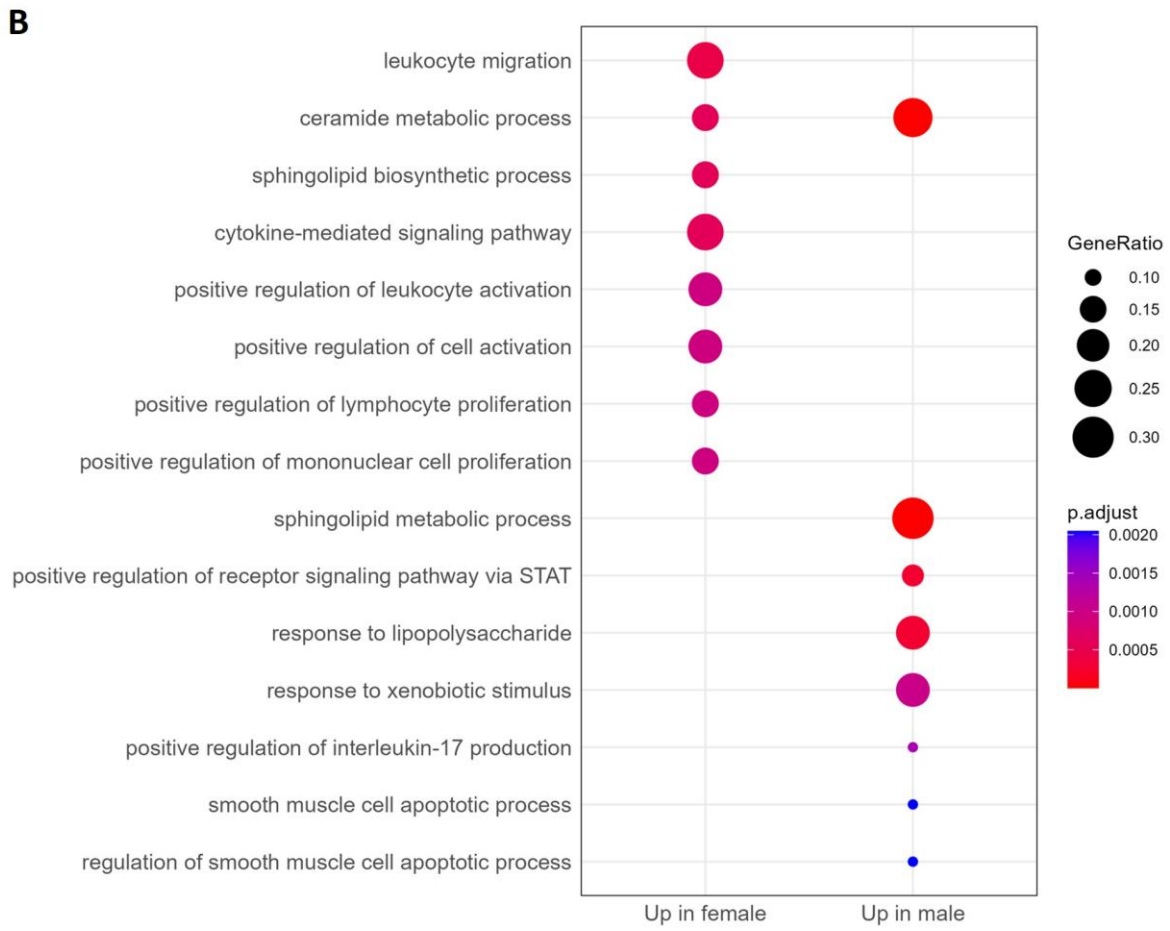
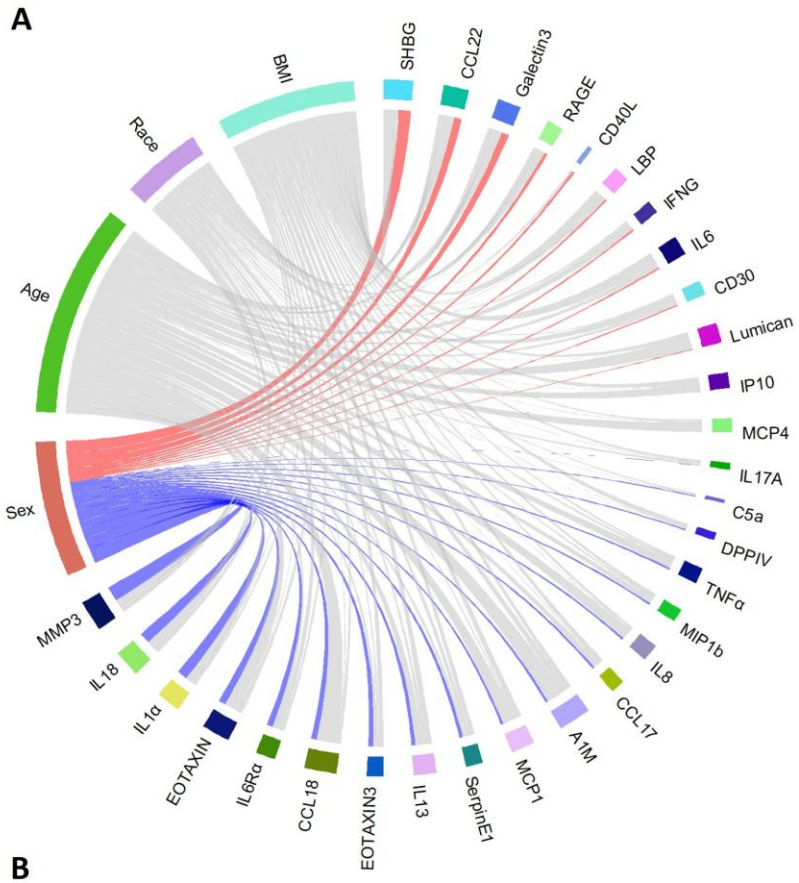

B
leukocyte migration
ceramide metabolic process
sphingolipid biosynthetic process
cytokine-mediated signaling pathway
positive regulation of leukocyte activation
positive regulation of cell activation
positive regulation of lymphocyte proliferation
positive regulation of mononuclear cell proliferation
sphingolipid metabolic process
positive regulation of receptor signaling pathway via STAT
response to lipopolysaccharide
response to xenobiotic stimulus
positive regulation of interleukin-17 production
smooth muscle cell apoptotic process
regulation of smooth muscle cell apoptotic process
Up in female
Up in male
GeneRatio
0.10
0.15
0.20
0.25
0.30
p.adjust
0.0020
0.0015
0.0010
0.0005

Figure 4.19 Sex differences in GO enrichment pathways in asthma with blood proteomics.

(A) GO enrichment pathways in mild to moderate asthma.

(B) GO enrichment pathways in severe asthma.

To further investigate sex-specific systemic signatures, circulating biomarkers were compared between females and males across healthy controls and asthma groups (Figure 4.20). The chord plot (Figure 4.20A) demonstrated that sex was strongly associated with variation in multiple immune- and inflammation-related proteins, including MMP3, IL1α, IL1β, IL6R, IL13, CCL17, CCL18, CCL22, Eotaxins 1 and 3, CD30, CD40L, DPPIV, IFNγ, TNFα, SerpinE1, and SHBG, highlighting broad sex-dependent differences in both cytokine signalling and tissue remodelling pathways. The forest plot (Figure 4.20B) quantified these associations. In healthy controls, females exhibited significantly higher levels of SHBG and SerpinE1, but lower levels of CCL22, MMP3, and TNFα compared with males. In mild to moderate asthma, females had lower expression of CD30, DPPIV, and MMP3, whereas males showed relatively higher levels of these markers. In severe asthma, females displayed increased levels of CCL22, SHBG, SerpinE1, and Lumican, alongside reduced levels of MMP3, IL18, and IL6, compared with males.



B

Biomarkers	Female vs male in healthy controls	Female vs male in mild/moderate asthma	Female vs male in severe asthma
A1M	-0.04 (-0.45 to 0.37)	-0.24 (-0.63 to 0.15)	0.08 (-0.11 to 0.27)
C5a	-0.09 (-0.52 to 0.35)	-0.18 (-0.60 to 0.24)	-0.13 (-0.35 to 0.08)
CCL17	-0.09 (-0.51 to 0.33)	0.05 (-0.40 to 0.51)	-0.17 (-0.39 to 0.05)
CCL18	-0.33 (-0.65 to -0.02)*	-0.38 (-0.70 to -0.07)*	0.01 (-0.20 to 0.22)
CCL22	0.28 (-0.16 to 0.73)	0.09 (-0.36 to 0.54)	0.33 (0.11 to 0.55)*
CD30	0.15 (-0.27 to 0.57)	-0.02 (-0.44 to 0.40)	0.12 (-0.09 to 0.33)
CD40L	-0.09 (-0.52 to 0.34)	0.31 (-0.11 to 0.73)	0.09 (-0.12 to 0.30)
DPPIV	-0.43 (-0.85 to -0.00)	-0.81 (-1.20 to -0.43)*	-0.11 (-0.32 to 0.10)
EOTAXIN	-0.05 (-0.50 to 0.40)	-0.42 (-0.84 to 0.01)	-0.11 (-0.32 to 0.11)
EOTAXIN3	-0.03 (-0.48 to 0.43)	-0.26 (-0.71 to 0.18)	0.18 (-0.04 to 0.41)
Galectin3	0.47 (0.05 to 0.89)*	0.24 (-0.16 to 0.64)	0.52 (0.32 to 0.73)*
IFNG	-0.12 (-0.57 to 0.33)	0.33 (-0.12 to 0.78)	0.17 (-0.06 to 0.39)
IL13	-0.23 (-0.68 to 0.22)	-0.36 (-0.83 to 0.12)	0.03 (-0.20 to 0.26)
IL17A	0.20 (-0.24 to 0.64)	-0.19 (-0.65 to 0.27)	-0.00 (-0.24 to 0.24)
IL18	-0.35 (-0.77 to 0.07)	-0.29 (-0.68 to 0.10)	-0.45 (-0.66 to -0.24)*
IL1α	-0.22 (-0.65 to 0.21)	-0.09 (-0.49 to 0.31)	-0.55 (-0.75 to -0.35)*
IL6	0.16 (-0.28 to 0.61)	-0.24 (-0.70 to 0.22)	0.07 (-0.16 to 0.29)
IL6Rα	0.16 (-0.26 to 0.58)	-0.84 (-1.22 to -0.46)*	-0.23 (-0.44 to -0.02)*
IL8	0.05 (-0.39 to 0.50)	0.41 (-0.03 to 0.86)	-0.13 (-0.36 to 0.10)
IP10	0.14 (-0.32 to 0.59)	0.18 (-0.26 to 0.62)	-0.11 (-0.33 to 0.12)
LBP	0.44 (0.02 to 0.86)*	0.55 (0.13 to 0.96)*	0.09 (-0.12 to 0.30)
Lumican	-0.11 (-0.49 to 0.28)	-0.86 (-1.23 to -0.48)*	0.21 (0.01 to 0.41)*
MCP1	-0.20 (-0.64 to 0.24)	-0.25 (-0.68 to 0.18)	0.00 (-0.22 to 0.22)
MCP4	0.01 (-0.41 to 0.43)	-0.39 (-0.79 to 0.00)	0.05 (-0.15 to 0.26)
MIP1b	-0.01 (-0.45 to 0.42)	-0.14 (-0.59 to 0.32)	0.09 (-0.14 to 0.32)
MMP3	-1.17 (-1.53 to -0.82)*	-1.20 (-1.52 to -0.88)*	-0.61 (-0.82 to -0.41)*
RAGE	0.19 (-0.21 to 0.58)	-0.30 (-0.64 to 0.05)	0.30 (0.09 to 0.50)*
SerpinE1	-0.16 (-0.59 to 0.28)	-0.18 (-0.60 to 0.25)	-0.21 (-0.42 to 0.00)
SHBG	1.03 (0.64 to 1.41)*	0.92 (0.57 to 1.27)*	0.57 (0.37 to 0.77)*
TNFα	-0.50 (-0.94 to -0.06)*	0.13 (-0.32 to 0.58)	-0.03 (-0.26 to 0.20)

Figure 4.20 Sex differences in blood biomarkers.

(A) Chord diagram illustrating associations between demographic variables and blood biomarkers. Red ribbons indicate positive correlations with female severe asthma, while blue ribbons indicate negative correlations with male severe asthma. The width of each ribbon reflects the strength of the association, as measured by Pearson correlation coefficients. **(B)** Linear regression analysis of sex differences in blood biomarkers in asthma and healthy controls. The forest plot displays standardised regression coefficients with 95% confidence intervals, adjusted for age, race, BMI, smoking status, and OCS use.

A schematic summary of sex-specific protein signatures in asthma is shown in Figure 4.21. Proteins that were differentially expressed between females and males were mapped onto key immune and inflammatory pathways. In female asthma, upregulated proteins included Galectin-3, CCL22, CCL11, IL-26, IL-6Rα, TSLP, and NGF, highlighting enhanced type 2 inflammatory responses, allergic sensitisation, eosinophil recruitment, and tissue remodelling. Several of these markers (Galectin-3, TSLP, CCL11, IL-26, IL-18) were particularly elevated in severe asthma, underscoring their contribution to advanced disease. In male asthma, proteins enriched relative to females included DPPIV, MMP3, Lumican, CRP, and IL-1α, consistent with stronger neutrophilic inflammation, extracellular matrix remodelling, and systemic inflammation. Notably, DPPIV was significantly altered in mild to moderate asthma, whereas MMP3 and Lumican were significantly elevated in severe asthma.

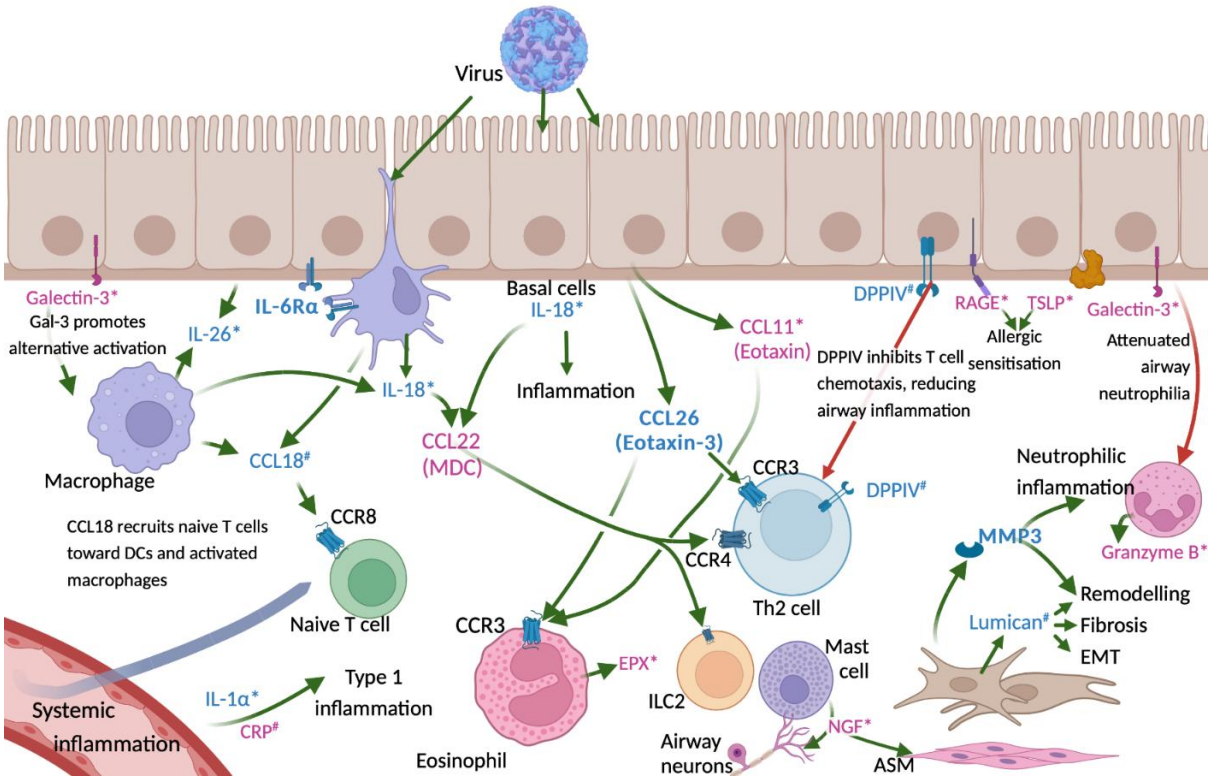


Figure 4.21 Schematic summary of differential immune pathways between males and females with asthma in serum and plasma proteomics.

Proteins in maroon increased in female asthma *vs.* male asthma; proteins in blue increased in male asthma *vs.* female asthma; proteins in bold are significantly differentially expressed in sex comparisons in both mild to moderate and severe asthma. *Indicates significant in severe asthma; #indicates significant in mild to moderate asthma.

Abbreviations: ASM: airway smooth muscle; CCL: chemokine (C-C motif) ligand; CCR: chemokine receptor; CRP: C-reactive protein; DPPIV: dipeptidyl peptidase-4 inhibitor; EMT: epithelial mesenchymal transition; EPX: eosinophil peroxidase; IL: interleukin; ILC2: type 2 innate lymphoid cell; MDC: macrophage derived chemokine; MMP: matrix metalloproteinase; NGF: nerve growth factor; RAGE: receptor for advanced glycation end products; TSLP: thymic stromal lymphopoietin.

Blood levels of biologic target proteins relevant to asthma were assessed for sex-specific differences (Figure 4.22). Across type 2 cytokines, no significant sex differences were observed for IL-4, IL-6, IL-13, or IL-25 in any group. However, in severe asthma, the IL-5 level was significantly higher in females compared with males ($p = 0.011$), while the TSLP level was significantly higher in males compared with females ($p = 0.001$). IL-33 levels were elevated in females compared to males in healthy controls ($p = 0.025$), although this difference was not significant in asthma groups. For non–type 2 cytokines, IL-17A showed a modest female bias in healthy controls ($p = 0.021$), but no difference was observed in asthma. TNF levels trended higher in males with severe asthma compared to females, although this difference did not reach statistical significance ($p = 0.079$) (Figure 4.22).

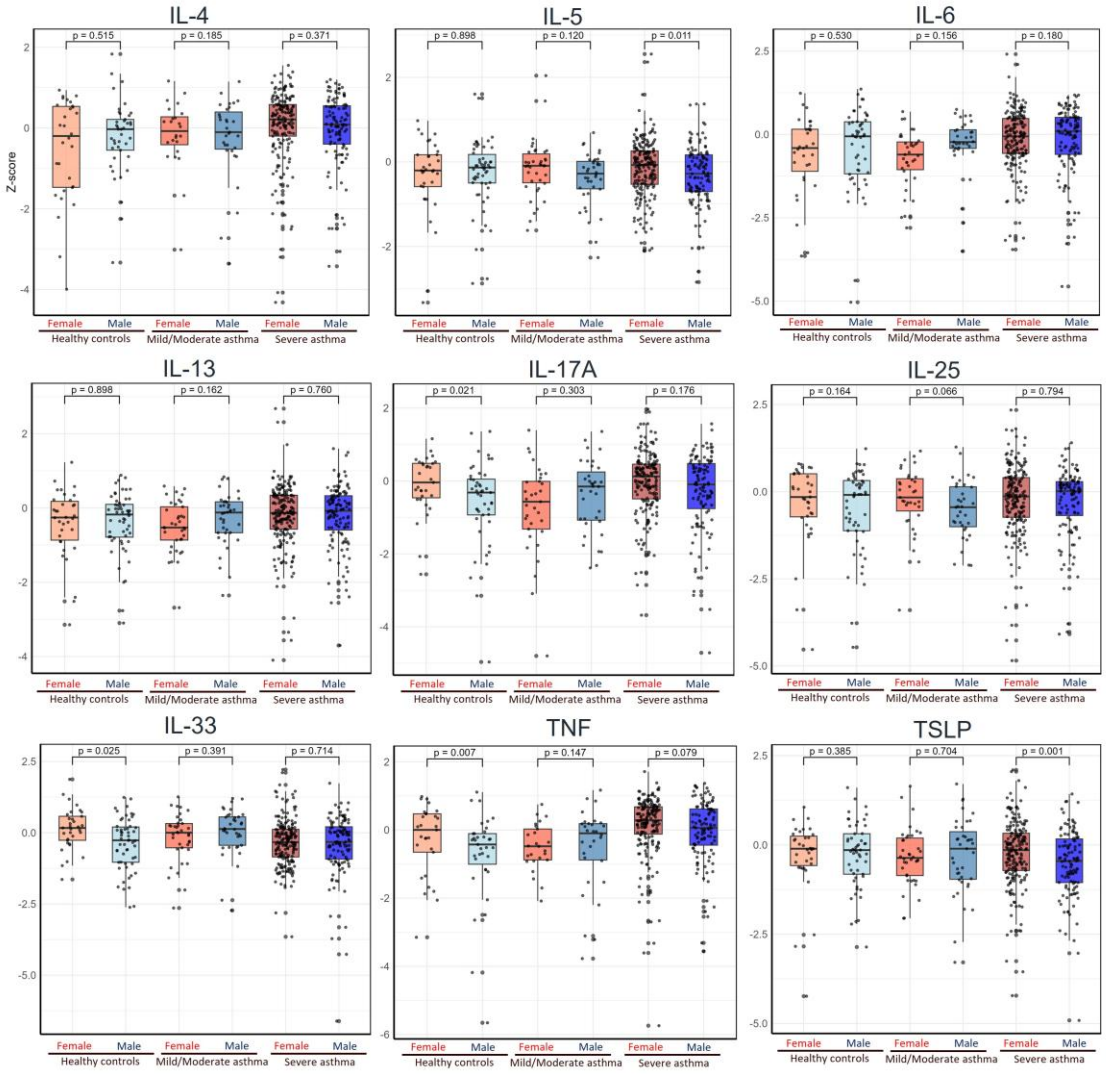


Figure 4.22 Sex differences in biologic target proteins in blood.

Z-scores of blood proteomic profiles highlighting sex-based differences in biologic target proteins.

4.4.5 Sex differences in urine metabolomics

Urine metabolomic analysis identified sex-related differences in several metabolites (Table S4.4 in appendix and Figure 4.23). In healthy controls (Figure 4.23A), males had higher levels of several dipeptides and amino acid derivatives, including N-acetylcarnosine, propionylcarnitine, carnosine, tyrosine, histidine, octopamine, and S-

adenosylhomocysteine. In contrast, females showed increased abundance of N-acetylputrescine, glutamic acid, aspartic acid, N-acetylglutamic acid, xanthine, caffeine, mannitol, sarcosine, and hippuric acid. In mild to moderate asthma (Figure 4.23B), males again exhibited enrichment of N-acetylcarnosine, carnosine, propionylcarnitine, taurine, lysine, and S-adenosylhomocysteine, while females had higher levels of sarcosine and N-acetylputrescine. In severe asthma (Figure 4.23C), the metabolic divergence became more pronounced. Females showed significant upregulation of hydroxyphenylacetic acid, sarcosine, proline, prolylhydroxyproline, glutamic acid, aspartic acid, O-acetylserine, N-acetylglutamic acid, 3-hydroxykynurenine, inosine, xanthine, and phosphoethanolamine, while males had higher levels of N-acetylcarnosine, propionylcarnitine, octopamine, tyrosine, histidine, taurine, methionine, and glucosamine.

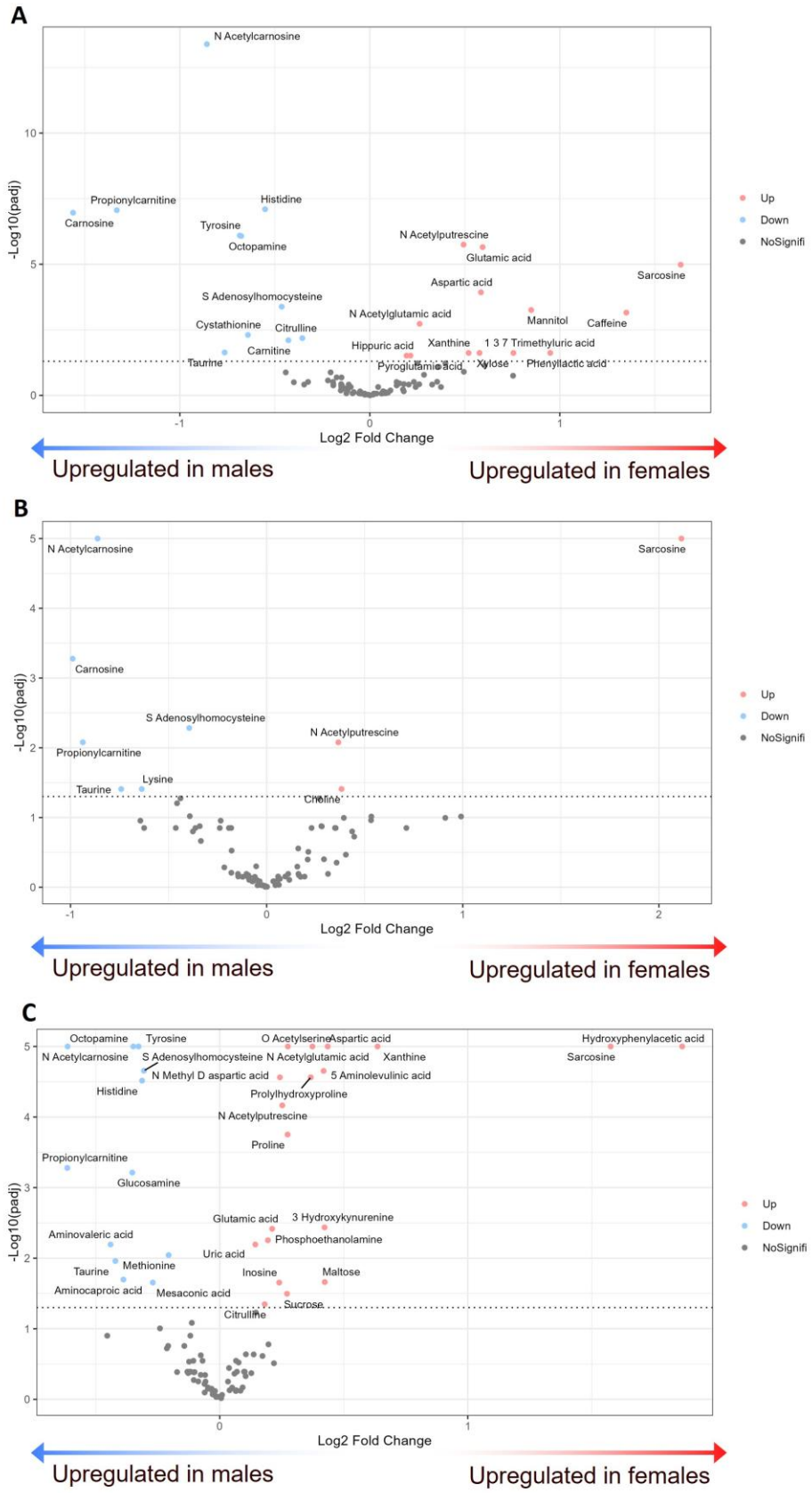


Figure 4.23 Sex differences in differentially abundant metabolites (DAMs) in

asthma with urine metabolomics.

(A) Volcano plot representing the DAMs between male healthy controls and female healthy controls.

(B) Volcano plot representing the DAMs between male mild to moderate asthma and female mild to moderate asthma.

(C) Volcano plot representing the DAMs between male severe asthma and female severe asthma.

Urinary steroid metabolite analysis revealed both disease severity–related and treatment-related patterns, with limited sex-specific differences (Table 4.9). For endogenous corticosteroids, the detection rates of cortisol were higher in females in the severe asthma group on daily OCS (35.2% vs. 18.5%, p = 0.04), which suggests that women are less likely to be adherent to prescribed systemic corticosteroids. Cortisone levels were modestly higher in females with mild to moderate asthma (229 vs. 193 ng/mL, p = 0.02), with no differences observed in those with severe asthma. For synthetic corticosteroids, concentrations of prednisolone, prednisone, and methylprednisolone showed no significant sex differences.

Table 4.8 Sex differences in urinary metabolites of corticosteroids.

	Mild/moderate asthma			Severe asthma (never prescribed OCS)			Severe asthma (daily prescribed OCS)		
	Female	Male	p-value	Female	Male	p-value	Female	Male	p-value
Cortisol, ng/mL	103 [63, 181]	88 [59, 119]	0.1	66 [42, 97]	58 [40, 117]	0.9	81 [38, 156]	86 [53, 163]	0.9
>LOD	32/39 (82.1%)	40/44 (90.9%)	0.4	69/94 (73.4%)	42/51 (82.4%)	0.3	31/88 (35.2%)	12/65 (18.5%)	0.04
Cortisone, ng/mL	229 [169, 275]	193 [116, 232]	0.02	161 [100, 231]	155 [97, 236]	0.7	102 [64, 239]	109 [65, 172]	0.8
>LOD	35/39 (89.7%)	41/44 (93.2%)	0.9	81/94 (86.2%)	46/51 (90.2%)	0.7	36/88 (40.9%)	23/65 (35.4%)	0.6
Methylprednisolone, ng/mL	41 [41, 41]	NA	NA	985 [985, 985]	68 [68, 68]	0.3	357 [259, 9002]	27 [27, 27]	0.1
>LOD	1/39 (2.6%)	0/44 (0.0%)	1.0	1/94 (1.1%)	1/51 (2.0%)	1.0	5/88 (5.7%)	1/65 (1.5%)	0.4
Prednisolone, ng/mL	1331 [692, 1572]	43 [43 43]	0.2	165 [112, 1380]	173 [161, 186]	1.0	1454 [587, 2749]	1659 [812, 3198]	0.4
>LOD	3/39 (7.7%)	1/44 (2.3%)	0.5	7/94 (7.4%)	2/51 (3.9%)	0.6	41/88 (46.6%)	36/65 (55.4%)	0.4
Prednisone, ng/mL	282 [210, 353]	38 [28, 98]	0.2	125 [118, 158]	128 [95, 162]	0.9	464 [213, 1665]	359 [266, 1641]	0.8
>LOD	2/39 (5.1%)	3/44 (6.8%)	1.0	5/94 (5.3%)	3/51 (5.9%)	1.0	15/88 (17.0%)	5/65 (7.7%)	0.1
Synthetic steroid# >LOD	6/39 (15.4%)	4/44 (9.1%)	0.5	13/94 (13.8%)	6/51 (11.8%)	0.9	61/88 (69.3%)	42/65 (64.6%)	0.7

*Hydrocortisone and triamcinolone doses were converted to equivalent prednisolone doses. #Synthetic steroid includes methylprednisolone, prednisolone, and prednisone.

Abbreviations: OCS: oral corticosteroid; LOD: limit of detection.

4.4.6 Sex differences in sputum microbiomics

Sputum microbiomic analysis indicated sex-specific microbiota profiles. Analysis of sputum microbiomes demonstrated broadly similar bacterial community structures between females and males across healthy controls and asthma groups (Table S4.5 in appendix and Figure 4.24). The dominant taxa included *Prevotella*, *Veillonella*, *Streptococcus*, *Neisseria*, *Fusobacterium*, *Rothia*, *Moraxella*, and *Haemophilus*. The severe asthma group generally have more abundance of pathogenic bacteria. Assessment of α-diversity using the Shannon index revealed no significant sex-based differences in sputum microbiome diversity across healthy controls, mild to moderate asthma, or severe asthma (Figure 4.24).

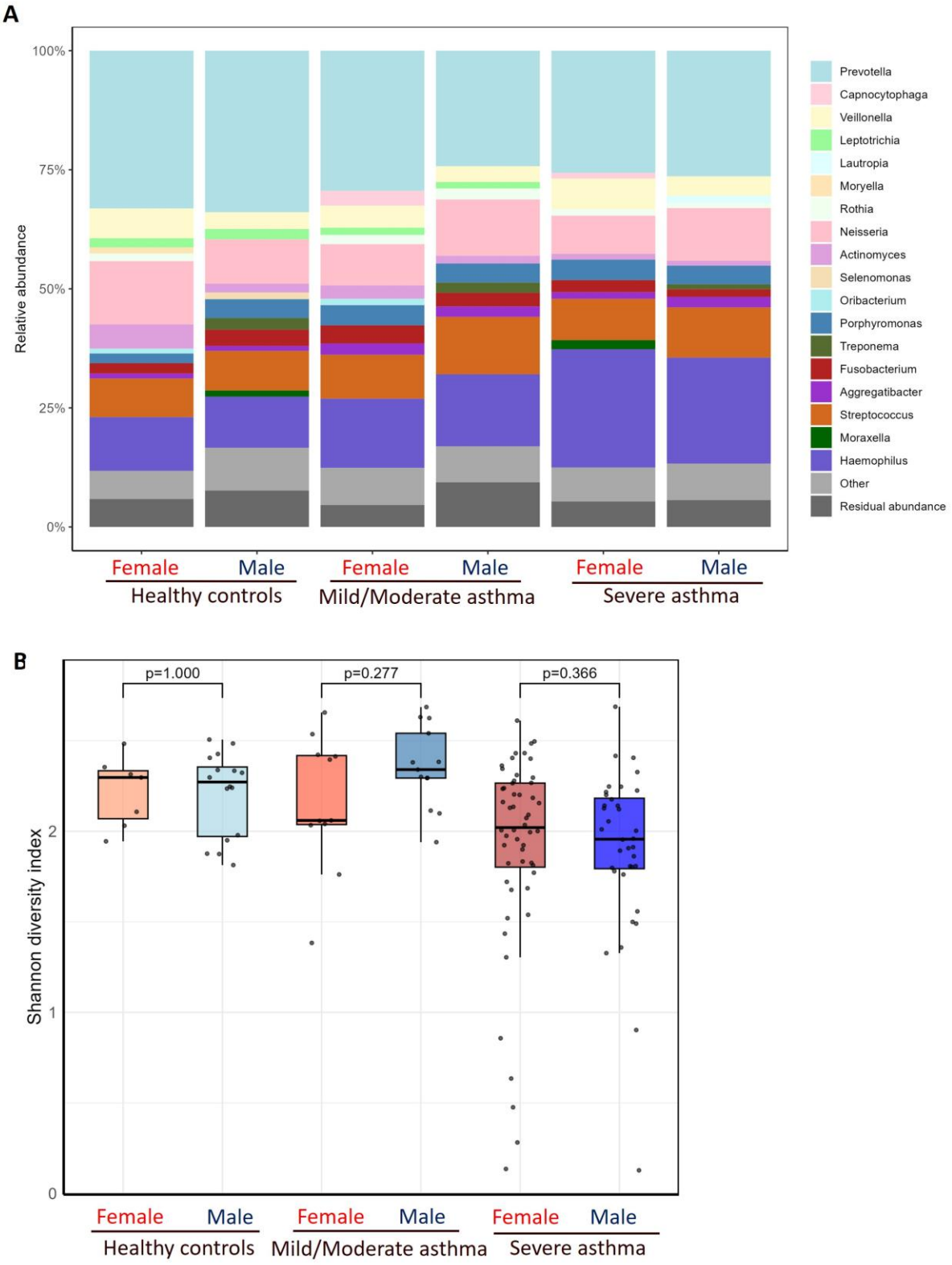


Figure 4.24 Airways microbiome in patients with asthma and healthy controls with sputum microbiomics.

(A) Relative abundance of bacterial genera.

(B) Sputum microbiome diversity assessed by Shannon index (α-diversity). Comparisons between groups were performed using the Mann-Whitney U test. Higher values indicate greater taxonomic richness and evenness in sputum microbiota at the sample level.

Differential abundance analysis of sputum microbiota revealed sex-specific signatures across health and asthma severity (Figure 4.25). In healthy controls (Figure 4.25A), several genera were enriched in males, including *Moraxella*, *Dialister*, *Mycoplasma*, *Tannerella*, *Parvimonas*, *Treponema*, *Bifidobacterium*, *Peptococcus*, and *Fusobacterium*, whereas *Actinomyces* was more abundant in females. In mild to moderate asthma (Figure 4.25B), male-enriched taxa included *Moraxella*, *Filifactor*, *Desulfobulbus*, *Bacteroides*, and *Treponema*, while females exhibited higher relative abundance of *Actinomyces*, *Propionibacterium*, *Alloscardovia*, *Megasphaera*, *Lactococcus*, *Eikenella*, *Steroidobacter*, and *Capnocytophaga*. In severe asthma (Figure 4.25C), distinct female-associated enrichment was observed for *Haemophilus*, *Stenotrophomonas*, *Sediminibacterium*, *Pseudomonas*, *Bacteroides*, *Moraxella*, and *Shewanella*, whereas only *Methylobacterium* and *Cellulomonas* were more abundant in males. A key observation was the significant female-associated enrichment of *Haemophilus*, *Pseudomonas*, and *Moraxella* in severe asthma, with fold changes of 5.6, 8.6, and 4.6, respectively, compared with men.

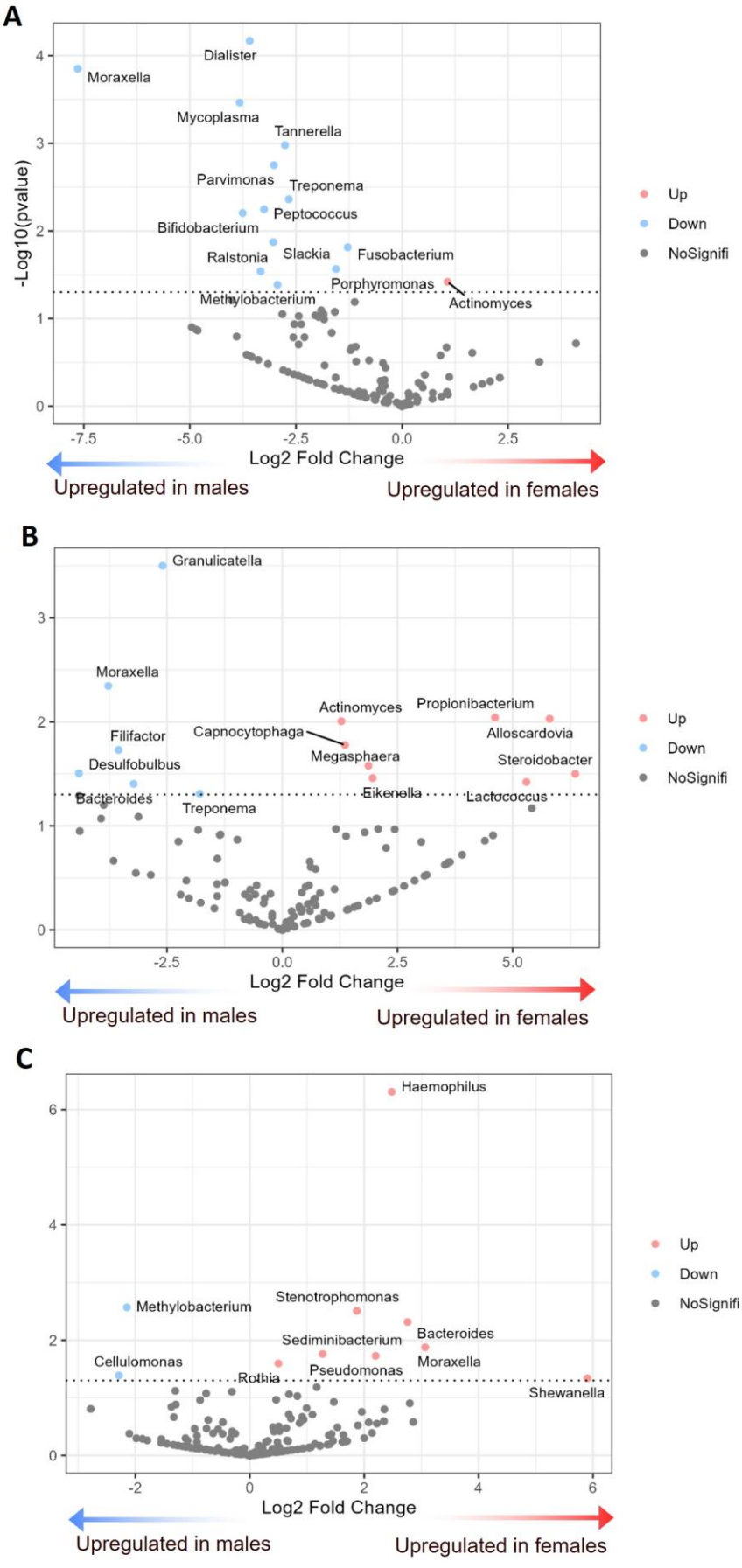

A
Dialister
Moraxella
Mycoplasma
Tannerella
Parvimonas
Treponema
Peptococcus
Bifidobacterium
Ralstonia
Slackia
Fusobacterium
Porphyromonas
Methylobacterium
Actinomyces
-Log10(pvalue)
Log2 Fold Change
Upregulated in males
Upregulated in females
Up
Down
NoSignifi
B
Granulicatella
Moraxella
Actinomyces
Propionibacterium
Alloscardovia
Capnocytophaga
Filifactor
Megasphaera
Steroidobacter
Desulfobulbus
Eikenella
Lactococcus
Bacteroides
Treponema
Log2 Fold Change
Upregulated in males
Upregulated in females
Up
Down
NoSignifi
C
Haemophilus
Methylobacterium
Stenotrophomonas
Sediminibacterium
Bacteroides
Cellulomonas
Rothia
Pseudomonas
Moraxella
Shewanella
Log2 Fold Change
Upregulated in males
Upregulated in females
Up
Down
NoSignifi

Figure 4.25 Sex differences in airways microbiome in patients with asthma and healthy controls with sputum microbiomics.

(A) Volcano plot representing the differentially abundant bacterial genera between male healthy controls and female healthy controls.

(B) Volcano plot representing the differentially abundant bacterial genera between male mild to moderate asthma and female mild to moderate asthma.

(C) Volcano plot representing the differentially abundant bacterial genera between male severe asthma and female severe asthma.

4.4.7 Sex hormones and asthma severity

Urine metabolomic analysis revealed marked sex differences in androgen-related metabolites across health and asthma severity (Figure 4.26). In general, a progressive decrease in six androgen metabolite levels was observed across the study groups: healthy controls, mild to moderate asthma, severe asthma without oral corticosteroids (OCS), and severe asthma with daily OCS. In healthy controls, males exhibited significantly higher concentrations of DHT-G, testosterone-G, DHEA-G, DHEA-S, androsterone-G, and androsterone-S compared with females. With increasing asthma severity, these metabolites exhibited a progressive decline in both sexes; however, the magnitude of reduction was greater in males. In mild to moderate asthma, androgen levels were already reduced in both sexes, with DHEA-S and Androsterone-G showing the most significant decreases. In severe asthma (never prescribed OCS), levels of DHT-G, DHEA-S, and Androsterone-S declined further, particularly in males. In patients with severe asthma who were daily prescribed OCS, concentrations of all six metabolites were profoundly suppressed, with male patients showing the most

pronounced reductions across all androgens measured (Figure 4.26).

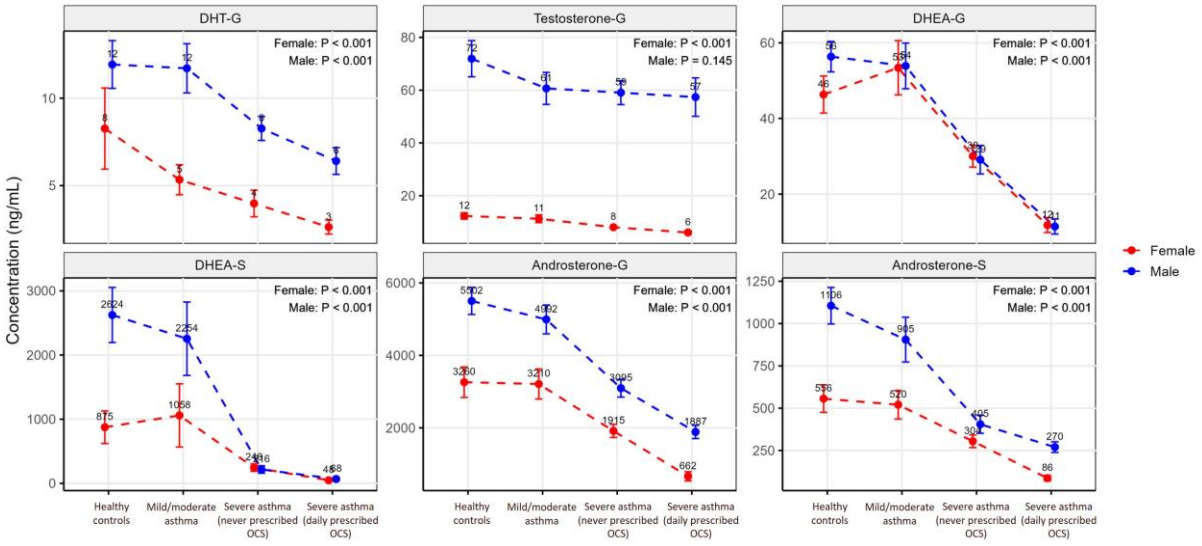


Figure 4.26 Sex differences in male sex hormones with urine metabolomics.
P-values were calculated using the Kruskal–Wallis test.

4.4.8 External validation

Validation of findings was undertaken in data from the RASP-UK cohort (Table S4.19 in appendix and Table S4.20 in appendix). Regarding clinical factors, significantly higher BMI in women was confirmed in RASP-UK (34.6 vs. 29.1, p = 0.006). A non-significant tendency towards a younger age of onset in women in UBIOPRED was significantly different in RASP-UK (18 vs. 34 years, p = 0.03). A similar trend was observed for higher symptom scores in women in RASP-UK, although this did not reach predefined statistical significance (ACQ-5 1.9 vs. 1.2, p = 0.06). Women were prescribed a significantly lower ICS dose than men in RASP-UK (1,600 mcg BDP equivalent vs. 2,000 mcg BDP equivalent, p = 0.01).

Notwithstanding the much smaller sample size, the following sex-specific DEGs

upregulated in women were replicated in the airway transcriptomic analysis of RASP-UK: *PNPLA4*, *RPS4X*, *ZFX*, *EIF1AX*, *EIF2S3* and the histone modifier genes *KDM6A*, *KDM5C* (Table S4.21 in appendix). Notably, *KDM5C* and *ZFX* were differentially expressed only in asthma patients and not in healthy individuals. Differential upregulation of KDM6A and KDM5C in healthy women compared with men receiving ICS in the HVS study was also confirmed, implying a sex-specific differential response to ICS.

4.5 Discussion

This study provided a comprehensive multi-omics analysis of sex differences in asthma, integrating clinical, physiological, immunological, transcriptomic, proteomic, metabolomic, microbiomic, and radiomic data. Our findings demonstrated that in severe asthma, men exhibited greater lung function impairments, while women experienced more severe symptoms, more exacerbations, as well as greater airways inflammation, remodelling, and a history of lung infections.

4.5.1 Sex difference in mild to moderate asthma

In mild to moderate asthma, women and men exhibited similar clinical characteristics. However, women displayed a higher prevalence of the T2-low phenotype, characterised by higher FeNO levels and lower blood eosinophil counts. Women also had higher CRP levels and medical histories of pneumonia or bronchitis. This observation is consistent with previous studies (Benson et al., 2022; Lola Loewenthal et al., 2024), suggesting that women may have a greater propensity for neutrophilic airways inflammation. Supporting this, earlier studies have demonstrated increased IL-17A secretion from Th17 cells in females, which are influenced by oestrogen and progesterone (Newcomb et al., 2015; Newcomb & Peebles, 2013). Moreover, differences in body composition may also contribute to this inflammatory profile. Specifically, a greater proportion of subcutaneous rather than abdominal adipose tissue is generally present in women, which secretes higher levels of leptin. Consistent with this, blood proteomics showed higher leptin levels in women. Leptin is a pro-inflammatory adipokine known to promote neutrophil recruitment to the airways, thereby enhancing neutrophilic inflammation (Baltieri et al., 2022).

Alternatively, it may be speculated that the predominance of neutrophilic inflammation in females could be driven by the increased abundance of pathogenic bacteria such as *Haemophilus*, *Moraxella*, and *Bacteroides*, potentially reflecting sex-specific susceptibility to colonisation by these organisms. However, the direction of causality in this association is not known. A further alternative might be that differences in airway inflammatory type might be driven simply by higher relative bioavailable dose of ICS in women: bioavailability of ICS may be affected by differential metabolism of steroids in women and men, but it is almost certainly also true that women receive a higher dose of ICS per kg body mass because adult asthma prescribing guidelines do not propose different dosing by sex or by weight, although in UK men are on average >18% heavier than women (England, 2021).

4.5.2 Sex difference in lung function and airways remodelling of severe asthma

Amongst patients with severe asthma, men exhibited poorer pulmonary function, consistent with previous reports (L. Loewenthal et al., 2024; Tessa et al., 2024). Airway narrowing was evident in males, characterised by reduced lumen area during inspiration and increased mucin and elastin deposition compared to healthy controls, contributing to air trapping. In contrast, women demonstrated a smaller diameter of the large conducting airways, both in health and in asthma, which would tend to predispose them to greater impairment in lung function during exacerbations, particularly when these are driven by mucus plugging. This is consistent with the previous findings that men have more emphysema and women have more small

airway disease (Martinez et al., 2007; Tam et al., 2016).

Transcriptomic data supported this more pronounced remodelling in women, which appeared to be at least partially driven by sex hormones. This finding aligns with previous studies showing stronger airway remodelling in females with severe COPD (Martinez et al., 2007). Experimental studies support a biological basis for these sex-related differences, which are linked to both hormonal influences and anatomical variations. For instance, in animal models, chronic cigarette smoke exposure induced small airway disease and airflow obstruction in female mice, whereas male animals developed emphysema. Reduction of female sex hormones by ovariectomy, or pharmacological blockade of oestrogen signalling with tamoxifen, shifted the phenotype towards emphysema, underscoring the protective role of oestrogen (Tam et al., 2016). At the molecular level, oestrogen contributes to airway remodelling via distinct receptor-mediated mechanisms: oestrogen receptor α (ERα) drives structural changes through an epidermal growth factor (EGF)-dependent, ligand-independent pathway, while oestrogen receptor β (ERβ) promotes airway smooth muscle proliferation and activates remodelling-associated signalling cascades (Qin et al., 2023). In line with these mechanistic insights, population-level data also indicate an airway-predominant phenotype in females. A 2010 US National Health and Wellness Survey found that 63% of individuals with chronic bronchitis were female (Allen-Ramey et al., 2012). Conversely, functional studies have shown that methacholine-induced air trapping, driven by small airway collapsibility, is more prominent in men than in women (Stengel et al., 2008).

4.5.3 Sex difference in symptom burden and comorbidities of severe asthma

Women with severe asthma had poorer symptom control, even after adjustment for potential confounders, including age, race, and BMI, consistent with previous epidemiological studies (L. Loewenthal et al., 2024; Tessa et al., 2024). Despite exhibiting greater markers of type 1 inflammation, women also have heightened somatosensory sensitivity, such as an increased cough reflex. This may contribute to the greater symptom burden observed in women. These findings suggest possible sex-related differences in symptom perception and reporting—an area currently under active investigation (Morice et al., 2014).

Women also often experienced a higher prevalence of comorbidities, such as allergic rhinitis, GORD, and psychiatric disorders, but had fewer nasal polyps, which aligns with previous studies (Idani et al., 2019). Fluctuations in oestrogen and progesterone may modulate mast cell activity and histamine release, thereby predisposing women to heightened atopy and reflux symptoms (Jacobson et al., 2008). The higher prevalence of psychiatric disorders in women with severe asthma may reflect both biological and psychosocial vulnerability. These comorbidities can, in turn, worsen asthma outcomes by amplifying symptom perception and reducing treatment adherence (Freitas & Novais, 2025). Conversely, the lower prevalence of nasal polyps in women is consistent with clinical observations that chronic rhinosinusitis with nasal polyps (CRSwNP) tends to be more common in men, often linked with type 2 eosinophilic inflammation (Langdon & Mullol, 2016). These conditions can exacerbate perceived respiratory symptoms or interfere with asthma management. However, in

addition to an increased daily symptom burden, higher rates of exacerbations were also observed in women with severe asthma, consistent with recent similar observations from robust, longitudinal analysis of 6,510 participants enrolled in the negative arms of clinical trials (Riemann et al., 2025). The management of asthma needs to be moderate according to differences in sex.

4.5.4 Sex difference in immunology of severe asthma

In severe asthma, women were observed to exhibit stronger Th2 responses, as evidenced by increased expression of Th2-associated cytokines and chemokines (TSLP, CCL11), as well as markers of mast cells and eosinophils (EPX, CMA1, GZMB) in proteomic analyses. Elevated numbers of $CD3^+$, $CD4^+$, and $CD8^+$ T cells accompanied these immunological changes. My findings were consistent with previous reports showing that women with severe asthma have higher T and B lymphocyte counts (Loza et al., 2010) and increased production of IL-5 and IL-13, which collectively drive eosinophilic inflammation and airway hyperresponsiveness (Okuyama et al., 2008).

Oestrogen has been associated with promoting eosinophil recruitment to the airway and enhanced type 2 inflammation, potentially leading to a higher frequency of asthma attacks (Riffo-Vasquez et al., 2007; Vijeyakumaran et al., 2023). In contrast, testosterone has been linked to reduced type 2 inflammation and a protective effect against asthma (Adeeb A Bulkhi et al., 2020; Y.-Y. Han et al., 2020). Oestrogen receptor α (ERα) signalling is known to amplify Th2 cytokine expression and upregulate upstream epithelial-derived mediators such as IL-33 and TSLP, thereby

enhancing type 2 immunity in women. In contrast, androgens have been shown to suppress type 2 inflammatory pathways by attenuating ILC2 proliferation, reducing IL-33 and TSLP expression, and limiting IL-17 production and neutrophilic infiltration (Nowrin U Chowdhury et al., 2021).

4.5.5 Sex difference in airways bacteria of severe asthma

Sputum microbiome analyses revealed additional sex differences. In healthy individuals, men typically exhibit a higher prevalence of bacterial colonisation in the lower airways, which may be partially attributed to anatomical differences (e.g., larger airway diameter) and behavioural factors (e.g., higher smoking rates). However, this trend appears to reverse in patients with severe asthma, where women demonstrate a higher relative abundance of potentially pathogenic genera, particularly *Haemophilus*, *Moraxella*, and *Pseudomonas*, which can exacerbate airways inflammation and contribute to disease severity and corticosteroid resistance. This observation aligns with our findings of impaired ciliary function in women with severe asthma. Oestrogen has been shown to reduce airway surface liquid height (ASLh) and suppress ciliary beat frequency, thereby impairing mucociliary clearance and increasing susceptibility to chronic bacterial colonisation and infection (Harvey & McElvaney, 2024; Jain et al., 2012; Somayaji & Chalmers, 2022). In addition, oestrogen signalling enhances neutrophilic inflammation via IL-17A production in asthma, especially among obese women. IL-17A stimulates airway epithelial cells to release pro-neutrophilic cytokines such as G-CSF and CXCL1, sustaining neutrophil recruitment and activation (Fuseini et al., 2019). This neutrophil-dominated environment supports the persistence of *Haemophilus* and *Moraxella*, which

preferentially thrive under such inflammatory conditions. Concurrently, epithelial barrier function may be compromised, facilitating bacterial colonisation and biofilm formation. Neutrophil-derived enzymes, including elastase and myeloperoxidase, further degrade epithelial integrity and impair mucociliary clearance, weakening host defence mechanisms (Rijken & Bruijnzeel, 2009). These processes collectively establish a self-reinforcing cycle of infection and inflammation, more frequently observed in females with severe, treatment-refractory asthma.

4.5.6 Sex hormones and asthma

Male sex hormone concentrations are negatively associated with asthma severity, likely due to their anti-inflammatory effects (Fuseini et al., 2018; Sathish et al., 2015), which is aligned with previous studies (Zein et al., 2021). In murine models, testosterone administration has been shown to attenuate dust mite–induced eosinophilic and neutrophilic inflammation in the lungs, an effect mediated at least in part through androgen receptor signalling pathways (Y. Y. Han et al., 2020). According to a national survey, higher serum testosterone levels were associated with a dose-dependent reduction in asthma prevalence and with increased FEV_1 in both adult men and women (A. A. Bulkhi et al., 2020). DHEA-S levels, which are already low in women, may be further reduced by systemic glucocorticoids, potentially impairing asthma control (Zein et al., 2020).

4.5.7 Study limitations

This study has several limitations. First, although I leveraged multi-omics profiling from a large, well-characterised cohort, the cross-sectional design limits causal inference

regarding the observed sex-related molecular and clinical differences. Second, while I integrated transcriptomic, proteomic, metabolomic, and microbiomic layers, the samples were obtained from different samples (airway tissue, blood, urine, sputum), and not all participants contributed material for each assay, which may introduce sampling bias and reduce power for some analyses. Third, the findings were derived from a predominantly European population within the U-BIOPRED cohort, which may limit generalizability to more diverse ethnic groups. Fourth, while corticosteroid use and adherence were considered, residual confounding from treatment history cannot be entirely excluded, especially in severe asthma, where cumulative steroid exposure may affect immune, metabolic, and microbiome readouts. Finally, although validation was attempted using the RASP-UK cohort, the sample size of that cohort is limited and longitudinal data are lacking, with future studies needed to confirm whether the identified sex-specific molecular pathways predict disease progression or treatment response.

4.5.8 Clinical implications

This study highlighted significant sex differences in severe asthma that have direct clinical relevance. From a disease management perspective, women experienced a greater symptom burden, more comorbidities, and higher levels of pathogenic airway colonisation, whereas men exhibited more pronounced airflow limitation. Recognising these sex-specific patterns can inform more precise diagnostic strategies and personalised treatment approaches. For drug development, the distinct molecular pathways identified in each sex highlighted the need to account for sex differences when developing and testing novel biologics or targeted therapies. At the level of policy

making, these results supported the integration of sex-specific guidance into asthma management guidelines. This could include research into sex- or weight-specific dosing of ICSe to reduce the risk of airway infections, thereby promoting precision medicine approaches that addresse disease heterogeneity and improve outcomes across patient populations.

4.6 Conclusions

To conclude, this study underscored the multifaceted impact of biological sex on asthma across clinical, structural, immunological, and molecular domains. My findings suggested that men with severe asthma are more prone to functional impairments, including significantly reduced lung function and increased airflow limitation. In contrast, female patients experienced a greater symptom burden, characterised by poorer symptom control and more frequent exacerbations. Moreover, women with severe asthma exhibited stronger airways inflammation, remodelling, and infection. These findings had important implications for asthma management. Sex-based stratification may enhance diagnostic accuracy, inform treatment decisions, and guide future research. Further studies should elucidate the underlying mechanisms and determine whether sex-specific therapies can improve clinical outcomes.

Chapter 5 General discussion and future work

5.1 General discussion and future work

5.1.1 Type 2 phenotype in asthma: an airways transcriptomic analysis

By integrating detailed clinical phenotyping with transcriptomic profiling of bronchial biopsies and brushes in a well-characterised multi-centre severe asthma cohort, I provided a comprehensive analysis of molecular pathways underpinning different phenotypes. Importantly, by incorporating healthy controls before and after inhaled corticosteroid treatment, our findings demonstrated transcriptomic signatures of asthma which were independent of corticosteroid effects. The FeNO suppression testing and biomarker-guided ICS treatment ensure the appropriate ICS dosage for each patient. This study found that corticosteroid-resistant T2-high disease was characterised by T2-dependent genes, adaptive immune responses, impaired ciliary function, and epithelial development, while T2-low asthma was driven by Th1, IL-17, interferon-γ, and neuroimmune pathways, highlighting alternative immune drivers. Moreover, this study challenged the traditional binary T2-high/T2-low classification of asthma and instead supported a spectrum model by proposing a partly corticosteroid-responsive T2-intermediate asthma. These endotype-specific molecular signatures underscored the heterogeneity of severe asthma and supported the need for more precise, phenotype-driven therapeutic strategies.

In the future, several aspects remain to be detected. First, the integration of multi-

omics approaches could provide a more comprehensive understanding of the biological mechanisms underlying T2 heterogeneity (Zhang et al., 2024). Such integrative analyses may reveal upstream regulators and downstream effectors of the observed transcriptomic changes, offering new opportunities for therapeutic targeting. Second, spatial and single-cell transcriptomic analyses represent a promising direction for future research (Aldridge & Teichmann, 2020; Williams et al., 2022), which will enable a higher-resolution dissection of cell type–specific contributions to T2-high, T2-intermediate, and T2-low phenotypes. These approaches may reveal novel subpopulations of epithelial or immune cells, as well as spatial niches of inflammation and remodelling. Moreover, functional studies are warranted to experimentally validate key candidate genes and pathways highlighted in this work. *In vitro* models of airway epithelial cells or organoid systems could be used to test causal relationships, while *in vivo* studies may clarify their contribution to airways inflammation and remodelling (Zhou et al., 2023). Furthermore, the clinical utility of the transcriptomic profiles should be investigated, with a focus on developing biomarker panels or predictive models that can support patient stratification and guide precision medicine approaches in asthma management.

5.1.2 Bacteria and antibiotic resistance in asthma: a 27-year longitudinal analysis

This study obtained a large, longitudinal electronic health record (EHR) dataset spanning nearly three decades, which enabled comprehensive characterisation of respiratory pathogens and antimicrobial resistance in asthma. By integrating detailed microbiological data with clinical and biological factors, we were able to identify both

long-term trends, such as the decline in *H. influenzae* and rise in *P. aeruginosa*, and important associations with age, neutrophilic inflammation, and inhaled corticosteroid use. The systematic assessment of antimicrobial susceptibility across multiple species also provided novel insights into the burden of resistance in asthma, addressing a key knowledge gap and highlighting the need for careful antimicrobial stewardship and tailored management strategies in patients with asthma.

Future studies should aim to build on these findings by integrating microbiological surveillance more systematically into asthma research and clinical management. Advanced sequencing approaches, such as shotgun metagenomics and metatranscriptomics, can provide deeper insights into dynamic pathogen profiles, strain-level diversity, and functional resistance mechanisms that are missed by culture-based techniques (Tyagi & Katara, 2024). Mechanistic studies using *in vitro* airway models or animal systems should be pursued to test how specific bacterial taxa, such as *P. aeruginosa* or *S. aureus*, interact with host immunity and treatment exposure to shape asthma trajectories. These approaches could also reveal ecological interactions within polymicrobial communities and their impact on airways inflammation (Welp & Bomberger, 2020). Translational research should focus on developing biomarkers for infection-prone asthma phenotypes and evaluating antimicrobial stewardship protocols, including targeted antibiotics, non-antibiotic anti-infective strategies, and microbiome-modulating interventions, to optimise outcomes while mitigating resistance.

5.1.3 Sex differences in asthma: a large-scale multi-omics analysis

By analyzing the multi-omics data from a large-scale, well-characterised adult asthma of cohort, I investigated sex differences across molecular, microbial, and physiological domains. By combining transcriptomic, proteomic, metabolomic, and microbiomic data, I were able to delineate distinct biological pathways underpinning the higher symptom burden, exacerbation risk, and comorbidity prevalence observed in women with severe asthma, in contrast to the greater airflow limitation seen in men. Importantly, the use of independent validation in the RASP-UK cohort increased the robustness and generalisability of these findings. This comprehensive systems approach provided novel insights into the sex-specific pathophysiology of asthma and highlighted potential avenues for more personalised, gender-sensitive treatment strategies. These findings suggested that sex is not merely a demographic descriptor but a biological modifier with direct relevance for disease mechanisms, clinical presentation, and treatment outcomes.

Future studies should aim to dissect further the role of sex-specific immunity in shaping asthma heterogeneity and promote sex-specific asthma management. Spatial and single-cell transcriptomic approaches will be particularly valuable for resolving cell-type– and tissue–context–specific immune differences between men and women, potentially uncovering sex-linked pathways of airways inflammation, remodelling, and microbial colonisation that are masked in bulk analyses. Longitudinal cohort studies are needed to validate the persistence and evolution of sex-related differences across disease progression, including the impact of hormonal transitions such as puberty,

pregnancy, and menopause, thereby moving beyond cross-sectional associations. Complementary experimental approaches using *in vitro* and *in vivo* models will also be critical to test directly how sex hormones modulate immune cell activity, mitochondrial function, and epithelial–microbial interactions, providing mechanistic insight into differential disease expression.

Moreover, it is very important to perform sex-stratified analyses of all outputs from randomised controlled trials (RCTs) in asthma. Reanalysing sex-specific data from many clinical trials together could uncover important differences in how men and women respond to treatments and experience side effects, which may be hidden when results are only reported in aggregate. Finally, the potential of harnessing massive real-world electronic patient record (EPR) data should be explored. This includes the use of free-text clinical letters and hospital notes through natural language processing (NLP). These approaches will support the development of sex-informed predictive models and precision treatment strategies, embedding sex as a core dimension of asthma research and care.

5.1.4 Summary

Asthma is a complex, heterogeneous syndrome with the interplay of diverse immunological, microbial, and clinical factors. These three studies address critical aspects of airways inflammation in asthma with multi-omics analysis and longitudinal analysis: molecular mechanisms across T2 biomarker phenotype, the long-term pattern of bacterial infection and antibiotic resistance, and sex differences in characteristics and mechanisms. These findings provided a multidimensional

perspective on asthma with important implications for precision diagnosis, personalised treatment, and the identification of novel therapeutic targets.

5.2 Future plans

5.2.1 Short-term plans

Bioinformatics is an emerging and powerful approach for investigating the mechanisms of chronic respiratory diseases. Compared with traditional epidemiological studies, bioinformatics enables the identification of mechanistic pathways; compared with single-layer biological experiments, it offers an integrative and systems-level perspective. This makes it particularly suitable for disentangling the complexity and heterogeneity of chronic respiratory diseases.

Current asthma research has focused mainly on genomics and blood-based transcriptomics (Bunyavanich et al., 2024). Future studies should extend to airway-specific transcriptomics and proteomics, which are more directly linked to asthma pathology. In addition, integrating other molecular layers, such as metabolomics, lipidomics, microbiomics, breathomics, exposomics, and radiomics, will provide a more holistic understanding of disease processes and help identify potential therapeutic targets. Epidemiological research has already identified many patient characteristics and risk factors associated with the development of asthma (Merin E Kuruvilla et al., 2019). Advanced bioinformatics methods are crucial for uncovering the biological mechanisms underlying these associations. U-BIOPRED, a comprehensive multi-omics database, provides a powerful platform to enable such studies.

Age is a critical determinant of asthma, with manifestations, severity, and underlying biology differing markedly across the lifespan (Kaplan et al., 2019). However, most asthma studies have disproportionately focused on adults, and some results cannot

be generalised to other age groups, including preschool wheeze, paediatric asthma, and asthma in elderly people. A comprehensive lifetime study of asthma is therefore necessary to identify age-related differences in clinical features, biomarkers, and transcriptomic signatures. This approach would provide insights into the mechanistic differences of different asthma age groups and support the development of personalised treatment strategies.

Obesity is a major public health challenge and is strongly linked to asthma incidence, severity, and treatment resistance. Patients with obesity-related asthma often show impaired lung function, greater symptom burden, and reduced responses to corticosteroids (Muc et al., 2016). However, the mechanisms remain poorly defined and may involve altered lung mechanics, systemic and airways inflammation, metabolic and lipid dysregulation, and microbiome changes (Miethe et al., 2020). Multi-omics approach will be applied to address it. Lung function and airway structure will be assessed by spirometry and quantitative CT, while transcriptomic and proteomic profiling of airway and blood samples will identify molecular signatures. Metabolomic and lipidomic analyses will characterise metabolic alterations, and metagenomic sequencing will be applied to study airway microbiomes. This study is expected to provide a multidimensional characterisation of obesity-related asthma, uncover novel biomarkers, and yield mechanistic insights to guide precision medicine for this high-burden subgroup.

Beyond asthma, chronic respiratory diseases such as bronchiectasis remain underexplored. Spatial transcriptomics enables the mapping of gene expression within intact airway tissue, thereby linking molecular changes to structural pathology. In

bronchiectasis, this approach can reveal how epithelial damage, immune cell infiltration, and tissue remodelling are organised in space. It may also clarify how pathogens such as *P. aeruginosa* or *H. influenzae* shape local immune responses and how biologics control the disease progression. By integrating spatial data with other omics, spatial transcriptomics has the potential to identify new disease endotypes and therapeutic targets.

Translational medicine is a rapidly developing area, with many monoclonal antibodies and small-molecule inhibitors already developed or under investigation (Imran Howell et al., 2023). Advanced data analytic approaches can facilitate this process by integrating multi-omics, clinical trial, and real-world evidence to identify novel therapeutic targets and biomarkers. Machine learning and computational modelling can further support the stratification of patients into molecularly defined subgroups, predict treatment responsiveness, and inform optimal dosing and safety monitoring strategies. These applications not only accelerate drug discovery and repurposing but also enhance precision medicine approaches, bridging the gap between mechanistic insights and clinical practice. These efforts converge to improve patient outcomes, reduce disease burden, and promote long-term respiratory health at both individual and population levels.

Systematic reviews and meta-analyses are undergoing a paradigm shift, moving from manual extraction and evaluation towards automatic, AI-assisted approaches in the coming decades (Delgado-Chaves et al., 2025). My goal is to accelerate this transformation, and I have already proposed a large language model–based framework to support systematic reviews and meta-analyses. Furthermore, in my

ongoing Cochrane network meta-analysis on biologics for chronic severe asthma, I tested AI tools to streamline data processing, and the outcomes were highly promising. In respiratory medicine, meta-analyses play a pivotal role in integrating evidence across diverse clinical trials, guiding treatment recommendations, and shaping international guidelines. Given the rapid expansion of clinical studies in asthma, COPD, and other airway diseases, AI-driven methods can enhance both the efficiency and reliability of evidence synthesis, substantially reducing the human resources and costs traditionally required. This will enable faster and more cost-effective translation of research into clinical practice.

5.2.2 Long-term plans

In the longer term, the mechanistic understanding and clinical decision-making aspects are two key questions to address in studying chronic respiratory diseases. On the mechanistic side, cutting-edge technologies such as single-cell RNA sequencing, spatial transcriptomics, and T-cell receptor (TCR) profiling are being developed to elucidate the molecular and cellular processes underlying disease development and progression (Aldridge & Teichmann, 2020; Williams et al., 2022). In addition, longitudinal multi-omics integration, network-based systems biology, and AI–driven modelling will be used to identify key regulatory pathways, predictive biomarkers, and therapeutic targets, advancing precision medicine. The integrated evidence from three complementary levels: clinical data (patient- and population-level evidence), biological experiments (molecular, cellular, and animal-level evidence), and bioinformatics analyses (multi-omics level evidence) will provide a comprehensive understanding of the mechanisms of chronic respiratory diseases.

On the clinical decision-making side, large-scale datasets such as the UK Biobank and the Clinical Practice Research Datalink (CPRD) require more extensive exploration. While most studies using these resources have focused on cancer or cardiovascular disease, respiratory medicine remains underrepresented. Leveraging these datasets could advance understanding of disease prevention, progression, treatment patterns, and comorbidities, as well as environmental and socioeconomic influences. The rise of AI and multimodal large language models (MLLMs) now enables integration of diverse clinical data, including EHRs, imaging, omics, and patient-reported outcomes, into a comprehensive view of patient health (Liu et al., 2025). These tools can improve diagnosis, prognosis, treatment selection, and risk stratification, while also informing clinical trial design, drug discovery, and digital health interventions. These advances could transform respiratory medicine by enabling more proactive, precise, and equitable care.

Chronic respiratory medicine, positioned at the intersection of specialised clinical practice and biomedical research, reflects the complexity of modern healthcare. These diseases impose a substantial global burden, yet effective prevention and treatment remain limited. Their progression is further shaped by biological, environmental, and social factors, demanding multidisciplinary, patient-centred approaches. Advances in data science enable the integration of clinical records, imaging, omics, and real-world evidence at an unprecedented scale and depth. Combining these strands can offer a deeper understanding of disease mechanisms and identify potential therapeutic targets. This aspiration is not merely to generate knowledge, but to transform it into tangible improvements in patient outcomes, reduce disease burden, and promote healthier lives for future generations.

Appendix

Table S2.1 All differentially expressed genes between patients with asthma and healthy controls in bronchial biopsies and brushings.

All differentially expressed genes between patients with asthma and healthy controls without receiving ICS; patients with asthma and healthy controls receiving ICS. Differential expression analysis was performed using the *DESeq2* package.

Bronchial biopsies transcriptome							
Patients with asthma v Healthy controls without ICS				Patients with asthma v Healthy controls with ICS			
Number of upregulated genes			70	Number of upregulated genes			54
Number of downregulated genes			416	Number of downregulated genes			4
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**	**Gene**	**Log2 fold change**	***P* value**	**FDR *P* value**
CYP1A1	4.79	1.50E-09	4.45E-08	SYNCRIP	12.11	4.21E-21	7.32E-17
CTAG2	3.83	1.45E-02	4.40E-02	PDE8B	8.79	5.80E-20	6.68E-16
MTCO1P7	3.65	2.75E-04	1.62E-03	POLR2J4	8.52	2.09E-19	1.45E-15
CTAG1A	3.44	1.65E-02	4.89E-02	SFTA3	8.03	2.49E-21	7.32E-17
SLCO1B7	3.39	2.96E-08	6.14E-07	PINX1	7.45	7.68E-20	6.68E-16
SPRR3	3.28	4.56E-07	6.62E-06	CST1	4.78	1.12E-06	3.79E-05
SPRR1A	3.17	1.12E-06	1.44E-05	CST2	4.16	1.14E-06	3.84E-05
GHRHR	3.09	2.78E-07	4.30E-06	HERC3	4.13	1.86E-18	1.08E-14
MUC7	3.01	6.60E-07	9.11E-06	CPA4	3.98	8.82E-08	5.90E-06
NKX2-6	2.97	7.48E-04	3.78E-03	CST4	3.60	1.41E-05	2.46E-04
CLCA1	2.94	2.93E-04	1.71E-03	B3GNT6	3.56	5.59E-12	4.05E-09
CCL26	2.89	1.12E-07	1.95E-06	CT75	3.56	4.00E-16	1.99E-12
PRSS41	2.83	8.34E-06	8.08E-05	SPRR3	3.47	1.68E-04	1.60E-03
PPDPFL	2.82	5.97E-06	6.08E-05	CEACAM5	3.35	1.50E-12	1.69E-09
SPRR2D	2.81	1.46E-05	1.32E-04	CLCA1	3.08	3.06E-04	2.55E-03
KLK8	2.77	5.45E-03	1.98E-02	CLDN2	3.05	1.56E-14	4.18E-11
SLCO1B3	2.68	5.87E-17	1.34E-14	FETUB	3.01	4.77E-05	6.18E-04
LINC02303	2.65	8.59E-06	8.29E-05	SFTPA2	2.82	1.10E-03	6.86E-03
MTCO1P58	2.64	1.66E-04	1.05E-03	PTPRH	2.74	2.24E-10	7.23E-08
ODAM	2.62	2.81E-05	2.32E-04	MGAM2	2.72	1.76E-07	9.64E-06
FLVCR2-AS1	2.61	4.48E-07	6.52E-06	EPHA5	2.69	4.79E-09	6.98E-07
RPL32P9	2.61	1.53E-02	4.60E-02	LOC105375826	2.68	1.29E-07	7.76E-06
C8orf74	2.59	1.25E-05	1.15E-04	LOC401589	2.67	1.65E-03	9.27E-03
SLCO1B3-SLCO1B7	2.55	1.30E-03	6.03E-03	SLCO1A2	2.66	4.42E-06	1.06E-04
SPRR2A	2.55	3.51E-05	2.81E-04	DMBT1	2.64	9.54E-06	1.85E-04
SPRR2E	2.54	8.06E-05	5.68E-04	TFF1	2.54	5.28E-11	2.39E-08
CPA4	2.54	3.73E-05	2.96E-04	CD1A	2.49	7.19E-11	3.02E-08
SPRR2B	2.52	1.17E-04	7.81E-04	ITPRID1	2.49	4.98E-07	2.08E-05
CSN3	2.51	6.72E-04	3.45E-03	CACNA1E	2.47	2.56E-12	2.47E-09
RPL6P8	2.50	2.69E-04	1.59E-03	BPIFB6	2.43	7.63E-07	2.86E-05
ECEL1P1	2.48	5.57E-04	2.94E-03	SCEL	2.43	1.54E-06	4.81E-05
OTX2	2.47	9.78E-04	4.72E-03	TCN1	2.40	4.45E-09	6.59E-07
CD200LP	2.46	7.49E-04	3.78E-03	CGREF1	2.37	1.23E-08	1.37E-06
PIP	2.42	1.51E-06	1.87E-05	C6orf58	2.37	5.85E-03	2.46E-02
PABPC1L2A	2.41	4.84E-06	5.08E-05	LOC105372984	2.33	1.80E-13	3.30E-10
HTR2C	2.41	5.80E-03	2.08E-02	NXPE2	2.33	1.48E-06	4.68E-05
CAPN14	2.40	4.94E-08	9.66E-07	SFTPA1	2.33	2.66E-03	1.33E-02
TCN1	2.40	3.53E-13	2.90E-11	SPRR1A	2.28	6.31E-03	2.60E-02
SLCO1B1	2.39	9.74E-05	6.69E-04	ACAN	2.28	9.50E-06	1.85E-04
LINC02474	2.38	6.90E-04	3.53E-03	LYZ	2.26	8.31E-05	9.43E-04
EGR4	2.37	2.65E-03	1.09E-02	FAM111B	2.24	3.41E-08	2.95E-06
EREG	2.33	1.09E-06	1.41E-05	SPRR1B	2.19	1.01E-02	3.71E-02
MT1B	2.31	9.70E-03	3.17E-02	NPY5R	2.17	4.02E-06	9.80E-05

Bronchial biopsies transcriptome

Patients with asthma v Healthy controls without ICS			
Number of upregulated genes			70
Number of downregulated genes			416
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
AZGP1	2.30	2.51E-08	5.34E-07
RNU6-554P	2.28	5.84E-04	3.06E-03
MANCR	2.27	5.80E-04	3.04E-03
BPIFB2	2.27	1.79E-08	3.96E-07
PRICKLE2-AS2	2.25	6.83E-04	3.50E-03
KRT24	2.23	1.61E-03	7.19E-03
RNU6-944P	2.22	2.46E-03	1.02E-02
SPRR2G	2.21	2.20E-03	9.30E-03
CRISP3	2.20	3.55E-05	2.84E-04
IL36G	2.18	8.58E-04	4.24E-03
CACNG1	2.18	5.66E-05	4.21E-04
C5orf46	2.18	1.70E-03	7.53E-03
SPRR2F	2.17	1.23E-03	5.72E-03
PABPC1L2B	2.14	4.29E-05	3.32E-04
BRD7P6	2.13	3.07E-03	1.23E-02
MIR449C	2.12	1.17E-03	5.48E-03
EEF1DP5	2.10	6.24E-04	3.24E-03
NANOGP7	2.09	2.08E-11	1.01E-09
DLX2-DT	2.08	5.35E-03	1.96E-02
RPL35AP28	2.07	5.16E-06	5.36E-05
LMOD2	2.07	8.32E-04	4.13E-03
RN7SL803P	2.07	1.37E-03	6.30E-03
CNIH3-AS1	2.06	3.07E-03	1.23E-02
PHACTR3	2.06	5.90E-16	1.01E-13
PRB4	2.05	2.19E-03	9.29E-03
MUC6	2.02	5.62E-10	1.87E-08
NOVA1-DT	2.00	1.49E-03	6.76E-03
CD248	-2.00	2.15E-22	2.55E-19
TRAJ27	-2.00	9.37E-03	3.08E-02
MIR497HG	-2.00	6.09E-09	1.51E-07
FREM1	-2.01	3.97E-17	9.82E-15
IGHV1-67	-2.01	7.28E-06	7.20E-05
TRAV34	-2.01	6.78E-03	2.37E-02
ROBO2	-2.01	7.82E-21	5.89E-18
IGHV3-43	-2.01	1.59E-06	1.96E-05
TRAV16	-2.01	1.28E-06	1.62E-05
MIR4537	-2.01	2.44E-04	1.46E-03
TRAV26-2	-2.01	2.57E-03	1.06E-02
SLCO5A1-AS1	-2.02	5.66E-06	5.81E-05
C1QTNF2	-2.02	3.15E-16	5.79E-14
CD40LG	-2.02	1.43E-15	2.16E-13
CD22	-2.02	7.69E-12	4.19E-10
GPR25	-2.03	2.25E-08	4.88E-07
MMP2	-2.03	5.39E-19	2.25E-16
NKX6-1	-2.03	9.21E-13	6.79E-11
GOLGA5P1	-2.03	2.63E-04	1.56E-03
IGKV1OR-3	-2.03	3.74E-11	1.70E-09
CLEC4C	-2.04	2.20E-03	9.31E-03
HAPLN1	-2.04	1.70E-09	4.95E-08
IGHV1-69-2	-2.04	1.40E-05	1.27E-04
SLC1A7	-2.04	1.02E-13	1.00E-11
PNOC	-2.05	5.10E-13	4.00E-11
PENK	-2.05	5.09E-10	1.70E-08
IGHV3-54	-2.05	2.48E-04	1.48E-03
SEMA5B	-2.06	5.43E-16	9.42E-14
RPS16P2	-2.06	1.22E-04	8.13E-04
TNR	-2.06	7.81E-06	7.63E-05
GRID1-AS1	-2.06	3.35E-03	1.32E-02
BLK	-2.06	5.80E-15	7.86E-13
TRBV21-1	-2.07	3.08E-04	1.78E-03

Patients with asthma v Healthy controls with ICS			
Number of upregulated genes			54
Number of downregulated genes			4
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
SLC26A4	2.16	2.22E-03	1.17E-02
IL1RL1	2.15	5.06E-06	1.16E-04
POSTN	2.15	2.95E-08	2.61E-06
MMP12	2.12	3.39E-04	2.75E-03
PRR4	2.12	8.82E-04	5.77E-03
NANOGP7	2.10	2.32E-11	1.26E-08
RNU6-652P	2.06	5.44E-11	2.40E-08
HRH4	2.04	1.42E-08	1.53E-06
ODAM	2.03	6.00E-03	2.51E-02
DEPDC1P2	2.00	1.61E-07	9.11E-06
ASPM	2.00	3.85E-07	1.75E-05
HTRA3	-2.09	7.34E-08	5.20E-06
RBP4	-2.14	1.07E-06	3.69E-05
HIF3A	-2.15	3.12E-08	2.74E-06
SCGB1A1	-2.25	5.46E-09	7.54E-07

Bronchial biopsies transcriptome

Patients with asthma v Healthy controls without ICS			
Number of upregulated genes			70
Number of downregulated genes			416
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
FCRL3	-2.07	7.92E-09	1.91E-07
IGHV3-20	-2.07	4.05E-06	4.38E-05
CALB2	-2.07	6.78E-09	1.67E-07
KIAA1755	-2.08	4.29E-17	1.04E-14
ECMXP	-2.08	3.41E-04	1.94E-03
PI16	-2.08	3.73E-12	2.24E-10
HBG2	-2.08	5.28E-03	1.93E-02
SYNDIG1	-2.08	1.16E-11	6.01E-10
IGKV6D-41	-2.09	2.69E-03	1.10E-02
NRXN2	-2.10	6.03E-16	1.03E-13
OLFML1	-2.10	2.29E-20	1.43E-17
OGN	-2.10	1.64E-10	6.33E-09
GJD2	-2.10	7.52E-04	3.79E-03
COX4I2	-2.10	8.72E-13	6.47E-11
CILP2	-2.10	1.09E-10	4.41E-09
GLIS1	-2.10	2.81E-14	3.21E-12
IGHV1-18	-2.10	5.70E-06	5.84E-05
FAM162B	-2.10	1.59E-16	3.18E-14
LINC01827	-2.10	9.53E-04	4.62E-03
VSTM4	-2.11	1.43E-18	5.14E-16
FBLN1	-2.11	3.48E-25	7.68E-22
TRBV15	-2.11	4.67E-05	3.57E-04
TRAJ18	-2.11	6.02E-03	2.15E-02
C1QTNF7	-2.11	3.85E-12	2.31E-10
MRGPRX2	-2.12	3.35E-03	1.32E-02
HCG22	-2.12	1.20E-04	7.99E-04
TRBV7-4	-2.13	1.93E-05	1.67E-04
LINC00968	-2.13	4.02E-13	3.26E-11
SCTR-AS1	-2.14	4.31E-04	2.37E-03
IGHV3OR16-12	-2.14	2.27E-05	1.93E-04
IGLV3-30	-2.14	1.21E-03	5.65E-03
IGHV3OR16-15	-2.15	3.80E-07	5.63E-06
CORIN	-2.16	2.94E-20	1.75E-17
PYDC1	-2.16	1.48E-03	6.71E-03
IGHV1-68	-2.16	2.56E-04	1.52E-03
IGHV3OR16-10	-2.16	4.90E-07	7.05E-06
IGKV7-3	-2.17	1.66E-03	7.36E-03
IGHV1-69	-2.17	2.73E-06	3.12E-05
FCRL1	-2.17	3.21E-07	4.90E-06
LINC02812	-2.18	2.46E-05	2.06E-04
LINC02227	-2.18	4.38E-03	1.66E-02
IGHV1OR16-1	-2.18	1.12E-07	1.95E-06
SCARA5	-2.18	1.65E-14	2.00E-12
TRAJ10	-2.18	5.20E-04	2.77E-03
CALCR	-2.18	1.13E-08	2.63E-07
IGKV1OR22-1	-2.18	1.90E-05	1.65E-04
CD5L	-2.18	2.10E-03	8.98E-03
HMGCLL1	-2.19	1.57E-18	5.53E-16
LAMP5	-2.19	6.17E-14	6.29E-12
IGHV3-74	-2.19	1.54E-07	2.60E-06
IGHV1OR15-6	-2.19	4.44E-05	3.42E-04
IGKV1D-35	-2.19	3.20E-04	1.84E-03
GFRA1	-2.19	2.93E-19	1.31E-16
TNFRSF17	-2.20	3.36E-17	8.38E-15
CACNA1G	-2.20	6.50E-16	1.10E-13
IGLV2-34	-2.21	6.37E-08	1.20E-06
IGHA2	-2.21	5.39E-10	1.79E-08
BGN	-2.21	1.88E-16	3.76E-14
IGHV3OR16-11	-2.21	2.73E-07	4.25E-06
THY1	-2.22	8.72E-17	1.91E-14

Patients with asthma v Healthy controls with ICS			
Number of upregulated genes			54
Number of downregulated genes			4
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**

Bronchial biopsies transcriptome

Patients with asthma v Healthy controls without ICS			
Number of upregulated genes			70
Number of downregulated genes			416
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
IGLV3-27	-2.22	4.46E-06	4.75E-05
COL1A2	-2.22	1.30E-22	1.75E-19
COL6A6	-2.22	3.40E-14	3.83E-12
TCL1A	-2.22	8.80E-04	4.32E-03
IGLV1-41	-2.22	2.15E-09	6.08E-08
TRBV11-3	-2.23	3.45E-03	1.36E-02
SLCO1C1	-2.23	2.35E-13	2.05E-11
IGLV3-21	-2.24	8.27E-10	2.63E-08
LINC01857	-2.24	4.39E-09	1.15E-07
GNG8	-2.24	3.28E-07	4.99E-06
DRD5	-2.25	5.83E-12	3.29E-10
COL3A1	-2.26	3.21E-17	8.20E-15
IGLV3-32	-2.26	1.33E-04	8.76E-04
IGLL1	-2.26	1.14E-09	3.51E-08
FOXS1	-2.27	5.55E-15	7.55E-13
APLNR	-2.27	1.54E-15	2.30E-13
MIR4539	-2.27	3.81E-03	1.47E-02
IGHV1OR15-2	-2.28	5.92E-06	6.03E-05
LINC01989	-2.28	7.71E-03	2.63E-02
IGKV1OR2-1	-2.28	7.32E-11	3.08E-09
IGHV5-78	-2.28	2.43E-08	5.19E-07
TRAV8-4	-2.28	2.21E-13	1.94E-11
TRBV10-3	-2.28	2.38E-10	8.67E-09
IGLV2-14	-2.29	7.07E-09	1.73E-07
IGHV3-62	-2.30	1.14E-09	3.51E-08
LTB	-2.30	3.44E-13	2.85E-11
IGKV1OR9-1	-2.30	4.58E-10	1.55E-08
LILRA4	-2.31	7.12E-08	1.32E-06
TPBGL-AS1	-2.31	7.51E-05	5.34E-04
CSF2	-2.32	7.87E-05	5.56E-04
IGHV4-31	-2.32	1.39E-08	3.16E-07
IGHV3OR16-13	-2.32	1.00E-08	2.37E-07
IGHV3-63	-2.32	3.27E-06	3.66E-05
IGLV7-35	-2.32	2.94E-04	1.72E-03
OSTN	-2.33	2.58E-05	2.15E-04
GRID2	-2.33	1.70E-09	4.95E-08
CD79A	-2.34	1.30E-13	1.22E-11
IGLJ2	-2.34	3.63E-07	5.43E-06
IGHV1-14	-2.35	1.12E-09	3.47E-08
CRYBA4	-2.35	1.65E-05	1.46E-04
IGHV4-28	-2.35	1.05E-09	3.29E-08
IGLV2-18	-2.35	1.78E-09	5.14E-08
TRBV7-9	-2.36	4.84E-11	2.12E-09
TRBV7-3	-2.36	2.30E-09	6.48E-08
IGKV1-6	-2.36	9.84E-08	1.73E-06
LINC02104	-2.36	1.28E-09	3.85E-08
IGHV7-81	-2.37	3.38E-06	3.75E-05
IGHA1	-2.37	1.15E-12	8.18E-11
IGKV1D-43	-2.37	1.21E-06	1.54E-05
IGHV3-73	-2.37	2.26E-09	6.37E-08
IGKV1OR2-108	-2.38	6.90E-11	2.93E-09
IGKV1-5	-2.38	1.93E-08	4.25E-07
IGHV3-30	-2.39	5.63E-10	1.87E-08
IGHV1OR15-4	-2.39	2.69E-08	5.64E-07
IGKV6D-21	-2.39	4.66E-05	3.56E-04
IGDCC4	-2.39	4.74E-26	1.83E-22
TRAJ12	-2.39	2.49E-04	1.49E-03
IGHV3-75	-2.39	1.48E-06	1.84E-05
IGHV4-55	-2.40	6.17E-12	3.46E-10
KLK4	-2.40	6.01E-10	1.98E-08

Patients with asthma v Healthy controls with ICS			
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Number of downregulated genes			4
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Bronchial biopsies transcriptome

Patients with asthma v Healthy controls without ICS			
Number of upregulated genes			70
Number of downregulated genes			416
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
IGHV3-38	-2.40	4.87E-13	3.84E-11
RBP4	-2.41	6.78E-09	1.67E-07
CDX1	-2.41	1.36E-05	1.24E-04
SHANK1	-2.41	4.08E-13	3.29E-11
IGHV5-51	-2.41	3.95E-09	1.05E-07
DAZL	-2.42	1.65E-02	4.87E-02
IGHV1OR15-1	-2.42	2.15E-16	4.09E-14
TMEM119	-2.42	2.16E-28	1.66E-24
IGLC5	-2.42	1.71E-08	3.81E-07
IGHV3-66	-2.42	3.23E-10	1.13E-08
IGLV6-57	-2.44	2.45E-10	8.88E-09
IL2	-2.44	8.95E-08	1.60E-06
FOXF2-DT	-2.44	9.20E-10	2.90E-08
GATA3-AS1	-2.44	2.26E-03	9.52E-03
JCHAIN	-2.45	8.31E-15	1.08E-12
TRAV8-2	-2.45	5.74E-20	3.28E-17
ATP1B2	-2.45	2.64E-20	1.60E-17
IGKV1OR1-1	-2.45	3.50E-05	2.80E-04
IGHV3-7	-2.46	2.63E-09	7.32E-08
IGHV4-34	-2.46	1.53E-11	7.70E-10
XCR1	-2.46	1.77E-19	8.69E-17
DCSTAMP	-2.46	2.98E-05	2.44E-04
IGHJ5	-2.46	2.43E-10	8.85E-09
IGKV1OR10-1	-2.47	4.60E-08	9.06E-07
IGLV2-11	-2.47	5.40E-12	3.08E-10
IGKV1-27	-2.47	5.74E-09	1.44E-07
KLK2	-2.47	1.11E-08	2.60E-07
IGHV3-47	-2.47	4.74E-08	9.29E-07
IGKV1OR22-5	-2.47	3.32E-05	2.68E-04
PRND	-2.48	4.82E-11	2.12E-09
TMEM132C	-2.48	1.47E-16	3.05E-14
KLK5	-2.48	8.08E-05	5.69E-04
IGLC6	-2.48	2.65E-14	3.06E-12
MIR4538	-2.48	2.19E-03	9.28E-03
IGHV3-33	-2.49	2.35E-11	1.12E-09
IGLV1-51	-2.49	7.71E-13	5.78E-11
IGHV4-4	-2.50	2.96E-10	1.05E-08
IGHV2OR16-5	-2.50	5.27E-09	1.34E-07
LINC01724	-2.50	5.55E-03	2.01E-02
TRAV13-1	-2.51	1.84E-12	1.24E-10
IGLC2	-2.51	7.89E-15	1.04E-12
IGHV3-19	-2.51	2.08E-07	3.35E-06
LINC01502	-2.52	4.19E-05	3.26E-04
TDO2	-2.52	1.20E-13	1.14E-11
LINC01055	-2.52	3.36E-08	6.84E-07
IGHV3-53	-2.52	5.13E-11	2.23E-09
IGKV1-12	-2.52	1.36E-09	4.08E-08
HTRA3	-2.53	2.03E-20	1.31E-17
NMRK2	-2.53	2.79E-05	2.30E-04
IGKV1D-22	-2.53	1.96E-04	1.22E-03
IGKV1OR2-6	-2.53	9.13E-06	8.73E-05
IGHV4OR15-8	-2.53	4.20E-14	4.57E-12
IGLV3-13	-2.53	1.06E-04	7.20E-04
IGHV1-2	-2.54	1.78E-10	6.78E-09
IGKV1D-12	-2.54	6.72E-10	2.19E-08
IGHV1-45	-2.54	2.95E-07	4.53E-06
IGLV1-40	-2.55	2.94E-12	1.84E-10
IGKV1D-27	-2.55	6.81E-10	2.22E-08
IGLC4	-2.55	1.95E-03	8.41E-03
IGHV3OR16-8	-2.55	1.24E-11	6.32E-10

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Bronchial biopsies transcriptome

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Number of downregulated genes			416
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
DPT	-2.56	2.03E-19	9.79E-17
IGHV3-21	-2.57	1.79E-10	6.79E-09
BHLHE22-AS1	-2.58	8.56E-09	2.04E-07
IGKV1D-37	-2.58	1.27E-10	5.03E-09
FCRL5	-2.58	9.51E-16	1.50E-13
IGHV4-61	-2.58	1.51E-14	1.85E-12
FAM30A	-2.59	9.68E-16	1.52E-13
IGLV1-50	-2.59	1.53E-14	1.87E-12
IGHV7-27	-2.59	1.20E-05	1.11E-04
IGKV1-17	-2.59	1.80E-10	6.84E-09
IGKV1-8	-2.60	3.99E-12	2.36E-10
IGHV4-59	-2.60	7.28E-15	9.65E-13
IGHV1-17	-2.60	4.03E-05	3.16E-04
TMEM244	-2.60	2.37E-03	9.91E-03
IGLC3	-2.60	3.47E-15	4.85E-13
FCER2	-2.60	3.23E-10	1.13E-08
IGLV5-48	-2.61	2.35E-05	1.98E-04
IGLV9-49	-2.61	6.93E-05	4.99E-04
LCNL1	-2.61	5.80E-18	1.76E-15
IGKV1D-17	-2.61	7.87E-11	3.29E-09
IGKV1-9	-2.61	1.42E-12	9.88E-11
IGHV1OR15-3	-2.61	1.09E-15	1.68E-13
IGKJ4	-2.62	4.18E-10	1.43E-08
PLPPR4	-2.62	5.90E-42	1.82E-37
SFRP4	-2.62	1.37E-19	7.06E-17
IGHJ4	-2.62	1.62E-12	1.10E-10
IGHV1-46	-2.62	2.20E-12	1.45E-10
IGHJ3P	-2.63	3.19E-06	3.57E-05
IGHV3OR16-17	-2.63	3.33E-13	2.77E-11
IGKV3D-7	-2.63	1.61E-11	8.04E-10
IGKV3OR2-268	-2.64	1.20E-11	6.16E-10
IGHV3-42	-2.64	1.94E-06	2.32E-05
IGLV4-69	-2.64	2.65E-10	9.52E-09
IGKV6-21	-2.64	3.07E-06	3.46E-05
IGHV3-72	-2.64	2.63E-10	9.47E-09
IGHV1-12	-2.64	1.70E-09	4.95E-08
IGHV7-4-1	-2.64	2.35E-08	5.06E-07
IGKV1D-8	-2.64	5.26E-12	3.01E-10
LINC01394	-2.65	4.64E-05	3.55E-04
IGLV5-45	-2.65	1.38E-07	2.35E-06
APOC1	-2.65	1.70E-17	4.74E-15
TNN	-2.66	1.72E-10	6.60E-09
IGHV3-69-1	-2.66	5.03E-13	3.96E-11
IGHV3-25	-2.67	7.34E-07	9.98E-06
IGHV3-11	-2.67	1.54E-11	7.73E-10
IGKV3-7	-2.67	1.30E-12	9.10E-11
IGHV3-60	-2.67	3.85E-10	1.32E-08
TRBV24-1	-2.67	1.71E-07	2.84E-06
BHLHE22	-2.68	2.54E-24	4.62E-21
IGKV1D-42	-2.68	5.40E-08	1.05E-06
IGHV2-5	-2.68	4.72E-11	2.07E-09
AMPD1	-2.68	5.74E-26	1.97E-22
IGLV3-9	-2.69	1.19E-08	2.77E-07
IGHV3-48	-2.69	1.12E-12	8.04E-11
IGLV2-8	-2.70	1.27E-12	8.96E-11
IGKV1-37	-2.70	9.20E-13	6.79E-11
IQSEC3-AS2	-2.70	2.47E-06	2.86E-05
TMEM215	-2.71	6.01E-17	1.37E-14
IGKV1D-13	-2.72	1.10E-11	5.77E-10
IGKV3-15	-2.72	5.27E-12	3.01E-10

Patients with asthma v Healthy controls with ICS			
Number of upregulated genes			54
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Gene	**Log2 fold change**	***P* value**	**FDR *P* value**

Bronchial biopsies transcriptome

Patients with asthma v Healthy controls without ICS			
Number of upregulated genes			70
Number of downregulated genes			416
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
IGKV3-20	-2.72	3.53E-12	2.15E-10
IGHV3-71	-2.72	2.34E-14	2.75E-12
IGLV2-28	-2.72	2.02E-11	9.85E-10
IGHD3-9	-2.73	9.64E-03	3.15E-02
COPDA1	-2.73	4.72E-06	4.98E-05
IGHV7-34-1	-2.74	7.74E-05	5.48E-04
IGKV1-39	-2.74	4.65E-12	2.71E-10
IGKV3D-20	-2.74	1.11E-12	8.02E-11
IGHV3-41	-2.74	3.70E-12	2.23E-10
IGHV3-52	-2.75	2.14E-10	7.94E-09
TRBV6-5	-2.75	3.33E-15	4.67E-13
IGHV1OR16-4	-2.76	9.88E-09	2.34E-07
IGHV3OR15-7	-2.76	8.47E-12	4.57E-10
IGKV1D-39	-2.76	3.35E-12	2.06E-10
IGKV3D-15	-2.76	1.15E-12	8.18E-11
IGLV3-17	-2.76	6.54E-03	2.30E-02
IGHV1OR16-3	-2.76	4.58E-12	2.68E-10
IGKV2D-30	-2.76	1.33E-08	3.05E-07
CR2	-2.76	4.83E-09	1.24E-07
IGHV3OR16-9	-2.77	2.65E-13	2.28E-11
FCRLA	-2.77	7.91E-14	7.88E-12
IGKV3D-11	-2.78	1.80E-12	1.22E-10
LRRC15	-2.78	5.48E-17	1.26E-14
IGKV2-30	-2.78	1.35E-08	3.09E-07
IGKV1OR2-3	-2.79	1.00E-08	2.37E-07
SLITRK1	-2.79	5.87E-04	3.07E-03
IGKV4-1	-2.79	6.38E-12	3.55E-10
IGKV1-13	-2.80	2.27E-12	1.48E-10
IGKV1D-16	-2.80	9.65E-12	5.13E-10
IGHJ3	-2.80	4.76E-12	2.76E-10
TRBV11-2	-2.80	1.78E-09	5.14E-08
SERPINA9	-2.81	1.89E-05	1.65E-04
IGLV3-16	-2.81	5.10E-17	1.20E-14
PTGDS	-2.81	1.02E-25	3.14E-22
FSTL5	-2.81	2.49E-06	2.88E-05
IGKJ1	-2.82	2.33E-11	1.11E-09
IGKV2OR2-1	-2.83	2.73E-11	1.27E-09
IGHV3-64	-2.83	1.65E-13	1.49E-11
IGKV1-22	-2.83	8.62E-06	8.31E-05
IGHV1OR16-2	-2.83	8.99E-08	1.60E-06
IGHV2-26	-2.83	8.48E-08	1.53E-06
IGHJ6	-2.85	1.99E-16	3.94E-14
IGKV5-2	-2.85	6.99E-04	3.56E-03
IGHJ2	-2.85	1.13E-12	8.11E-11
IGKV3-11	-2.85	1.24E-14	1.56E-12
IGLV3-19	-2.85	6.09E-10	2.00E-08
CHRNA4	-2.86	4.42E-14	4.79E-12
PAX5	-2.86	1.64E-13	1.49E-11
MARCO	-2.86	2.02E-08	4.43E-07
IGLL5	-2.87	1.35E-13	1.26E-11
LINC00582	-2.87	3.39E-12	2.08E-10
IGKV2-14	-2.88	4.09E-05	3.20E-04
IGLC7	-2.88	2.07E-14	2.47E-12
IGLV5-37	-2.89	2.18E-06	2.57E-05
IGKV2D-14	-2.89	1.07E-04	7.23E-04
IGHV1OR21-1	-2.89	1.55E-13	1.42E-11
IGHV1-58	-2.90	6.52E-12	3.62E-10
IGHV4-39	-2.90	3.00E-17	7.73E-15
IGKV1OR2-11	-2.91	8.89E-11	3.67E-09
IGHV4-80	-2.92	2.19E-13	1.93E-11

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Gene	**Log2 fold change**	***P* value**	**FDR *P* value**

Bronchial biopsies transcriptome

Patients with asthma v Healthy controls without ICS			
Number of upregulated genes			70
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Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
IGLV10-54	-2.93	2.33E-06	2.72E-05
IGLV3-6	-2.94	3.17E-03	1.27E-02
IGHV3OR16-16	-2.94	3.11E-12	1.94E-10
IGKC	-2.94	2.61E-17	6.94E-15
LRRTM4	-2.95	5.51E-22	5.87E-19
IGKV1-16	-2.96	4.47E-12	2.63E-10
IGKV2OR22-3	-2.96	1.09E-08	2.56E-07
IGKV1OR2-9	-2.96	2.31E-10	8.47E-09
ARHGAP36	-2.98	7.93E-11	3.31E-09
IGHV3-23	-2.98	2.51E-18	8.15E-16
IGLV3-29	-2.98	8.29E-04	4.11E-03
IGHV3-64D	-3.00	1.90E-13	1.70E-11
IGKJ5	-3.00	8.61E-12	4.64E-10
IGHV6-1	-3.00	2.54E-12	1.63E-10
IGHV1-3	-3.03	7.61E-16	1.25E-13
IGLV3-25	-3.03	6.57E-23	9.67E-20
IGKJ3	-3.05	4.08E-13	3.29E-11
IGHJ1	-3.05	1.09E-12	7.92E-11
IGHV3-49	-3.06	2.99E-11	1.38E-09
IGKV3D-31	-3.07	3.71E-03	1.44E-02
IGHV3-13	-3.07	2.99E-17	7.73E-15
IGHV3-16	-3.08	2.82E-18	8.97E-16
IGHV3-22	-3.09	1.35E-18	5.01E-16
IGKV2-24	-3.09	2.30E-12	1.50E-10
IGKV2D-40	-3.09	2.10E-12	1.39E-10
MS4A1	-3.09	3.90E-15	5.36E-13
CCL19	-3.10	7.38E-18	2.21E-15
IGHV3-65	-3.10	1.17E-18	4.42E-16
IGKV2-40	-3.11	2.62E-12	1.67E-10
IGKV3-31	-3.12	5.28E-03	1.93E-02
IGHG4	-3.12	1.01E-14	1.28E-12
FAM30B	-3.13	5.08E-10	1.70E-08
IGHV5-10-1	-3.13	2.12E-14	2.52E-12
IGLV3-22	-3.14	5.19E-06	5.39E-05
NR1H4	-3.14	1.46E-09	4.34E-08
IGKV2D-26	-3.14	1.76E-08	3.91E-07
IGHV3-35	-3.15	5.65E-19	2.28E-16
IGHV3-15	-3.15	1.25E-16	2.62E-14
FAM30C	-3.17	3.44E-10	1.20E-08
IGKV2-36	-3.18	1.49E-04	9.63E-04
IGKV1D-32	-3.20	2.54E-05	2.12E-04
IGHV3OR16-6	-3.20	8.44E-16	1.37E-13
IGLV2-5	-3.21	1.21E-15	1.84E-13
FCRL4	-3.21	2.80E-09	7.72E-08
LINC03110	-3.24	1.98E-07	3.20E-06
IGHV7-56	-3.27	9.07E-08	1.61E-06
IGKV1D-33	-3.28	1.61E-20	1.10E-17
IGKV1-33	-3.31	6.82E-21	5.40E-18
CBLN2	-3.32	1.62E-07	2.71E-06
IGLV7-46	-3.34	1.21E-19	6.47E-17
IGKV2D-24	-3.34	1.19E-13	1.13E-11
TACR3	-3.36	1.93E-09	5.52E-08
IGHV3OR16-7	-3.36	2.88E-16	5.39E-14
IGHG2	-3.36	8.12E-18	2.41E-15
IGKV2-26	-3.37	1.11E-11	5.80E-10
IGKV2D-18	-3.38	1.42E-09	4.23E-08
IGHGP	-3.39	4.53E-17	1.09E-14
IGHG3	-3.40	3.37E-16	6.16E-14
IGKV1-32	-3.41	3.15E-08	6.46E-07
IGLV4-60	-3.42	1.11E-10	4.48E-09

Patients with asthma v Healthy controls with ICS			
Number of upregulated genes			54
Number of downregulated genes			4
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**

Bronchial biopsies transcriptome

Patients with asthma v Healthy controls without ICS			
Number of upregulated genes			70
Number of downregulated genes			416
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
IGLV2-33	-3.42	2.87E-12	1.80E-10
TNFRSF13B	-3.42	1.00E-24	1.93E-21
IGKV2-29	-3.42	7.65E-11	3.21E-09
IGHG1	-3.44	2.25E-18	7.56E-16
IGHV2-70	-3.46	1.25E-19	6.56E-17
IGLV3-10	-3.47	4.28E-28	2.65E-24
IGKV1OR2-118	-3.51	2.35E-11	1.12E-09
WNT2	-3.52	3.02E-11	1.40E-09
IGHV2-70D	-3.52	2.30E-19	1.08E-16
IGLV7-43	-3.52	2.32E-18	7.70E-16
IGKV2OR22-4	-3.54	6.95E-12	3.83E-10
IGKV2-18	-3.57	1.59E-12	1.09E-10
IGKV2D-29	-3.61	1.88E-14	2.25E-12
IGKV2D-36	-3.64	5.45E-07	7.71E-06
IGHV7-40	-3.75	3.55E-05	2.84E-04
PLA2G2D	-3.75	1.02E-13	1.00E-11
LINC02154	-3.76	3.50E-08	7.09E-07
IGKV2-28	-3.77	7.55E-22	7.48E-19
IGKV2D-28	-3.77	7.04E-22	7.26E-19
IGKV2-4	-4.17	3.56E-14	3.97E-12
KERA	-4.24	2.32E-10	8.50E-09
IGLV11-55	-4.44	4.92E-07	7.07E-06
AGTR2	-4.69	5.33E-19	2.25E-16

Patients with asthma v Healthy controls with ICS			
Number of upregulated genes			54
Number of downregulated genes			4
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**

Table S2.2 All differentially expressed genes between patients with asthma and healthy controls in bronchial brushings.

All differentially expressed genes between patients asthma and healthy controls without receiving ICS; patients with asthma and healthy controls receiving ICS. Differential expression analysis was performed using the *DESeq2* package.

Bronchial brushings transcriptome							
Patients with asthma v Healthy controls without ICS				Patients with asthma v Healthy controls with ICS			
Number of upregulated genes			100	Number of upregulated genes			46
Number of downregulated genes			24	Number of downregulated genes			26
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**	**Gene**	**Log2 fold change**	***P* value**	**FDR *P* value**
HBA1	5.64	7.70E-16	1.09E-11	SYNCRIP	11.78	1.58E-15	1.32E-11
HBA2	5.63	8.40E-16	1.09E-11	PDE8B	9.36	9.51E-15	3.25E-11
PPBP	5.11	2.81E-05	4.98E-04	POLR2J4	7.82	5.03E-16	1.15E-11
PRSS33	4.54	4.45E-06	1.25E-04	PINX1	7.66	2.58E-15	1.72E-11
CLCA1	4.52	3.10E-06	9.62E-05	SFTA3	7.61	1.00E-14	3.25E-11
FETUB	4.45	1.28E-08	1.94E-06	CST1	4.88	1.04E-04	3.66E-03
CST1	4.44	6.29E-07	3.11E-05	CST4	4.67	4.01E-05	1.81E-03
LDHC	4.44	1.19E-08	1.86E-06	CLCA1	4.00	6.55E-04	1.38E-02
HBD	4.40	6.37E-10	2.24E-07	CST2	3.99	2.23E-04	6.42E-03
CST2	4.36	5.34E-07	2.80E-05	FETUB	3.65	1.10E-05	6.44E-04
CST4	4.25	7.71E-07	3.62E-05	HBA2	3.61	2.73E-04	7.42E-03
ALAS2	4.20	4.46E-08	4.53E-06	HBA1	3.61	2.78E-04	7.55E-03
PRB1	4.14	9.83E-07	4.27E-05	HERC3	3.60	1.55E-13	2.40E-10
MYL4	4.11	2.07E-07	1.41E-05	CT75	3.48	4.64E-11	2.98E-08
DES	4.09	1.72E-04	1.96E-03	B3GNT6	3.13	3.32E-06	2.41E-04
VSTM1	3.96	3.07E-05	5.34E-04	LOC105375826	2.73	1.54E-05	8.36E-04
TCF21	3.93	5.58E-04	4.77E-03	CEACAM5	2.64	3.64E-08	6.84E-06
HBB	3.92	1.86E-10	9.29E-08	CD1A	2.62	8.61E-07	8.31E-05
CTSG	3.83	3.02E-05	5.27E-04	GATA2	2.57	1.65E-06	1.43E-04
HBG1	3.74	4.27E-04	3.89E-03	IGHA2	2.57	9.04E-04	1.72E-02
RD3	3.69	2.05E-10	9.87E-08	SIGLEC6	2.53	1.54E-05	8.36E-04
DEFA1B	3.65	5.56E-06	1.47E-04	C22orf42	2.53	8.38E-06	5.09E-04
DEFA1	3.61	6.90E-06	1.74E-04	CPA3	2.52	1.08E-04	3.75E-03
PRB2	3.53	1.31E-05	2.83E-04	IGHA1	2.48	2.35E-03	3.32E-02
LINC02829	3.42	1.93E-03	1.21E-02	MS4A2	2.41	1.23E-04	4.16E-03
DEFA3	3.41	5.15E-05	7.87E-04	GCSAML	2.34	3.68E-05	1.69E-03
CYP1A1	3.39	4.92E-03	2.45E-02	CPA4	2.31	4.04E-04	1.00E-02
SLC4A1	3.38	1.11E-06	4.58E-05	PTGDR2	2.30	8.86E-08	1.38E-05
MTCO1P7	3.37	6.61E-03	3.05E-02	TPSD1	2.29	6.28E-05	2.55E-03
CPA4	3.27	8.26E-08	7.20E-06	COL3A1	2.27	6.32E-07	6.47E-05
KLK7	3.09	2.72E-05	4.86E-04	CD1E	2.26	4.22E-08	7.59E-06
GHRHR	3.08	1.56E-03	1.03E-02	ZNF683	2.26	3.70E-05	1.70E-03
CCL7	3.02	8.72E-03	3.76E-02	IL1RL1	2.26	4.02E-04	9.98E-03
CLC	3.00	2.36E-03	1.41E-02	CD207	2.25	4.02E-07	4.54E-05
FAM245A	2.92	1.71E-04	1.94E-03	TPSB2	2.24	2.66E-04	7.28E-03
LGALS12	2.91	3.18E-05	5.48E-04	TESPA1	2.24	8.82E-08	1.38E-05
FOSB	2.89	1.83E-11	1.64E-08	MUC3A	2.22	1.45E-04	4.69E-03
HTR3C	2.81	1.17E-02	4.67E-02	TPSAB1	2.20	3.98E-04	9.91E-03
FCN1	2.81	2.36E-08	2.91E-06	TAL1	2.18	1.28E-05	7.18E-04
HS6ST3	2.81	4.45E-04	4.02E-03	ZNF831	2.12	4.16E-06	2.84E-04
IGHV3-49	2.79	6.23E-03	2.92E-02	CD1B	2.11	4.05E-06	2.78E-04
SLCO1B3	2.78	9.83E-14	3.20E-10	AHSG	2.10	7.55E-05	2.87E-03
CT83	2.73	1.01E-04	1.32E-03	NTRK2	2.09	2.16E-06	1.72E-04
SLC32A1	2.69	1.56E-06	5.84E-05	CDK15	2.04	4.29E-04	1.04E-02
FFAR3	2.68	2.60E-04	2.66E-03	IGKV1-9	2.02	1.64E-03	2.58E-02
MS4A3	2.66	5.61E-03	2.70E-02	TPRXL	2.00	6.35E-08	1.05E-05
PNMT	2.64	3.04E-07	1.88E-05	GRP	-2.00	1.73E-05	9.27E-04
VN1R21P	2.64	1.89E-04	2.11E-03	BPIFA1	-2.01	2.25E-03	3.22E-02
KRT24	2.63	7.77E-05	1.08E-03	CHGA	-2.03	1.84E-05	9.69E-04

Bronchial brushings transcriptome

Patients with asthma v Healthy controls without ICS

Number of upregulated genes	100
Number of downregulated genes	24

Gene	Log2 fold change	*P* value	FDR *P* value
AHSG	2.59	1.68E-03	1.09E-02
SNTG2	2.59	2.34E-12	5.07E-09
CHIT1	2.58	5.87E-05	8.75E-04
CCK	2.58	1.56E-04	1.82E-03
MTND5P32	2.57	1.26E-02	4.94E-02
HOXB13	2.57	1.12E-03	8.06E-03
LINC00160	2.55	1.03E-04	1.34E-03
LINC01398	2.55	2.09E-03	1.28E-02
GAL	2.53	8.32E-03	3.62E-02
MIR6822	2.52	6.75E-03	3.10E-02
RNASE3	2.50	5.47E-03	2.65E-02
NNMT	2.49	8.67E-08	7.43E-06
DAND5	2.45	1.09E-03	7.91E-03
LY6L	2.45	3.55E-06	1.06E-04
ALOX15B	2.44	7.95E-11	4.92E-08
SLC27A6	2.41	2.61E-05	4.71E-04
CCL13	2.38	4.11E-03	2.14E-02
ERVFRD-1	2.36	5.65E-04	4.82E-03
LINC02664	2.35	4.09E-03	2.14E-02
PRB4	2.33	1.76E-03	1.13E-02
IL1RL1	2.31	7.24E-06	1.80E-04
MMP19	2.31	3.37E-05	5.72E-04
LINC02474	2.29	8.10E-03	3.55E-02
LINC02600	2.27	4.92E-03	2.45E-02
CCT4P2	2.27	1.09E-02	4.44E-02
LINC02207	2.27	7.88E-06	1.92E-04
DIPK2B	2.26	6.83E-03	3.12E-02
SCUBE1-AS2	2.24	1.18E-04	1.47E-03
MUC6	2.23	4.65E-07	2.54E-05
MS4A10	2.23	1.47E-03	9.90E-03
NT5CP2	2.23	1.05E-02	4.33E-02
PHACTR3	2.21	1.60E-08	2.24E-06
MEG9	2.21	6.46E-03	3.00E-02
PRB3	2.21	1.76E-04	1.99E-03
KRT72	2.20	4.96E-03	2.46E-02
SLC9A9-AS2	2.18	3.68E-03	1.97E-02
PCDH10	2.17	5.33E-03	2.60E-02
BRINP1	2.12	2.85E-04	2.86E-03
BCAN-AS1	2.09	1.81E-05	3.57E-04
PDE2A	2.07	1.13E-05	2.53E-04
RNA5SP466	2.05	6.18E-03	2.90E-02
OLIG1	2.05	2.81E-03	1.61E-02
ANGPTL4	2.03	1.06E-07	8.57E-06
SLC5A5	2.01	6.92E-05	9.92E-04
RNASE2	2.01	3.73E-04	3.50E-03
SNTG2-AS1	2.01	5.88E-11	4.14E-08
FLNC	2.01	4.43E-08	4.53E-06
MLXP1	2.00	1.11E-02	4.51E-02
PTGDR2	2.00	7.76E-07	3.63E-05
B3GNT6	2.00	4.13E-05	6.67E-04
FOSL1	2.00	5.26E-06	1.42E-04
HAS2-AS1	-2.01	4.77E-03	2.39E-02
LINC02406	-2.10	1.22E-02	4.82E-02
TRBV6-1	-2.11	2.95E-05	5.17E-04
CR2	-2.14	1.39E-03	9.46E-03
INSRR	-2.15	1.34E-05	2.88E-04
STMN2	-2.16	4.65E-03	2.35E-02
TRAV2	-2.19	9.71E-03	4.07E-02
GPM6A	-2.23	2.35E-05	4.36E-04

Patients with asthma v Healthy controls with ICS

Number of upregulated genes	46
Number of downregulated genes	26

Gene	Log2 fold change	*P* value	FDR *P* value
TERB2	-2.03	1.73E-06	1.49E-04
CD163	-2.06	1.49E-05	8.14E-04
LOC105370185	-2.08	2.02E-07	2.63E-05
LPL	-2.09	1.18E-03	2.06E-02
IGKV2OR2-10	-2.12	8.51E-07	8.27E-05
IGKV2OR2-8	-2.17	2.25E-07	2.85E-05
KCNQ3	-2.17	8.10E-05	3.03E-03
NELL1	-2.22	2.53E-05	1.26E-03
SNAP91	-2.23	2.56E-08	5.34E-06
PHACTR3	-2.24	3.56E-06	2.54E-04
SCGB3A1	-2.26	1.10E-05	6.44E-04
DDC	-2.31	3.87E-06	2.69E-04
APOC1	-2.31	9.24E-05	3.35E-03
INSRR	-2.31	8.47E-09	2.38E-06
LINC03007	-2.32	5.50E-05	2.30E-03
SCGN	-2.39	8.37E-07	8.18E-05
SERPINA3	-2.41	2.43E-07	3.01E-05
IGKV2OR2-2	-2.46	2.10E-10	1.06E-07
APELA	-2.53	4.63E-05	2.01E-03
FABP4	-2.83	1.57E-04	4.95E-03
HIF3A	-2.84	5.38E-08	9.30E-06
GP2	-2.99	4.50E-09	1.49E-06
GABRG1	-3.02	5.58E-05	2.31E-03

Bronchial brushings transcriptome

Patients with asthma v Healthy controls without ICS			
Number of upregulated genes			100
Number of downregulated genes			24
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
OR51E1	-2.24	1.99E-09	5.12E-07
HTR1E	-2.24	6.37E-04	5.27E-03
FCRL5	-2.26	1.81E-04	2.03E-03
LRRC55	-2.28	3.13E-04	3.08E-03
LINC01616	-2.30	2.50E-07	1.61E-05
PAGE4	-2.30	3.93E-05	6.43E-04
TRAV26-2	-2.37	6.53E-03	3.03E-02
NRSN1	-2.45	4.59E-05	7.23E-04
SCARNA5	-2.47	1.19E-03	8.39E-03
ST8SIA3	-2.50	3.57E-04	3.39E-03
MS4A1	-2.65	2.97E-09	6.79E-07
TNFRSF13B	-2.73	1.35E-03	9.22E-03
FCRLA	-2.74	4.51E-05	7.11E-04
WNT2	-3.01	4.07E-05	6.61E-04
TRAV35	-3.42	6.28E-04	5.21E-03
GABRG1	-4.07	1.65E-06	6.12E-05

Patients with asthma v Healthy controls with ICS			
Number of upregulated genes			46
Number of downregulated genes			26
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**

Table S2.3 All differentially expressed genes across T2 phenotypes in bronchial biopsies.

All differentially expressed genes between T2-high asthma and T2-low asthma; between T2-high asthma and T2-intermediate asthma. Differential expression analysis was performed using the *DESeq2* package.

Bronchial biopsies transcriptome

T2-high asthma v T2-low asthma			
Number of upregulated genes			115
Number of downregulated genes			38
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
CST1	10.89	1.03E-17	2.62E-13
CST4	10.33	2.79E-16	3.54E-12
CST2	9.78	2.03E-14	1.72E-10
CLC	8.26	2.74E-08	1.74E-04
CLCA1	6.77	3.90E-06	4.50E-03
PRB2	6.41	1.07E-06	2.72E-03
PRB1	6.34	1.67E-06	3.44E-03
CEBPE	5.79	2.26E-06	3.44E-03
FETUB	5.63	4.08E-07	1.29E-03
CTSG	5.39	2.35E-04	4.21E-02
KRT1	5.12	5.56E-05	1.62E-02
CCL26	4.85	6.97E-08	2.95E-04
ITLN1	4.57	1.97E-07	7.16E-04
HBD	4.54	1.81E-05	8.12E-03
LGALS12	4.32	2.73E-05	1.05E-02
HBA1	4.21	7.37E-05	1.91E-02
HBA2	4.21	7.54E-05	1.93E-02
HBB	4.03	1.19E-05	7.59E-03
SLC4A1	3.99	1.26E-04	2.84E-02
ITLN2	3.55	4.81E-08	2.44E-04
C1QTNF1	3.03	8.88E-05	2.23E-02
CCR3	3.01	2.49E-05	9.89E-03
SERPINB2	3.01	4.39E-05	1.40E-02
SIRLNT	2.99	9.06E-05	2.23E-02
POSTN	2.94	3.64E-06	4.40E-03
TAL1	2.85	1.75E-05	8.12E-03
SIGLEC6	2.76	7.32E-05	1.91E-02
LINC02207	2.73	1.46E-04	3.11E-02
ZNF385D	2.66	1.31E-04	2.89E-02
CEACAM21	2.56	1.60E-04	3.37E-02
NTRK2	2.44	4.08E-06	4.50E-03
ORM1	2.42	1.97E-05	8.12E-03
ZMAT4	2.24	1.17E-04	2.71E-02
ANGPTL4	2.23	3.74E-05	1.25E-02
GATA2	2.19	2.94E-04	4.89E-02
SERPINB10	2.09	5.37E-05	1.59E-02
ITGA2B	2.00	2.49E-04	4.37E-02
CD36	1.97	4.26E-05	1.37E-02
MT-TT	1.88	2.98E-05	1.10E-02
LINC01127	1.84	4.79E-05	1.47E-02
MTND4LP1	1.80	1.19E-04	2.71E-02
KRT4	1.75	2.03E-04	3.82E-02
TNNI3	1.75	1.85E-05	8.12E-03
MTCYBP35	1.74	1.40E-05	7.59E-03
CAMK2B	1.72	9.10E-05	2.23E-02
MT-TQ	1.69	4.57E-05	1.42E-02
GATA1	1.69	2.16E-04	3.95E-02
SLC24A3	1.67	2.68E-05	1.05E-02
ROR1	1.67	1.33E-04	2.89E-02

T2-high asthma v T2-intermediate asthma			
Number of upregulated genes			17
Number of downregulated genes			8
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
FDCSP	5.52	7.91E-08	1.10E-03
GPM6A	4.30	3.48E-07	3.23E-03
OLIG2	3.73	4.26E-05	4.74E-02
PGC	3.10	4.57E-06	2.12E-02
ITLN1	3.05	3.82E-06	2.12E-02
ITLN2	2.76	9.60E-09	2.67E-04
OLIG1	2.71	4.11E-05	4.74E-02
CCR3	2.40	5.70E-06	2.27E-02
COL4A3	2.09	3.25E-05	4.74E-02
CLDN18	2.03	3.87E-05	4.74E-02
MUCL3	2.02	2.68E-05	4.74E-02
KRT13	1.84	2.41E-06	1.68E-02
LINC01127	1.39	3.05E-05	4.74E-02
RTKN2	1.13	1.23E-05	4.10E-02
MYO15A	0.94	1.32E-05	4.10E-02
MTATP8P2	0.88	4.00E-05	4.74E-02
MT-TY	0.87	3.91E-05	4.74E-02
BBOX1	-0.63	2.63E-05	4.74E-02
RPS3AP15	-0.73	4.20E-05	4.74E-02
CHST13	-0.98	3.33E-05	4.74E-02
GBP1	-1.46	1.47E-05	4.10E-02
C4B	-1.50	2.78E-05	4.74E-02
C4A	-1.50	2.39E-05	4.74E-02
IFIT3	-1.84	2.16E-05	4.74E-02
BPIFA1	-2.44	1.99E-05	4.74E-02

Bronchial biopsies transcriptome

T2-high asthma v T2-low asthma			
Number of upregulated genes			115
Number of downregulated genes			38
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**
LINC00896	1.65	6.14E-06	5.46E-03
MT-TE	1.65	1.34E-05	7.59E-03
MTCO2P2	1.59	1.63E-06	3.44E-03
TEX29	1.59	2.81E-05	1.07E-02
DOK1	1.57	2.73E-04	4.65E-02
MTND6P3	1.56	1.69E-05	8.12E-03
MT-TI	1.55	8.65E-05	2.20E-02
MTND6P22	1.54	1.15E-05	7.59E-03
MTCO2P12	1.53	1.87E-06	3.44E-03
SPEG	1.50	1.30E-04	2.89E-02
MTND1P23	1.47	1.51E-05	7.82E-03
MTND6P35	1.47	3.57E-05	1.23E-02
MT-ND3	1.46	3.23E-06	4.10E-03
MT-CO2	1.44	2.37E-06	3.44E-03
MTND6P4	1.43	1.01E-05	7.30E-03
MT-TS1	1.43	2.20E-04	3.97E-02
TNNC1	1.42	1.13E-04	2.64E-02
MT-RNR1	1.40	5.14E-06	5.08E-03
MT-ND6	1.39	1.16E-05	7.59E-03
MT-TK	1.38	1.01E-06	2.72E-03
MT-RNR2	1.37	2.37E-06	3.44E-03
MTCO1P40	1.35	1.38E-05	7.59E-03
ITGA10	1.35	4.58E-05	1.42E-02
MT-CYB	1.35	4.89E-06	5.08E-03
MTND4LP30	1.34	1.39E-05	7.59E-03
SLC9B2	1.34	6.79E-05	1.85E-02
MTCO1P2	1.33	6.91E-05	1.87E-02
MT-TH	1.32	6.73E-05	1.85E-02
MT-ND1	1.32	1.31E-05	7.59E-03
MT-ND4	1.30	3.02E-06	4.04E-03
MT-TY	1.28	5.95E-06	5.46E-03
MT-ATP6	1.27	6.23E-06	5.46E-03
MT-TC	1.25	1.26E-05	7.59E-03
OR2A1-AS1	1.24	1.86E-04	3.60E-02
MT-CO3	1.24	8.23E-06	6.74E-03
MT-TL1	1.24	9.12E-05	2.23E-02
MTATP6P1	1.22	1.10E-05	7.59E-03
CDH26	1.22	9.83E-06	7.30E-03
MT-ND2	1.22	5.20E-06	5.08E-03
PROCR	1.20	1.13E-04	2.64E-02
MTCO1P12	1.19	1.37E-05	7.59E-03
MTATP8P2	1.19	3.07E-05	1.10E-02
MTATP6P2	1.18	3.04E-05	1.10E-02
MTCO3P12	1.17	6.63E-06	5.61E-03
MT-TN	1.16	1.39E-04	3.00E-02
HDC	1.16	6.97E-05	1.87E-02
KYAT1	1.15	1.72E-04	3.44E-02
MT-CO1	1.14	1.90E-05	8.12E-03
MT-ND5	1.14	1.49E-05	7.82E-03
MTND2P28	1.13	9.47E-06	7.29E-03
SNORD3A	1.12	1.70E-04	3.44E-02
MTND5P11	1.12	1.96E-05	8.12E-03
SNORD3B-2	1.11	1.66E-04	3.44E-02
MT-ND4L	1.11	1.98E-05	8.12E-03
MTCO1P22	1.11	7.31E-05	1.91E-02
CD44	1.11	3.30E-05	1.15E-02
MT-ATP8	1.04	1.92E-05	8.12E-03
ARHGEF35-AS1	1.00	2.07E-04	3.86E-02
MTATP8P1	0.98	9.42E-05	2.26E-02

T2-high asthma v T2-intermediate asthma			
Number of upregulated genes			17
Number of downregulated genes			8
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**

Bronchial biopsies transcriptome							
T2-high asthma v T2-low asthma				T2-high asthma v T2-intermediate asthma			
Number of upregulated genes			115	Number of upregulated genes			17
Number of downregulated genes			38	Number of downregulated genes			8
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**	**Gene**	**Log2 fold change**	***P* value**	**FDR *P* value**
POLR2J3.1	0.77	2.15E-04	3.95E-02				
C9orf40	0.74	1.89E-04	3.63E-02				
SNHG3	0.69	2.69E-04	4.61E-02				
FAM133DP	0.67	1.33E-04	2.89E-02				
C1QTNF6	0.66	2.54E-04	4.42E-02				
WDR91	0.59	1.18E-04	2.71E-02				
ZNF436-AS1	0.54	1.68E-04	3.44E-02				
KAZN	-0.35	2.65E-04	4.58E-02				
AGAP1	-0.42	1.47E-04	3.11E-02				
ESRRAP2	-0.43	1.06E-04	2.52E-02				
PIK3IP1	-0.47	4.06E-05	1.32E-02				
CYB561A3	-0.54	2.86E-04	4.79E-02				
BTC	-0.59	3.66E-05	1.24E-02				
LXN	-0.61	6.57E-05	1.83E-02				
RFX5	-0.70	1.80E-05	8.12E-03				
FBLN5	-0.76	2.03E-04	3.82E-02				
ACE2	-0.87	5.79E-05	1.67E-02				
B3GALT2	-0.94	1.70E-04	3.44E-02				
RPS3AP15	-1.01	1.38E-05	7.59E-03				
TDRD1	-1.13	1.86E-04	3.60E-02				
TRIM55	-1.14	9.34E-05	2.26E-02				
M1AP	-1.20	1.80E-04	3.54E-02				
COSMOC	-1.24	6.06E-05	1.73E-02				
NCOA7	-1.33	8.57E-06	6.80E-03				
NRCAM	-1.34	3.00E-05	1.10E-02				
SLC34A2	-1.38	2.43E-06	3.44E-03				
WSCD1	-1.42	2.84E-04	4.79E-02				
WARS1	-1.45	2.48E-04	4.37E-02				
SCG2	-1.50	3.82E-05	1.26E-02				
LAG3	-1.53	2.39E-04	4.25E-02				
CIITA	-1.56	2.03E-05	8.20E-03				
GBP1	-1.63	2.87E-04	4.79E-02				
CALCA	-1.78	1.63E-05	8.12E-03				
SH3TC2	-1.95	4.90E-05	1.48E-02				
C4A	-2.03	1.74E-05	8.12E-03				
C4B	-2.03	1.81E-05	8.12E-03				
RND1	-2.39	2.08E-06	3.44E-03				
KLHDC7B	-2.40	5.11E-05	1.53E-02				
CSF3	-2.94	2.18E-04	3.96E-02				
KCNJ4	-2.96	3.30E-05	1.15E-02				
MOGAT2	-3.01	1.95E-04	3.73E-02				
CCL25	-3.44	2.16E-04	3.95E-02				
CXCL10	-3.48	1.75E-04	3.48E-02				
TRAV19	-3.54	1.71E-04	3.44E-02				
IDO1	-3.83	6.30E-05	1.78E-02				

Table S2.4 All differentially expressed genes across T2 phenotypes in bronchial brushings.

All differentially expressed genes between T2-high asthma and T2-low asthma; between T2-high asthma and T2-intermediate asthma. Differential expression analysis was performed using the *DESeq2* package.

Bronchial brushings transcriptome							
T2-high asthma v T2-low asthma				T2-high asthma v T2-intermediate asthma			
Number of upregulated genes			0	Number of upregulated genes			0
Number of downregulated genes			0	Number of downregulated genes			6
Gene	**Log2 fold change**	***P* value**	**FDR *P* value**	**Gene**	**Log2 fold change**	***P* value**	**FDR *P* value**
				ZNF385B	-0.85	7.94E-06	4.34E-02
				ENPEP	-1.30	1.50E-07	1.64E-03
				HLA-DQA1	-1.48	1.34E-06	8.82E-03
				MTND5P10	-1.79	3.97E-07	3.26E-03
				CXCL10	-2.99	2.57E-09	4.21E-05
				ARL14EPL	-5.25	1.10E-09	3.61E-05

Table S3.1 Taxonomic classification of bacterial species.

Item	Bacteria	Pathogenic bacterium	Group
Streptococcus pyogenes	Yes	Yes	Beta-haemolytic streptococci
Streptococcus agalactiae	Yes	Yes	Beta-haemolytic streptococci
Streptococcus constellatus	Yes	Yes	Beta-haemolytic streptococci
Coliforms	Yes	Yes	Escherichia coli
Escherichia coli	Yes	Yes	Escherichia coli
Enterobacter cloacae	Yes	Yes	Enterobacter cloacae
Haemophilus influenzae	Yes	Yes	Haemophilus influenzae
Klebsiella pneumoniae	Yes	Yes	Klebsiella species
Klebsiella species	Yes	Yes	Klebsiella species
Moraxella catarrhalis	Yes	Yes	Moraxella species
Moraxella species	Yes	Yes	Moraxella species
Mycobacterium tuberculosis	Yes	Yes	Mycobacterium tuberculosis
Mycobacterium	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium abscessus	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium avium	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium chelonae	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium chimaera	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium colombiense	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium fortuitum	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium gordonae	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium intracellulare	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium kansasii	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium mageritense	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium peregrinum	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium species	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium szulgai	Yes	Yes	Non-tuberculous mycobacteria
Mycobacterium xenopi	Yes	Yes	Non-tuberculous mycobacteria
Achromobacter ruhlandii	Yes	Yes	Other bacteria
Achromobacter species	Yes	Yes	Other bacteria
Actinobacillus suis	Yes	Yes	Other bacteria
Bordetella bronchiseptica	Yes	Yes	Other bacteria
Citrobacter species	Yes	Yes	Other bacteria
Diphtheroids	Yes	Yes	Other bacteria
Mycoplasma pneumoniae	Yes	Yes	Other bacteria
Pasteurella multocida	Yes	Yes	Other bacteria
Proteus mirabilis	Yes	Yes	Proteus species
Proteus species	Yes	Yes	Proteus species
Pseudomonas aeruginosa	Yes	Yes	Pseudomonas species
Pseudomonas fluorescens	Yes	Yes	Pseudomonas species
Pseudomonas libanensis	Yes	Yes	Pseudomonas species
Pseudomonas putida	Yes	Yes	Pseudomonas species
Pseudomonas species	Yes	Yes	Pseudomonas species
Serratia marcescens	Yes	Yes	Serratia marcescens
Staphylococcus aureus	Yes	Yes	Staphylococcus aureus
Stenotrophomonas maltophilia	Yes	Yes	Stenotrophomonas maltophilia
Streptococcus pneumoniae	Yes	Yes	Streptococcus pneumoniae

Table S3.2 Classification of antibiotics.

Antibiotic name	Antibiotic class
Amikacin	Aminoglycosides
Gentamicin	Aminoglycosides
Netilmicin	Aminoglycosides
Streptomycin	Aminoglycosides
Tobramycin	Aminoglycosides
Augmentin	Broad-spectrum penicillins
Co-Amoxiclav	Broad-spectrum penicillins
Piperacillin	Broad-spectrum penicillins
Piperacillin-tazobactam	Broad-spectrum penicillins
Pivmecillinam	Broad-spectrum penicillins
Temocillin	Broad-spectrum penicillins
Ertapenem	Carbapenems
Imipenem	Carbapenems
Meropenem	Carbapenems
Cefalexin	Cephalosporins
Cefazolin	Cephalosporins
Cefepime	Cephalosporins
Cefiderocol	Cephalosporins
Cefixime	Cephalosporins
Cefotaxime	Cephalosporins
Cefoxitin	Cephalosporins
Cefradine	Cephalosporins
Ceftazidime	Cephalosporins
Ceftazidime + Avibactam	Cephalosporins
Ceftolozane-tazobactam	Cephalosporins
Ceftriaxone	Cephalosporins
Cefuroxime	Cephalosporins
Chloramphenicol	Chloramphenicols
Azithromycin	Macrolides
Clarithromycin	Macrolides
Erythromycin	Macrolides
Quinupristin/dalfopristin (Synercid)	Macrolides
Aztreonam	Monobactams
Amoxicillin	Narrow-spectrum penicillins
Ampicillin	Narrow-spectrum penicillins
Benzylpenicillin	Narrow-spectrum penicillins
Flucloxacillin	Narrow-spectrum penicillins
Oxacillin	Narrow-spectrum penicillins
Ampicillin/Amoxicillin	Narrow-spectrum penicillins
Penicillin	Narrow-spectrum penicillins
Penicillin G	Narrow-spectrum penicillins
Phenoxymethylpenicillin	Other antibiotics
Clindamycin	Other antibiotics
Dalbavancin	Other antibiotics
Daptomycin	Other antibiotics
Ethambutol	Other antibiotics
Fidaxomicin	Other antibiotics
Fosfomycin	Other antibiotics
Isoniazid	Other antibiotics
Linezolid	Other antibiotics
Nitrofurantoin	Other antibiotics
Optochin	Diagnostic use only
Pyrazinamide	Other antibiotics
Rifampicin	Other antibiotics
Fusidic acid	Steroid antibiotics
Teicoplanin	Glycopeptides
Tigecycline	Tetracyclines
Colistin (colistimethate sodium)	Polypeptides
Colistin	Polypeptides
Fusidic Acid	Polypeptides
Mupirocin	Other antibiotics
Vancomycin	Glycopeptides
Ciprofloxacin	Quinolones
Delafloxacin	Quinolones

Antibiotic name	Antibiotic class
Levofloxacin	Quinolones
Moxifloxacin	Quinolones
Norfloxacin	Quinolones
Ofloxacin	Quinolones
Quinolone	Sulfonamides
Co-trimoxazole	Sulfonamides
Trimethoprim	Sulfonamides
Trimethoprim-sulfamethoxazole	Tetracyclines
Doxycycline	Tetracyclines
Lymecycline	Tetracyclines
Minocycline	Tetracyclines
Oxytetracycline	Tetracyclines
Tetracycline	Tetracyclines

Table S4.1 All differentially expressed genes between females and males among patients with asthma and healthy controls in bronchial biopsies transcriptomes.

Bronchial biopsies transcriptome

Female healthy controls (n=10) v Male healthy controls (n=16)			
Number of upregulated genes			7
Number of downregulated genes			13
Gene	**Fold change (log2)**	***P* value**	**FDR**
TSIX	6.10	6.89E-211	3.75E-207
XIST	6.03	8.92E-307	1.46E-302
HEMGN	2.05	1.82E-05	1.56E-02
KDM6A	0.63	6.56E-06	6.68E-03
EIF1AX	0.62	2.12E-09	3.15E-06
ARCN1	0.50	1.74E-05	1.56E-02
RPS4X	0.29	3.60E-09	4.90E-06
PRKY	-0.76	1.68E-06	1.83E-03
ARSE	-0.81	1.61E-05	1.54E-02
NCRNA00185	-0.89	2.07E-05	1.69E-02
UTY	-1.27	1.72E-20	2.80E-17
CYorf15A	-2.45	7.28E-26	1.32E-22
USP9Y	-2.58	5.10E-26	1.04E-22
CYorf15B	-2.66	9.09E-44	2.47E-40
DDX3Y	-2.82	5.97E-56	2.43E-52
KDM5D	-3.55	2.40E-38	5.60E-35
COL2A1	-3.73	5.44E-07	6.33E-04
EIF1AY	-4.00	1.24E-46	4.06E-43
CYTL1	-4.13	6.01E-08	7.55E-05
RPS4Y1	-4.75	7.12E-294	5.81E-290

Female mild/moderate asthma (n=16) v Male mild/moderate asthma (n=12)			
Number of upregulated genes			26
Number of downregulated genes			30
Gene	**Fold change (log2)**	***P* value**	**FDR**
XIST	6.16	2.57E-262	1.54E-258
TSIX	5.82	1.28E-238	5.11E-235
ODAM	2.77	1.46E-05	7.94E-03
SCGB3A2	2.60	1.66E-04	4.23E-02
C7	1.10	1.91E-04	4.35E-02
LOC644246	0.99	9.05E-05	3.19E-02
OMD	0.97	2.00E-04	4.35E-02
FIBIN	0.96	1.24E-04	3.71E-02
SCN7A	0.84	1.96E-04	4.35E-02
KDM6A	0.77	5.71E-10	5.26E-07
LOC100506517	0.76	1.29E-04	3.71E-02
COX7A1	0.68	5.57E-05	2.08E-02
EIF1AX	0.68	3.20E-10	3.30E-07
CYS1	0.65	1.02E-04	3.39E-02
ZFX	0.58	3.97E-05	1.70E-02
LOC100507303	0.54	7.57E-06	4.77E-03
LMCD1	0.52	1.33E-04	3.71E-02
RHOBTB3	0.51	2.22E-05	1.14E-02
KDM5C	0.49	1.38E-04	3.75E-02
ARSD	0.46	1.38E-06	9.19E-04
DDX3X	0.41	7.05E-11	8.44E-08
RPS4X	0.40	1.30E-17	1.73E-14
EIF2S3	0.39	9.51E-08	7.59E-05
CNBP	0.25	1.53E-04	4.07E-02
WAC	0.24	6.33E-05	2.30E-02
RPL34	0.20	3.57E-05	1.59E-02
ARPC2	-0.28	1.70E-04	4.23E-02
PLAGL2	-0.48	5.27E-05	2.04E-02
HLA-A	-0.50	1.85E-04	4.34E-02
HLA-DRB6	-0.56	1.43E-05	7.94E-03

Female severe asthma (n=23) v Male severe asthma (n=23)			
Number of upregulated genes			27
Number of downregulated genes			60
Gene	**Fold change (log2)**	***P* value**	**FDR**
TSIX	6.16	1.56E-171	1.27E-167
XIST	6.00	8.63E-185	1.41E-180
CYTL1	2.42	1.42E-08	1.36E-05
IGHG1	1.63	9.33E-06	5.96E-03
IGHD	1.47	4.53E-06	3.08E-03
COL3A1	0.92	2.17E-04	4.54E-02
IL7R	0.88	7.96E-05	2.70E-02
CCDC3	0.87	1.13E-04	3.20E-02
COL14A1	0.83	1.62E-04	4.06E-02
RASD2	0.79	2.29E-04	4.55E-02
TCEAL2	0.78	8.33E-05	2.70E-02
DPT	0.72	6.85E-05	2.60E-02
KDM5C	0.65	1.53E-09	1.78E-06
LOC100507345	0.60	2.39E-05	1.08E-02
COL5A1	0.60	1.60E-04	4.06E-02
GSTT2	0.59	2.25E-04	4.54E-02
COL1A1	0.57	1.31E-04	3.55E-02
KDM6A	0.57	3.35E-09	3.64E-06
NAP1L5	0.54	1.17E-04	3.23E-02
NCRNA00183	0.50	4.23E-07	3.83E-04
SEPT6	0.50	6.24E-05	2.42E-02
OLFML2B	0.49	1.06E-04	3.08E-02
MARVELD1	0.45	1.49E-05	7.84E-03
ZFX	0.37	1.92E-04	4.41E-02
COL16A1	0.36	1.06E-04	3.08E-02
DDX3X	0.32	1.39E-09	1.74E-06
RPS4X	0.29	1.18E-08	1.20E-05
CHP	-0.24	2.57E-04	4.88E-02
TOP1	-0.28	2.00E-04	4.52E-02
TMOD3	-0.31	8.45E-05	2.70E-02

Bronchial biopsies transcriptome

Female healthy controls (n=10) v Male healthy controls (n=16)			
Number of upregulated genes			7
Number of downregulated genes			13
Gene	**Fold change (log2)**	***P* value**	**FDR**

Female mild/moderate asthma (n=16) v Male mild/moderate asthma (n=12)			
Number of upregulated genes			26
Number of downregulated genes			30
Gene	**Fold change (log2)**	***P* value**	**FDR**
LYPD5	-0.58	1.96E-04	4.35E-02
CASP1	-0.64	2.45E-05	1.18E-02
HLA-DRB1	-0.67	1.09E-04	3.54E-02
TAP1	-0.67	2.25E-04	4.81E-02
PARP14	-0.70	2.66E-05	1.23E-02
RARRES3	-0.77	1.29E-04	3.71E-02
RTP4	-0.79	1.80E-04	4.34E-02
SLAMF8	-0.80	1.12E-04	3.54E-02
PRKY	-0.80	6.63E-10	5.67E-07
NLRC5	-0.82	1.68E-04	4.23E-02
IFI27	-0.97	4.74E-05	1.96E-02
C1QC	-1.01	5.08E-05	2.03E-02
PSMB9	-1.03	5.57E-07	3.92E-04
NCRNA00185	-1.05	3.31E-10	3.30E-07
ALDH3A1	-1.07	1.31E-04	3.71E-02
MMP10	-1.10	1.83E-04	4.34E-02
HLA-DQB1	-1.14	2.29E-05	1.14E-02
UTY	-1.17	3.07E-23	4.60E-20
LY6D	-1.21	1.34E-05	7.94E-03
CXCL10	-1.44	9.49E-05	3.25E-02
CXCL9	-2.19	4.22E-07	3.16E-04
DDX3Y	-2.52	1.40E-75	4.19E-72
CYorf15B	-2.66	1.20E-72	2.86E-69
KDM5D	-3.65	4.96E-42	8.49E-39
EIF1AY	-3.88	1.35E-56	2.69E-53
RPS4Y1	-5.09	0.00E+00	0.00E+00

Female severe asthma (n=23) v Male severe asthma (n=23)			
Number of upregulated genes			27
Number of downregulated genes			60
Gene	**Fold change (log2)**	***P* value**	**FDR**
ERLIN1	-0.34	7.88E-05	2.70E-02
LGALS3	-0.34	1.14E-04	3.20E-02
TWF1	-0.35	9.80E-06	5.96E-03
ANXA2P2	-0.35	1.46E-04	3.80E-02
BAZ1A	-0.37	2.17E-04	4.54E-02
CNOT6	-0.37	2.22E-04	4.54E-02
HEBP2	-0.38	7.88E-05	2.70E-02
C16orf61	-0.38	1.88E-04	4.41E-02
RBM47	-0.39	1.38E-04	3.68E-02
B4GALT2	-0.40	2.43E-04	4.72E-02
CSTB	-0.41	5.58E-05	2.22E-02
ENOPH1	-0.41	5.25E-05	2.14E-02
KRT8	-0.41	2.25E-04	4.54E-02
SNX7	-0.42	2.15E-04	4.54E-02
KRT18	-0.45	2.35E-06	1.74E-03
NME7	-0.46	7.76E-05	2.70E-02
HMGB3	-0.47	2.63E-04	4.93E-02
EPN3	-0.48	2.11E-04	4.54E-02
PVRL4	-0.49	2.25E-04	4.54E-02
MYO5B	-0.50	1.83E-05	9.06E-03
LRRC8E	-0.50	2.02E-04	4.52E-02
POF1B	-0.52	1.47E-04	3.80E-02
ST14	-0.54	4.96E-05	2.14E-02
ATP1B1	-0.55	1.61E-05	8.19E-03
TRIM16	-0.57	8.29E-05	2.70E-02
NUP37	-0.57	1.91E-04	4.41E-02
CLDN4	-0.57	1.85E-04	4.41E-02
AP1S3	-0.63	1.31E-05	7.14E-03
TTC9	-0.64	8.99E-05	2.77E-02
SERPINB1	-0.67	1.12E-05	6.50E-03
ECT2	-0.67	2.36E-04	4.63E-02
PHLDA2	-0.68	9.39E-05	2.83E-02
ARSE	-0.70	2.70E-06	1.91E-03
MAL2	-0.73	1.06E-06	9.14E-04

Bronchial biopsies transcriptome												
Female healthy controls (n=10) v Male healthy controls (n=16)				Female mild/moderate asthma (n=16) v Male mild/moderate asthma (n=12)				Female severe asthma (n=23) v Male severe asthma (n=23)			
Number of upregulated genes			7	Number of upregulated genes			26	Number of upregulated genes			27
Number of downregulated genes			13	Number of downregulated genes			30	Number of downregulated genes			60
Gene	**Fold change (log2)**	***P* value**	**FDR**	**Gene**	**Fold change (log2)**	***P* value**	**FDR**	**Gene**	**Fold change (log2)**	***P* value**	**FDR**
								TPRXL	-0.78	1.31E-05	7.14E-03
								PRKY	-0.82	8.96E-11	1.22E-07
								S100P	-0.87	5.10E-05	2.14E-02
								LRRC4	-0.94	9.86E-06	5.96E-03
								LCN2	-0.94	1.73E-04	4.27E-02
								CDC20B	-0.97	2.50E-04	4.79E-02
								ALDH3A1	-0.98	2.20E-06	1.71E-03
								NCRNA00185	-0.99	1.19E-13	1.77E-10
								UTY	-1.11	6.80E-28	1.11E-24
								CCNB1	-1.17	5.17E-05	2.14E-02
								C15orf48	-1.25	1.87E-04	4.41E-02
								ZWINT	-1.30	1.27E-06	1.03E-03
								CLCA4	-1.42	8.76E-05	2.75E-02
								SPRR3	-1.48	2.03E-05	9.76E-03
								KRT24	-1.57	8.27E-05	2.70E-02
								KRT4	-1.82	2.12E-05	9.89E-03
								CYorf15A	-1.88	2.09E-30	3.79E-27
								CYorf15B	-2.19	2.24E-49	6.09E-46
								USP9Y	-2.30	9.76E-35	1.99E-31
								DDX3Y	-2.37	1.49E-62	8.08E-59
								KDM5D	-3.02	3.63E-44	8.45E-41
								EIF1AY	-3.54	1.03E-58	4.18E-55
								RPS4Y1	-4.10	3.31E-57	1.08E-53

Table S4.2 All differentially expressed genes between females and males among patients with asthma and healthy controls in blood transcriptomes.

Blood transcriptome

Female healthy controls (n=34) v Male healthy controls (n=53)			
Number of upregulated genes			24
Number of downregulated genes			23
Gene	**Log2 fold change**	***P* value**	**FDR**
XIST	6.99	0.00E+00	0.00E+00
TSIX	6.10	0.00E+00	0.00E+00
CD177	1.49	1.52E-06	7.01E-04
DACT1	0.84	4.15E-06	1.80E-03
LOC441528	0.80	1.72E-18	1.41E-15
FAM133A	0.73	1.83E-05	6.91E-03
LOC389906	0.71	4.40E-25	4.05E-22
EIF1AX	0.67	1.00E-11	7.41E-09
KDM6A	0.55	1.06E-08	6.23E-06
HDHD1	0.53	1.14E-24	9.91E-22
BEND7	0.48	1.27E-04	3.54E-02
PRKX	0.47	5.08E-11	3.56E-08
NCRNA00183	0.47	1.25E-09	8.01E-07
ZFX	0.42	2.35E-07	1.15E-04
TXLNG	0.41	5.54E-08	3.03E-05
CACNA1E	0.41	7.26E-05	2.23E-02
ZRSR2	0.38	1.21E-10	8.12E-08
KDM5C	0.35	2.49E-12	1.94E-09
SYAP1	0.33	5.39E-05	1.81E-02
ARSD	0.32	1.89E-06	8.43E-04
INE1	0.30	1.74E-05	6.75E-03
CA5BP	0.28	8.47E-08	4.30E-05
DDX3X	0.28	2.19E-05	7.97E-03
PNPLA4	0.26	5.00E-05	1.71E-02
CALHM2	-0.29	2.22E-05	7.97E-03
TUBG2	-0.30	1.36E-04	3.71E-02
ZBED1	-0.30	2.61E-05	9.16E-03
FAM83H	-0.30	1.69E-05	6.74E-03
CX3CR1	-0.32	6.11E-05	2.00E-02
ASCL2	-0.33	1.16E-04	3.29E-02
PIK3R3	-0.35	1.07E-04	3.16E-02
PLEKHF1	-0.35	1.59E-05	6.52E-03
TMSB4Y	-0.35	1.40E-09	8.61E-07
PTGDS	-0.52	6.34E-05	2.03E-02
BCORP1	-1.71	4.10E-26	4.03E-23

Female mild/moderate asthma (n=37) v Male mild/moderate asthma (n=40)			
Number of upregulated genes			21
Number of downregulated genes			22
Gene	**Log2 fold change**	***P* value**	**FDR**
XIST	7.05	0.00E+00	0.00E+00
TSIX	5.92	0.00E+00	0.00E+00
MAP7D2	0.84	3.85E-13	4.10E-10
DACT1	0.84	9.35E-06	4.85E-03
LOC441528	0.67	2.49E-12	2.29E-09
LOC389906	0.61	8.70E-13	8.80E-10
EIF1AX	0.57	7.30E-10	5.09E-07
PRKX	0.54	3.18E-11	2.68E-08
HDHD1	0.48	1.37E-17	1.54E-14
ZRSR2	0.44	2.02E-12	1.94E-09
NCRNA00183	0.42	7.21E-08	4.42E-05
KDM6A	0.41	2.10E-06	1.12E-03
PNPLA4	0.40	1.98E-10	1.43E-07
CISH	0.39	1.28E-08	8.38E-06
KDM5C	0.39	2.21E-28	2.63E-25
ZFX	0.37	1.50E-08	9.51E-06
TXLNG	0.36	4.40E-09	2.97E-06
CA5BP	0.34	1.81E-10	1.36E-07
CA5B	0.31	1.02E-05	5.14E-03
ARSD	0.31	1.51E-06	8.43E-04
SEPT6	0.30	1.14E-10	8.88E-08
S100Z	-0.29	5.48E-05	2.47E-02
TMSB4Y	-0.30	1.54E-06	8.43E-04
MPO	-0.47	4.98E-05	2.34E-02
SASH1	-0.48	1.85E-05	8.92E-03
CTSG	-0.70	8.53E-05	3.75E-02
BPI	-0.82	1.05E-04	4.53E-02
LOC100506003	-0.86	5.22E-12	4.60E-09
CEACAM6	-0.92	6.14E-07	3.55E-04
TTTY15	-1.42	1.50E-35	2.03E-32
BCORP1	-1.60	9.98E-35	1.26E-31
PRKY	-2.15	3.67E-116	6.75E-113
TTTY10	-2.22	9.81E-95	1.53E-91
UTY	-2.26	1.06E-168	2.38E-165
ZFY	-2.55	5.26E-56	7.60E-53

Female severe asthma (n=182) v Male severe asthma (n=114)			
Number of upregulated genes			75
Number of downregulated genes			81
Gene	**Log2 fold change**	***P* value**	**FDR**
XIST	6.86	0.00E+00	0.00E+00
TSIX	5.71	0.00E+00	0.00E+00
DACT1	0.87	2.17E-11	8.08E-09
MAP7D2	0.80	1.87E-34	1.53E-31
LOC441528	0.66	1.96E-30	1.52E-27
EIF1AX	0.64	3.06E-23	1.92E-20
LOC389906	0.64	6.48E-42	5.57E-39
RSAD2	0.63	5.12E-05	4.66E-03
NLRP2	0.60	1.82E-11	7.05E-09
PRKX	0.53	1.63E-16	8.06E-14
KDM6A	0.50	1.73E-18	9.08E-16
LPAR4	0.50	3.56E-05	3.58E-03
ZFX	0.48	1.31E-24	9.29E-22
HDHD1	0.47	3.75E-48	3.39E-45
TXLNG	0.43	1.09E-20	6.33E-18
KDM5C	0.43	3.46E-83	3.32E-80
ZRSR2	0.40	1.69E-23	1.15E-20
FAM133A	0.39	9.69E-04	3.43E-02
ALAS2	0.38	9.71E-05	7.58E-03
OAS3	0.37	9.53E-04	3.40E-02
SLC14A1	0.37	2.07E-04	1.28E-02
NCRNA00183	0.36	2.01E-19	1.09E-16
CD274	0.36	2.59E-04	1.50E-02
SEPT6	0.35	3.11E-16	1.49E-13
IFI6	0.35	4.13E-04	2.05E-02
ICOS	0.35	2.50E-05	2.58E-03
NRN1	0.34	1.73E-03	4.96E-02
TDRD12	0.34	6.99E-06	9.19E-04
PNPLA4	0.34	7.40E-20	4.16E-17
GAL3ST4	0.34	2.76E-08	7.13E-06
NT5E	0.34	2.59E-06	4.10E-04
TMEM158	0.33	2.08E-04	1.28E-02
RGPD1	0.33	3.87E-04	1.96E-02
TRAJ17	0.33	1.48E-05	1.72E-03
SOCS2	0.33	4.06E-05	3.87E-03

Blood transcriptome

Female healthy controls (n=34) v Male healthy controls (n=53)

Number of upregulated genes	24
Number of downregulated genes	23

Gene	Log2 fold change	*P* value	FDR
PRKY	-2.01	6.69E-144	1.10E-140
UTY	-2.15	7.97E-131	1.07E-127
ZFY	-2.34	9.08E-40	9.57E-37
TTTY10	-2.41	8.10E-100	9.18E-97
NCRNA00185	-2.72	1.99E-127	2.45E-124
CYorf15A	-3.96	1.29E-143	1.90E-140
DDX3Y	-4.24	1.51E-245	3.19E-242
CYorf15B	-4.51	0.00E+00	0.00E+00
USP9Y	-5.17	4.54E-189	8.37E-186
EIF1AY	-5.33	0.00E+00	0.00E+00
KDM5D	-6.80	0.00E+00	0.00E+00
RPS4Y1	-7.41	0.00E+00	0.00E+00

Female mild/moderate asthma (n=37) v Male mild/moderate asthma (n=40)

Number of upregulated genes	21
Number of downregulated genes	22

Gene	Log2 fold change	*P* value	FDR
NCRNA00185	-2.62	2.94E-112	4.96E-109
CYorf15A	-3.90	1.72E-163	3.48E-160
DDX3Y	-4.16	3.10E-272	8.97E-269
CYorf15B	-4.46	0.00E+00	0.00E+00
USP9Y	-5.23	1.46E-258	3.70E-255
EIF1AY	-5.39	0.00E+00	0.00E+00
KDM5D	-6.72	0.00E+00	0.00E+00
RPS4Y1	-7.36	0.00E+00	0.00E+00

Female severe asthma (n=182) v Male severe asthma (n=114)

Number of upregulated genes	75
Number of downregulated genes	81

Gene	Log2 fold change	*P* value	FDR
GAGE1	0.32	1.25E-04	9.08E-03
LOC643648	0.32	2.22E-07	4.84E-05
CA5B	0.31	3.65E-11	1.32E-08
RGPD5	0.31	5.15E-04	2.34E-02
SPRYD5	0.31	5.04E-04	2.31E-02
NMT2	0.30	1.06E-06	1.91E-04
GPR171	0.30	7.46E-07	1.40E-04
GPR109B	0.30	1.27E-07	3.00E-05
INE1	0.30	3.29E-10	1.07E-07
ISM1	0.30	1.79E-04	1.18E-02
NELL2	0.30	7.76E-04	3.01E-02
LOC100288152	0.30	5.98E-04	2.54E-02
ITLN1	0.29	1.72E-03	4.96E-02
FOLR1	0.29	4.46E-04	2.15E-02
GPR174	0.29	3.43E-04	1.84E-02
PRSS16	0.29	1.22E-04	8.96E-03
MLLT3	0.28	2.23E-05	2.38E-03
CA5BP	0.28	1.73E-22	1.04E-19
LOC285972	0.28	3.92E-04	1.97E-02
ADAMTS17	0.27	2.07E-07	4.68E-05
AXIN2	0.27	1.05E-04	8.09E-03
LOC100289090	0.27	3.56E-04	1.86E-02
LOC100132741	0.27	2.12E-04	1.30E-02
GCET2	0.27	1.02E-05	1.22E-03
GPA33	0.27	3.54E-07	7.31E-05
KCNA3	0.27	1.83E-04	1.19E-02
LOC439949	0.27	3.99E-06	5.86E-04
RTKN2	0.27	5.11E-04	2.33E-02
CAMK4	0.26	4.53E-05	4.20E-03
LOC285965	0.26	1.29E-03	4.10E-02
BACH2	0.26	7.20E-04	2.89E-02
ANKRD36B	0.26	4.11E-05	3.90E-03
AP3M2	0.26	3.76E-06	5.63E-04
LOC100287616	0.26	9.59E-04	3.40E-02
IPW	0.26	1.16E-04	8.78E-03

Blood transcriptome

Female healthy controls (n=34) v Male healthy controls (n=53)			
Number of upregulated genes			24
Number of downregulated genes			23
Gene	**Log2 fold change**	***P* value**	**FDR**

Female mild/moderate asthma (n=37) v Male mild/moderate asthma (n=40)			
Number of upregulated genes			21
Number of downregulated genes			22
Gene	**Log2 fold change**	***P* value**	**FDR**

Female severe asthma (n=182) v Male severe asthma (n=114)			
Number of upregulated genes			75
Number of downregulated genes			81
Gene	**Log2 fold change**	***P* value**	**FDR**
FAM113B	0.25	5.14E-05	4.66E-03
C13orf15	0.25	1.72E-04	1.16E-02
EPHA4	0.25	3.66E-05	3.62E-03
LOC643008	0.25	1.13E-03	3.78E-02
BEX5	0.25	7.70E-04	3.01E-02
KIAA0101	-0.26	9.84E-05	7.64E-03
CSF2RA	-0.26	5.75E-07	1.14E-04
TP53I3	-0.26	6.82E-07	1.31E-04
TYMS	-0.26	2.65E-04	1.52E-02
CENPW	-0.26	5.30E-05	4.77E-03
STAB1	-0.27	1.92E-06	3.29E-04
UHRF1	-0.27	6.09E-04	2.57E-02
CLTCL1	-0.27	3.74E-05	3.68E-03
GGTA1	-0.28	1.20E-04	8.88E-03
RASSF4	-0.28	1.53E-09	4.81E-07
KIR2DL2	-0.28	3.48E-04	1.85E-02
KIF11	-0.28	1.71E-03	4.96E-02
FXYD6	-0.28	7.66E-07	1.40E-04
PTX3	-0.28	1.05E-03	3.59E-02
EPR1	-0.28	1.17E-03	3.82E-02
PFDN5	-0.29	4.67E-04	2.22E-02
MLC1	-0.29	3.36E-06	5.09E-04
EPB41L3	-0.29	7.62E-07	1.40E-04
NEIL3	-0.30	2.32E-04	1.38E-02
TACSTD2	-0.30	1.06E-04	8.15E-03
RAB13	-0.30	2.81E-06	4.37E-04
TMSB4Y	-0.31	2.58E-23	1.68E-20
PTGES	-0.31	9.03E-06	1.11E-03
CD24	-0.32	1.21E-03	3.92E-02
KIAA1598	-0.32	7.11E-05	5.95E-03
SCD	-0.32	1.84E-07	4.24E-05
TOP2A	-0.32	1.08E-05	1.29E-03
SLC22A16	-0.32	1.36E-04	9.71E-03
DPY19L1P1	-0.33	5.73E-05	5.02E-03
AKR1C3	-0.33	5.56E-05	4.97E-03

Blood transcriptome

Female healthy controls (n=34) v Male healthy controls (n=53)			
Number of upregulated genes			24
Number of downregulated genes			23
Gene	**Log2 fold change**	***P* value**	**FDR**

Female mild/moderate asthma (n=37) v Male mild/moderate asthma (n=40)			
Number of upregulated genes			21
Number of downregulated genes			22
Gene	**Log2 fold change**	***P* value**	**FDR**

Female severe asthma (n=182) v Male severe asthma (n=114)			
Number of upregulated genes			75
Number of downregulated genes			81
Gene	**Log2 fold change**	***P* value**	**FDR**
ANLN	-0.34	1.76E-06	3.08E-04
PRUNE2	-0.34	1.74E-04	1.16E-02
CLEC5A	-0.34	7.10E-05	5.95E-03
PCOLCE2	-0.35	2.63E-07	5.50E-05
CHIT1	-0.35	6.31E-05	5.45E-03
FAM19A2	-0.37	1.24E-04	9.04E-03
KIF18B	-0.37	1.85E-05	2.02E-03
RRM2	-0.38	4.76E-04	2.24E-02
SERPINB10	-0.38	5.47E-06	7.69E-04
CEP55	-0.38	6.72E-05	5.68E-03
SPON2	-0.41	7.15E-06	9.33E-04
ZNF788	-0.43	1.12E-08	3.19E-06
SLC28A3	-0.43	7.49E-08	1.85E-05
HIST1H3C	-0.45	6.80E-07	1.31E-04
TCN1	-0.48	1.38E-05	1.62E-03
RETN	-0.49	1.25E-07	3.00E-05
PTGDS	-0.50	3.75E-07	7.65E-05
INHBA	-0.51	1.25E-07	3.00E-05
RNASE3	-0.56	1.80E-04	1.18E-02
CAMP	-0.56	2.41E-07	5.10E-05
AZU1	-0.57	4.85E-11	1.72E-08
BEX1	-0.57	2.18E-11	8.08E-09
LTF	-0.77	1.92E-06	3.29E-04
MS4A3	-0.78	1.89E-08	5.13E-06
LCN2	-0.79	1.37E-07	3.19E-05
ABCA13	-0.81	1.55E-10	5.28E-08
CRISP3	-0.82	6.59E-07	1.29E-04
CEACAM8	-0.83	2.35E-07	5.04E-05
MMP8	-0.83	1.72E-06	3.05E-04
OLR1	-0.83	1.00E-10	3.47E-08
MPO	-0.93	4.44E-16	2.07E-13
BPI	-0.99	3.03E-10	1.01E-07
ELANE	-0.99	1.69E-14	7.32E-12
DEFA4	-1.04	2.39E-09	7.21E-07
OLFM4	-1.07	2.15E-07	4.79E-05

Blood transcriptome

Female healthy controls (n=34) v Male healthy controls (n=53)			
Number of upregulated genes			24
Number of downregulated genes			23
Gene	**Log2 fold change**	***P* value**	**FDR**

Female mild/moderate asthma (n=37) v Male mild/moderate asthma (n=40)			
Number of upregulated genes			21
Number of downregulated genes			22
Gene	**Log2 fold change**	***P* value**	**FDR**

Female severe asthma (n=182) v Male severe asthma (n=114)			
Number of upregulated genes			75
Number of downregulated genes			81
Gene	**Log2 fold change**	***P* value**	**FDR**
CEACAM6	-1.12	1.92E-12	7.84E-10
CTSG	-1.19	1.71E-14	7.32E-12
TTTY15	-1.26	1.80E-99	1.84E-96
BCORP1	-1.49	5.22E-122	5.68E-119
PRKY	-1.89	0.00E+00	0.00E+00
UTY	-2.16	0.00E+00	0.00E+00
TTTY10	-2.27	0.00E+00	0.00E+00
NCRNA00185	-2.31	0.00E+00	0.00E+00
ZFY	-2.52	3.68E-158	4.28E-155
CYorf15A	-3.74	0.00E+00	0.00E+00
CYorf15B	-4.14	0.00E+00	0.00E+00
DDX3Y	-4.15	0.00E+00	0.00E+00
USP9Y	-4.92	0.00E+00	0.00E+00
EIF1AY	-5.45	0.00E+00	0.00E+00
KDM5D	-6.46	0.00E+00	0.00E+00
RPS4Y1	-7.28	0.00E+00	0.00E+00

Table S4.3 All differentially expressed proteins between females and males among patients with asthma and healthy controls in blood proteomics.

Blood transcriptome											
Female healthy controls (n=34) v Male healthy controls (n=53)				Female mild/moderate asthma (n=37) v Male mild/moderate asthma (n=40)				Female severe asthma (n=182) v Male severe asthma (n=114)			
Number of upregulated genes			20	Number of upregulated genes			20	Number of upregulated genes			40
Number of downregulated genes			19	Number of downregulated genes			21	Number of downregulated genes			60
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
XIST	6.99	0.00E+00	0.00E+00	XIST	7.05	0.00E+00	0.00E+00	XIST	6.86	0.00E+00	0.00E+00
TSIX	6.10	0.00E+00	0.00E+00	TSIX	5.92	0.00E+00	0.00E+00	TSIX	5.71	0.00E+00	0.00E+00
CD177	1.49	1.52E-06	7.01E-04	MAP7D2	0.84	3.85E-13	4.10E-10	DACT1	0.87	2.17E-11	8.08E-09
DACT1	0.84	4.15E-06	1.80E-03	DACT1	0.84	9.35E-06	4.85E-03	MAP7D2	0.80	1.87E-34	1.53E-31
LOC441528	0.80	1.72E-18	1.41E-15	LOC441528	0.67	2.49E-12	2.29E-09	LOC441528	0.66	1.96E-30	1.52E-27
FAM133A	0.73	1.83E-05	6.91E-03	LOC389906	0.61	8.70E-13	8.80E-10	EIF1AX	0.64	3.06E-23	1.92E-20
LOC389906	0.71	4.40E-25	4.05E-22	EIF1AX	0.57	7.30E-10	5.09E-07	LOC389906	0.64	6.48E-42	5.57E-39
EIF1AX	0.67	1.00E-11	7.41E-09	PRKX	0.54	3.18E-11	2.68E-08	RSAD2	0.63	5.12E-05	4.66E-03
KDM6A	0.55	1.06E-08	6.23E-06	HDHD1	0.48	1.37E-17	1.54E-14	NLRP2	0.60	1.82E-11	7.05E-09
HDHD1	0.53	1.14E-24	9.91E-22	ZRSR2	0.44	2.02E-12	1.94E-09	PRKX	0.53	1.63E-16	8.06E-14
BEND7	0.48	1.27E-04	3.54E-02	NCRNA00183	0.42	7.21E-08	4.42E-05	KDM6A	0.50	1.73E-18	9.08E-16
PRKX	0.47	5.08E-11	3.56E-08	KDM6A	0.41	2.10E-06	1.12E-03	LPAR4	0.50	3.56E-05	3.58E-03
NCRNA00183	0.47	1.25E-09	8.01E-07	PNPLA4	0.40	1.98E-10	1.43E-07	ZFX	0.48	1.31E-24	9.29E-22
ZFX	0.42	2.35E-07	1.15E-04	CISH	0.39	1.28E-08	8.38E-06	HDHD1	0.47	3.75E-48	3.39E-45
TXLNG	0.41	5.54E-08	3.03E-05	KDM5C	0.39	2.21E-28	2.63E-25	TXLNG	0.43	1.09E-20	6.33E-18
CACNA1E	0.41	7.26E-05	2.23E-02	ZFX	0.37	1.50E-08	9.51E-06	KDM5C	0.43	3.46E-83	3.32E-80
ZRSR2	0.38	1.21E-10	8.12E-08	TXLNG	0.36	4.40E-09	2.97E-06	ZRSR2	0.40	1.69E-23	1.15E-20
KDM5C	0.35	2.49E-12	1.94E-09	CA5BP	0.34	1.81E-10	1.36E-07	FAM133A	0.39	9.69E-04	3.43E-02
SYAP1	0.33	5.39E-05	1.81E-02	CA5B	0.31	1.02E-05	5.14E-03	ALAS2	0.38	9.71E-05	7.58E-03
ARSD	0.32	1.89E-06	8.43E-04	ARSD	0.31	1.51E-06	8.43E-04	OAS3	0.37	9.53E-04	3.40E-02
CX3CR1	-0.32	6.11E-05	2.00E-02	TMSB4Y	-0.30	1.54E-06	8.43E-04	SLC14A1	0.37	2.07E-04	1.28E-02
ASCL2	-0.33	1.16E-04	3.29E-02	MPO	-0.47	4.98E-05	2.34E-02	NCRNA00183	0.36	2.01E-19	1.09E-16
PIK3R3	-0.35	1.07E-04	3.16E-02	SASH1	-0.48	1.85E-05	8.92E-03	CD274	0.36	2.59E-04	1.50E-02
PLEKHF1	-0.35	1.59E-05	6.52E-03	CTSG	-0.70	8.53E-05	3.75E-02	SEPT6	0.35	3.11E-16	1.49E-13
TMSB4Y	-0.35	1.40E-09	8.61E-07	BPI	-0.82	1.05E-04	4.53E-02	IFI6	0.35	4.13E-04	2.05E-02
PTGDS	-0.52	6.34E-05	2.03E-02	LOC100506003	-0.86	5.22E-12	4.60E-09	ICOS	0.35	2.50E-05	2.58E-03
BCORP1	-1.71	4.10E-26	4.03E-23	CEACAM6	-0.92	6.14E-07	3.55E-04	NRN1	0.34	1.73E-03	4.96E-02
PRKY	-2.01	6.69E-144	1.10E-140	TTTY15	-1.42	1.50E-35	2.03E-32	TDRD12	0.34	6.99E-06	9.19E-04
UTY	-2.15	7.97E-131	1.07E-127	BCORP1	-1.60	9.98E-35	1.26E-31	PNPLA4	0.34	7.40E-20	4.16E-17
ZFY	-2.34	9.08E-40	9.57E-37	PRKY	-2.15	3.67E-116	6.75E-113	GAL3ST4	0.34	2.76E-08	7.13E-06
TTTY10	-2.41	8.10E-100	9.18E-97	TTTY10	-2.22	9.81E-95	1.53E-91	NT5E	0.34	2.59E-06	4.10E-04

Blood transcriptome

Female healthy controls (n=34) v Male healthy controls (n=53)

Number of upregulated genes			20
Number of downregulated genes			19
Gene	**Log2 fold change**	***P* value**	**FDR**
NCRNA00185	-2.72	1.99E-127	2.45E-124
CYorf15A	-3.96	1.29E-143	1.90E-140
DDX3Y	-4.24	1.51E-245	3.19E-242
CYorf15B	-4.51	0.00E+00	0.00E+00
USP9Y	-5.17	4.54E-189	8.37E-186
EIF1AY	-5.33	0.00E+00	0.00E+00
KDM5D	-6.80	0.00E+00	0.00E+00
RPS4Y1	-7.41	0.00E+00	0.00E+00

Female mild/moderate asthma (n=37) v Male mild/moderate asthma (n=40)

Number of upregulated genes			20
Number of downregulated genes			21
Gene	**Log2 fold change**	***P* value**	**FDR**
UTY	-2.26	1.06E-168	2.38E-165
ZFY	-2.55	5.26E-56	7.60E-53
NCRNA00185	-2.62	2.94E-112	4.96E-109
CYorf15A	-3.90	1.72E-163	3.48E-160
DDX3Y	-4.16	3.10E-272	8.97E-269
CYorf15B	-4.46	0.00E+00	0.00E+00
USP9Y	-5.23	1.46E-258	3.70E-255
EIF1AY	-5.39	0.00E+00	0.00E+00
KDM5D	-6.72	0.00E+00	0.00E+00
RPS4Y1	-7.36	0.00E+00	0.00E+00

Female severe asthma (n=182) v Male severe asthma (n=114)

Number of upregulated genes			40
Number of downregulated genes			60
Gene	**Log2 fold change**	***P* value**	**FDR**
TMEM158	0.33	2.08E-04	1.28E-02
RGPD1	0.33	3.87E-04	1.96E-02
TRAJ17	0.33	1.48E-05	1.72E-03
SOCS2	0.33	4.06E-05	3.87E-03
GAGE1	0.32	1.25E-04	9.08E-03
LOC643648	0.32	2.22E-07	4.84E-05
CA5B	0.31	3.65E-11	1.32E-08
RGPD5	0.31	5.15E-04	2.34E-02
SPRYD5	0.31	5.04E-04	2.31E-02
TMSB4Y	-0.31	2.58E-23	1.68E-20
PTGES	-0.31	9.03E-06	1.11E-03
CD24	-0.32	1.21E-03	3.92E-02
KIAA1598	-0.32	7.11E-05	5.95E-03
SCD	-0.32	1.84E-07	4.24E-05
TOP2A	-0.32	1.08E-05	1.29E-03
SLC22A16	-0.32	1.36E-04	9.71E-03
DPY19L1P1	-0.33	5.73E-05	5.02E-03
AKR1C3	-0.33	5.56E-05	4.97E-03
ANLN	-0.34	1.76E-06	3.08E-04
PRUNE2	-0.34	1.74E-04	1.16E-02
CLEC5A	-0.34	7.10E-05	5.95E-03
PCOLCE2	-0.35	2.63E-07	5.50E-05
CHIT1	-0.35	6.31E-05	5.45E-03
FAM19A2	-0.37	1.24E-04	9.04E-03
KIF18B	-0.37	1.85E-05	2.02E-03
RRM2	-0.38	4.76E-04	2.24E-02
SERPINB10	-0.38	5.47E-06	7.69E-04
CEP55	-0.38	6.72E-05	5.68E-03
SPON2	-0.41	7.15E-06	9.33E-04
ZNF788	-0.43	1.12E-08	3.19E-06
SLC28A3	-0.43	7.49E-08	1.85E-05
HIST1H3C	-0.45	6.80E-07	1.31E-04
TCN1	-0.48	1.38E-05	1.62E-03
RETN	-0.49	1.25E-07	3.00E-05
PTGDS	-0.50	3.75E-07	7.65E-05

Blood transcriptome

Female healthy controls (n=34) v Male healthy controls (n=53)			
Number of upregulated genes			20
Number of downregulated genes			19
Gene	**Log2 fold change**	***P* value**	**FDR**

Female mild/moderate asthma (n=37) v Male mild/moderate asthma (n=40)			
Number of upregulated genes			20
Number of downregulated genes			21
Gene	**Log2 fold change**	***P* value**	**FDR**

Female severe asthma (n=182) v Male severe asthma (n=114)			
Number of upregulated genes			40
Number of downregulated genes			60
Gene	**Log2 fold change**	***P* value**	**FDR**
INHBA	-0.51	1.25E-07	3.00E-05
RNASE3	-0.56	1.80E-04	1.18E-02
CAMP	-0.56	2.41E-07	5.10E-05
AZU1	-0.57	4.85E-11	1.72E-08
BEX1	-0.57	2.18E-11	8.08E-09
LTF	-0.77	1.92E-06	3.29E-04
MS4A3	-0.78	1.89E-08	5.13E-06
LCN2	-0.79	1.37E-07	3.19E-05
ABCA13	-0.81	1.55E-10	5.28E-08
CRISP3	-0.82	6.59E-07	1.29E-04
CEACAM8	-0.83	2.35E-07	5.04E-05
MMP8	-0.83	1.72E-06	3.05E-04
OLR1	-0.83	1.00E-10	3.47E-08
MPO	-0.93	4.44E-16	2.07E-13
BPI	-0.99	3.03E-10	1.01E-07
ELANE	-0.99	1.69E-14	7.32E-12
DEFA4	-1.04	2.39E-09	7.21E-07
OLFM4	-1.07	2.15E-07	4.79E-05
CEACAM6	-1.12	1.92E-12	7.84E-10
CTSG	-1.19	1.71E-14	7.32E-12
TTTY15	-1.26	1.80E-99	1.84E-96
BCORP1	-1.49	5.22E-122	5.68E-119
PRKY	-1.89	0.00E+00	0.00E+00
UTY	-2.16	0.00E+00	0.00E+00
TTTY10	-2.27	0.00E+00	0.00E+00
NCRNA00185	-2.31	0.00E+00	0.00E+00
ZFY	-2.52	3.68E-158	4.28E-155
CYorf15A	-3.74	0.00E+00	0.00E+00
CYorf15B	-4.14	0.00E+00	0.00E+00
DDX3Y	-4.15	0.00E+00	0.00E+00
USP9Y	-4.92	0.00E+00	0.00E+00
EIF1AY	-5.45	0.00E+00	0.00E+00
KDM5D	-6.46	0.00E+00	0.00E+00
RPS4Y1	-7.28	0.00E+00	0.00E+00

Table S4.4 All differentially abundant metabolites between females and males among patients with asthma and healthy controls in urine metabolomics.

Urine metabolomic												
Female healthy controls (n=38) v Male healthy controls (n=62)				Female mild/moderate asthma (n=43) v Male mild/moderate asthma (n=44)				Female severe asthma (n=236) v Male severe asthma (n=140)			
Number of upregulated metabolites			18	Number of upregulated metabolites			14	Number of upregulated metabolites			20
Number of downregulated metabolites			13	Number of downregulated metabolites			17	Number of downregulated metabolites			15
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
Sarcosine	1.64	1.04E-06	1.04E-05	Sarcosine	2.11	5.93E-12	2.67E-10	Hydroxyphenylacetic acid	1.86	4.14E-12	9.31E-11
Caffeine	1.35	1.01E-04	6.99E-04	Hydroxyphenylacetic acid	0.99	1.57E-02	9.67E-02	Sarcosine	1.58	8.29E-19	3.73E-17
Phenyllactic acid	0.95	5.77E-03	2.41E-02	Phenyllactic acid	0.91	1.91E-02	1.01E-01	Xanthine	0.64	1.27E-15	3.82E-14
Mannitol	0.85	7.40E-05	5.55E-04	Caffeine	0.71	3.95E-02	1.41E-01	Aspartic acid	0.44	1.84E-08	2.33E-07
1 3 7 Trimethyluric acid	0.76	5.89E-03	2.41E-02	alpha Glutamyltyrosine	0.53	1.61E-02	9.67E-02	Maltose	0.42	6.53E-03	2.18E-02
1 7 Dimethyluric acid	0.60	2.16E-02	7.20E-02	Tryptamine	0.53	2.19E-02	1.09E-01	3 Hydroxykynurenine	0.42	7.73E-04	3.66E-03
Glutamic acid	0.59	1.98E-07	2.23E-06	5 Hydroxyindoleacetic acid	0.39	1.80E-02	1.01E-01	5 Aminolevulinic acid	0.42	2.71E-06	2.22E-05
Aspartic acid	0.58	1.31E-05	1.18E-04	Choline	0.38	3.84E-03	3.90E-02	O Acetylserine	0.37	2.07E-08	2.33E-07
Xylose	0.58	5.73E-03	2.41E-02	N Acetylputrescine	0.37	5.57E-04	8.35E-03	Prolylhydroxyproline	0.37	3.96E-06	2.74E-05
Xanthine	0.52	5.16E-03	2.41E-02	Guanosine	0.35	4.30E-02	1.41E-01	N Acetylglutamic acid	0.27	3.05E-10	5.48E-09
Theobromine	0.49	4.06E-02	1.26E-01	Serotonin	0.28	3.42E-02	1.34E-01	Proline	0.27	3.15E-05	1.77E-04
N Acetylputrescine	0.49	1.39E-07	1.79E-06	Aspartic acid	0.28	3.21E-02	1.33E-01	Sucrose	0.27	1.06E-02	3.19E-02
4 Pyridoxic acid	0.40	1.73E-02	5.98E-02	N Acetylglutamic acid	0.27	6.49E-03	5.31E-02	N Acetylputrescine	0.25	1.14E-05	6.82E-05
Tyramine	0.36	2.54E-02	8.18E-02	Glutamic acid	0.23	4.23E-02	1.41E-01	N Methyl D aspartic acid	0.24	3.70E-06	2.74E-05
N Acetylglutamic acid	0.26	2.91E-04	1.87E-03	Glutamine	-0.18	4.27E-02	1.41E-01	Inosine	0.24	7.13E-03	2.21E-02
Phenylacetylglutamine	0.25	1.71E-02	5.98E-02	Xanthosine	-0.19	4.97E-02	1.41E-01	Glutamic acid	0.21	8.51E-04	3.83E-03
Pyroglutamic acid	0.21	7.85E-03	3.05E-02	N Methylhistamine	-0.23	2.47E-02	1.11E-01	Phosphoethanolamine	0.19	1.30E-03	5.57E-03
Hippuric acid	0.19	8.12E-03	3.05E-02	Tyrosine	-0.24	4.58E-02	1.41E-01	Citrulline	0.18	1.54E-02	4.48E-02
Xanthosine	-0.21	4.58E-02	1.33E-01	Uracil	-0.34	3.25E-02	1.33E-01	Choline	0.15	2.10E-02	5.90E-02
Citrulline	-0.36	1.17E-03	6.56E-03	Methylthioadenosine	-0.36	5.00E-02	1.41E-01	Uric acid	0.14	1.62E-03	6.40E-03
Carnitine	-0.43	1.49E-03	7.89E-03	Prolylhydroxyproline	-0.39	1.38E-02	9.56E-02	Xanthosine	-0.11	3.02E-02	8.24E-02
Lysine	-0.44	4.51E-02	1.33E-01	S Adenosylhomocysteine	-0.39	2.31E-04	5.21E-03	Kynurenic acid	-0.12	4.92E-02	1.25E-01
S Adenosylhomocysteine	-0.46	5.09E-05	4.16E-04	Carnitine	-0.44	6.04E-03	5.31E-02	Methionine	-0.21	2.41E-03	9.03E-03
Histidine	-0.55	1.78E-09	8.01E-08	5 Aminolevulinic acid	-0.46	8.32E-03	6.24E-02	Cystathionine	-0.24	3.73E-02	9.87E-02
Cystathionine	-0.64	8.26E-04	4.96E-03	3 Methylhistidine	-0.46	4.39E-02	1.41E-01	Mesaconic acid	-0.27	7.10E-03	2.21E-02
Octopamine	-0.68	5.61E-08	8.42E-07	Lysine	-0.64	3.37E-03	3.90E-02	S Adenosylhomocysteine	-0.31	2.45E-06	2.21E-05
Tyrosine	-0.68	4.49E-08	8.08E-07	Acetylcarnitine	-0.64	2.35E-02	1.11E-01	Histidine	-0.31	4.75E-06	3.05E-05
Taurine	-0.76	4.63E-03	2.32E-02	Taurine	-0.74	3.90E-03	3.90E-02	Tyrosine	-0.33	1.53E-07	1.53E-06
N Acetylcarnosine	-0.86	4.55E-16	4.09E-14	N Acetylcarnosine	-0.86	2.13E-12	1.92E-10	Octopamine	-0.35	1.63E-08	2.33E-07
Propionylcarnitine	-1.33	2.91E-09	8.72E-08	Propionylcarnitine	-0.94	4.74E-04	8.35E-03	Glucosamine	-0.35	1.22E-04	6.12E-04

Urine metabolomic												
Female healthy controls (n=38) v Male healthy controls (n=62)				Female mild/moderate asthma (n=43) v Male mild/moderate asthma (n=44)				Female severe asthma (n=236) v Male severe asthma (n=140)			
Number of upregulated metabolites			18	Number of upregulated metabolites			14	Number of upregulated metabolites			20
Number of downregulated metabolites			13	Number of downregulated metabolites			17	Number of downregulated metabolites			15
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
Carnosine	-1.56	4.84E-09	1.09E-07	Carnosine	-0.99	1.76E-05	5.28E-04	Aminocaproic acid	-0.39	5.80E-03	2.01E-02
								Taurine	-0.42	3.05E-03	1.10E-02
								Aminovaleric acid	-0.44	1.63E-03	6.40E-03
								N Acetylcarnosine	-0.61	6.91E-25	6.22E-23
								Propionylcarnitine	-0.61	9.93E-05	5.26E-04

Table S4.5 All differentially abundant bacterial genera between females and males among patients with asthma and healthy controls in sputum microbiomics.

Sputum microbiome												
Female healthy controls (n=7) v Male healthy controls (n=16)				Female mild/moderate asthma (n=11) v Male mild/moderate asthma (n=13)				Female severe asthma (n=52) v Male severe asthma (n=36)			
Number of upregulated genera			1	Number of upregulated genera			8	Number of upregulated genera			8
Number of downregulated genera			13	Number of downregulated genera			6	Number of downregulated genera			2
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
Actinomyces	1.07	3.82E-02	3.85E-01	Actinomyces	1.28	9.87E-03	2.78E-01	Bacteroides	2.76	4.83E-03	2.12E-01
Bifidobacterium	-3.76	6.24E-03	1.02E-01	Alloscardovia	5.80	9.35E-03	2.78E-01	Haemophilus	2.48	4.92E-07	8.60E-05
Dialister	-3.59	6.77E-05	8.87E-03	Capnocytophaga	1.36	1.67E-02	3.76E-01	Moraxella	3.07	1.32E-02	4.63E-01
Fusobacterium	-1.28	1.54E-02	2.02E-01	Eikenella	1.96	3.47E-02	4.28E-01	Pseudomonas	2.20	1.87E-02	4.67E-01
Methylobacterium	-2.93	4.12E-02	3.86E-01	Lactococcus	5.30	3.78E-02	4.28E-01	Rothia	0.50	2.54E-02	5.55E-01
Moraxella	-7.64	1.41E-04	9.24E-03	Megasphaera	1.87	2.64E-02	4.28E-01	Sediminibacterium	1.27	1.73E-02	4.67E-01
Mycoplasma	-3.83	3.43E-04	1.50E-02	Propionibacterium	4.62	9.09E-03	2.78E-01	Shewanella	5.90	4.57E-02	8.00E-01
Parvimonas	-3.02	1.77E-03	4.65E-02	Steroidobacter	6.36	3.17E-02	4.28E-01	Stenotrophomonas	1.87	3.09E-03	1.80E-01
Peptococcus	-3.25	5.67E-03	1.02E-01	Bacteroides	-3.22	3.95E-02	4.28E-01	Cellulomonas	-2.29	4.09E-02	7.95E-01
Porphyromonas	-1.56	2.72E-02	3.16E-01	Desulfobulbus	-4.41	3.13E-02	4.28E-01	Methylobacterium	-2.15	2.69E-03	1.80E-01
Ralstonia	-3.33	2.89E-02	3.16E-01	Filifactor	-3.55	1.86E-02	3.76E-01				
Slackia	-3.03	1.34E-02	1.95E-01	Granulicatella	-2.60	3.16E-04	4.46E-02				
Tannerella	-2.76	1.05E-03	3.44E-02	Moraxella	-3.78	4.53E-03	2.78E-01				
Treponema	-2.67	4.34E-03	9.48E-02	Treponema	-1.79	4.91E-02	4.84E-01				

Table S4.6 All differentially expressed genes between mild to moderate asthma and healthy controls in females and males with bronchial biopsies transcriptomes.

Bronchial biopsies transcriptome							
Female mild/moderate asthma (n=16) v Female healthy controls (n=10)				Male mild/moderate asthma (n=12) v Male healthy controls (n=16)			
Number of upregulated genes			11	Number of upregulated genes			2
Number of downregulated genes			40	Number of downregulated genes			10
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
FKBP5	1.87	5.29E-09	6.34E-05	FKBP5	1.21	4.73E-05	3.47E-02
HIF3A	1.30	8.78E-08	2.10E-04	SLC45A4	0.46	5.47E-06	6.68E-03
PDK4	1.23	4.50E-05	2.00E-02	SLIT2	-0.56	7.35E-05	4.49E-02
APOD	0.92	2.42E-07	4.15E-04	PRRX1	-0.83	5.75E-05	3.83E-02
TSC22D3	0.91	1.04E-05	6.95E-03	APLNR	-1.02	4.78E-06	6.68E-03
MAOA	0.80	3.52E-07	4.68E-04	CHRDL1	-1.10	3.43E-05	2.80E-02
PHF17	0.65	1.37E-04	3.84E-02	SFRP2	-1.17	4.89E-06	6.68E-03
TCEAL4	0.61	3.03E-05	1.45E-02	LUM	-1.38	3.52E-06	6.68E-03
RHOBTB3	0.60	5.91E-07	7.08E-04	OGN	-1.69	1.68E-05	1.76E-02
LOC283788	0.53	9.07E-05	2.86E-02	COL9A3	-2.07	2.23E-05	2.04E-02
CCDC58	0.38	1.78E-04	4.48E-02	CYTL1	-3.45	2.49E-06	6.68E-03
HLA-A	-0.37	1.92E-04	4.68E-02	COL2A1	-3.79	2.28E-08	1.67E-04
SP110	-0.45	2.63E-05	1.37E-02				
SOX4	-0.45	1.89E-05	1.13E-02				
ALDH5A1	-0.49	8.65E-05	2.80E-02				
TNFSF10	-0.49	1.69E-05	1.06E-02				
NIPSNAP1	-0.50	1.35E-04	3.84E-02				
PHF13	-0.55	1.46E-04	3.97E-02				
DDX60L	-0.56	7.10E-05	2.74E-02				
PIM2	-0.60	1.54E-04	4.09E-02				
SLAMF8	-0.61	1.74E-04	4.48E-02				
HLA-DRB1	-0.61	8.39E-05	2.79E-02				
LYN	-0.62	8.40E-06	5.92E-03				
CASP1	-0.64	4.79E-06	3.59E-03				
SEPT6	-0.64	2.10E-05	1.20E-02				
SPOCK2	-0.66	2.25E-06	1.93E-03				
P2RY8	-0.67	1.80E-04	4.48E-02				
HLA-DMB	-0.67	7.93E-05	2.79E-02				
PSMB9	-0.69	2.01E-04	4.82E-02				
KIAA1949	-0.74	2.10E-04	4.93E-02				
CD2	-0.79	1.46E-06	1.59E-03				
F13A1	-0.80	8.15E-05	2.79E-02				
FAIM3	-0.80	3.41E-05	1.57E-02				
ADRA2A	-0.81	1.94E-07	3.88E-04				
LAPTM5	-0.83	9.93E-05	3.05E-02				
RAC2	-0.85	5.81E-05	2.40E-02				
CD48	-0.85	2.06E-06	1.93E-03				
HLA-DPB1	-0.89	3.22E-07	4.68E-04				
CPVL	-0.95	6.37E-08	1.91E-04				
IL2RG	-1.04	4.67E-05	2.00E-02				
C16orf54	-1.07	7.90E-05	2.79E-02				
C1QC	-1.09	2.58E-05	1.37E-02				
MPEG1	-1.12	2.85E-05	1.42E-02				
TRIM73	-1.14	1.38E-04	3.84E-02				
TRBC1	-1.14	6.08E-05	2.43E-02				
PTPRC	-1.17	8.12E-05	2.79E-02				
CSF2RB	-1.18	3.96E-06	3.16E-03				
CCL19	-1.34	2.19E-06	1.93E-03				
CD52	-1.36	1.38E-04	3.84E-02				
CH25H	-1.50	3.40E-08	1.36E-04				
MMP10	-1.53	2.90E-08	1.36E-04				

Table S4.7 All differentially expressed genes between severe asthma and healthy controls in females and males with bronchial biopsies transcriptomes.

Bronchial biopsies transcriptome							
Female severe asthma (n=23) v Female healthy controls (n=10)				Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			6	Number of upregulated genes			566
Number of downregulated genes			7	Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
KRT6A	2.84	2.06E-05	4.17E-02	KRT6A	3.56	4.52E-08	9.67E-06
FKBP5	1.78	9.94E-13	1.19E-08	SPRR1B	2.84	1.75E-06	1.79E-04
TFCP2L1	1.00	3.83E-05	4.17E-02	FGFBP1	2.40	5.07E-07	6.83E-05
PDK4	0.97	3.50E-05	4.17E-02	KRT13	2.14	1.04E-04	3.45E-03
TSC22D3	0.81	2.98E-05	4.17E-02	CEACAM5	1.99	3.72E-08	8.72E-06
MAOA	0.65	1.79E-05	4.17E-02	LY6D	1.66	2.32E-06	2.16E-04
NR3C1	-0.51	1.29E-05	4.17E-02	KRT24	1.62	5.67E-04	1.17E-02
OXCT1	-0.55	3.22E-05	4.17E-02	UPK1B	1.40	1.17E-05	6.99E-04
LSM10	-0.60	2.70E-05	4.17E-02	HIST1H3B	1.37	2.99E-03	3.51E-02
SMAD9	-0.64	4.84E-05	4.83E-02	SPRR1A	1.34	2.91E-04	7.40E-03
IFI6	-0.93	2.22E-05	4.17E-02	CSTA	1.33	3.01E-05	1.44E-03
SCD	-0.99	5.41E-05	4.98E-02	SPRR3	1.32	1.26E-03	2.05E-02
APOC1	-1.58	2.61E-05	4.17E-02	S100P	1.31	1.44E-09	6.64E-07
				SERPINB5	1.27	1.78E-04	5.08E-03
				CA12	1.24	1.51E-07	2.39E-05
				S100A2	1.23	2.16E-05	1.10E-03
				SDCBP2	1.22	5.34E-06	4.07E-04
				FKBP5	1.20	1.62E-05	8.75E-04
				ZWINT	1.19	3.29E-04	7.91E-03
				PSCA	1.17	1.89E-03	2.65E-02
				S100A14	1.15	4.90E-06	3.79E-04
				AKR1B10	1.10	7.39E-06	5.24E-04
				DEFB1	1.10	3.13E-07	4.68E-05
				CKS2	1.09	3.59E-03	3.99E-02
				TPRXL	1.09	3.33E-08	7.98E-06
				TPX2	1.08	1.81E-03	2.61E-02
				SCGB2A1	1.08	6.06E-04	1.23E-02
				GPX2	1.06	1.94E-04	5.43E-03
				AKR1C2	1.04	1.42E-09	6.64E-07
				LCN2	1.03	3.23E-04	7.83E-03
				ALDH3A1	1.03	2.74E-06	2.47E-04
				IL1R2	0.98	2.64E-03	3.25E-02
				LOC100292909	0.98	1.41E-03	2.20E-02
				CD163	0.98	4.75E-03	4.80E-02
				SLC6A14	0.97	2.05E-06	1.99E-04
				ALOX15	0.95	7.53E-06	5.27E-04
				KRT23	0.95	9.67E-08	1.70E-05
				LRRC4	0.94	2.79E-05	1.37E-03
				TFF3	0.94	4.40E-04	9.78E-03
				GPRC5A	0.94	8.37E-05	2.96E-03
				SFN	0.92	1.47E-04	4.34E-03
				MUC1	0.92	4.66E-09	1.64E-06
				IL8	0.92	3.45E-05	1.57E-03
				AGR2	0.91	3.18E-09	1.27E-06
				SERPINB4	0.91	7.98E-08	1.45E-05
				PTTG1	0.91	4.95E-03	4.93E-02
				CEACAM6	0.89	4.05E-04	9.21E-03
				DUOXA1	0.89	2.18E-09	9.31E-07
				TFCP2L1	0.89	1.22E-04	3.89E-03
				AKR1C1	0.88	6.79E-08	1.26E-05
				LYPD3	0.88	2.46E-04	6.52E-03
				RACGAP1	0.86	1.64E-03	2.43E-02
				ADH6	0.85	5.78E-04	1.18E-02
				CDC20B	0.85	3.42E-03	3.84E-02
				PAK6	0.84	6.89E-07	8.46E-05
				ATP1B1	0.84	8.84E-09	2.79E-06

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
SDR16C5	0.84	3.76E-06	3.10E-04
S100A16	0.84	2.77E-04	7.11E-03
RDH10	0.83	4.15E-07	5.78E-05
IMPA2	0.82	7.29E-09	2.36E-06
CLCA2	0.82	6.59E-04	1.29E-02
TMPRSS4	0.81	1.60E-06	1.69E-04
SERPINB3	0.81	6.12E-07	7.98E-05
B3GNT3	0.80	1.72E-06	1.78E-04
GPR109B	0.79	2.64E-05	1.30E-03
NQO1	0.79	3.95E-04	9.05E-03
KCNE3	0.79	1.61E-06	1.69E-04
OSTalpha	0.78	3.20E-04	7.81E-03
PIGW	0.78	3.17E-05	1.49E-03
TTC9	0.77	1.01E-05	6.46E-04
MAL2	0.77	1.47E-05	8.16E-04
PRR15	0.76	4.10E-06	3.23E-04
DNER	0.76	2.14E-03	2.87E-02
CLDN7	0.76	1.13E-05	6.86E-04
AKR1C3	0.75	1.48E-07	2.36E-05
HIST1H2BK	0.75	6.92E-04	1.33E-02
TIMP4	0.75	1.45E-03	2.23E-02
PHLDA2	0.75	3.22E-04	7.81E-03
TRIM16	0.74	1.99E-06	1.95E-04
CLDN10	0.74	1.86E-03	2.65E-02
SERPINB1	0.74	3.19E-05	1.49E-03
ITPKC	0.73	9.12E-04	1.63E-02
ANXA1	0.72	1.42E-04	4.26E-03
NME7	0.72	4.79E-09	1.64E-06
DTX2	0.72	6.76E-04	1.31E-02
HIST1H1A	0.71	3.14E-03	3.64E-02
FUT3	0.71	2.22E-05	1.12E-03
C19orf21	0.70	1.38E-04	4.20E-03
HN1	0.70	1.25E-04	3.92E-03
PNP	0.69	1.60E-04	4.64E-03
ST6GALNAC1	0.69	1.21E-07	2.03E-05
ALPL	0.69	6.97E-04	1.33E-02
C19orf33	0.69	3.36E-04	8.02E-03
C1orf116	0.69	1.51E-05	8.34E-04
TXN	0.68	4.31E-04	9.65E-03
KRT6B	0.68	3.64E-03	4.02E-02
B4GALT4	0.68	1.02E-05	6.47E-04
AK7	0.68	2.66E-04	6.89E-03
TRIP13	0.68	2.94E-03	3.46E-02
DUOX1	0.68	6.19E-07	7.98E-05
MAOA	0.67	1.40E-04	4.25E-03
GALNT5	0.67	2.82E-03	3.39E-02
VSIG2	0.67	2.09E-06	2.02E-04
PI3	0.67	4.83E-03	4.85E-02
EPS8L1	0.67	4.53E-06	3.55E-04
SLC16A9	0.67	1.62E-03	2.41E-02
TUFT1	0.67	7.92E-05	2.87E-03
CHST6	0.66	3.28E-05	1.52E-03
DUOXA2	0.66	2.67E-03	3.28E-02
LOC25845	0.66	5.74E-04	1.18E-02
GSTP1	0.66	1.44E-10	1.08E-07
TACSTD2	0.65	5.05E-05	2.10E-03
HMGB3	0.65	1.62E-05	8.75E-04
GJB5	0.65	5.58E-04	1.16E-02
PDK4	0.65	2.73E-03	3.33E-02
FA2H	0.64	7.40E-04	1.40E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
CHMP4C	0.64	1.59E-04	4.62E-03
P2RY2	0.64	2.78E-03	3.36E-02
LMNB2	0.63	6.50E-04	1.28E-02
FABP5	0.63	1.05E-03	1.81E-02
CD55	0.63	8.84E-04	1.59E-02
GJB3	0.63	4.87E-03	4.88E-02
HIST1H2AC	0.63	1.75E-03	2.54E-02
CLDN4	0.63	6.34E-05	2.46E-03
MACC1	0.62	4.88E-03	4.88E-02
TSPAN1	0.61	5.39E-04	1.13E-02
GDF15	0.61	4.49E-03	4.64E-02
GNA15	0.60	1.30E-03	2.08E-02
RUVBL1	0.60	2.32E-04	6.26E-03
KRT18	0.60	3.82E-06	3.14E-04
TRIM7	0.60	3.49E-04	8.25E-03
KRT8	0.59	3.85E-05	1.71E-03
PLA2G10	0.59	9.96E-05	3.37E-03
ATP6V1C2	0.59	6.83E-04	1.32E-02
ENPP4	0.59	2.19E-05	1.11E-03
RAPGEFL1	0.59	4.71E-03	4.79E-02
CXorf59	0.59	2.80E-03	3.38E-02
KRT19	0.59	3.69E-04	8.63E-03
C14orf129	0.59	3.03E-03	3.54E-02
HIST2H2BE	0.59	1.44E-03	2.22E-02
PROM2	0.58	9.40E-05	3.20E-03
FAM83B	0.58	1.44E-03	2.22E-02
SGMS2	0.58	1.41E-05	7.95E-04
NUP37	0.58	6.99E-04	1.33E-02
LRRC8A	0.58	3.82E-03	4.14E-02
POF1B	0.58	1.57E-05	8.64E-04
B4GALT5	0.58	3.87E-04	8.92E-03
TP53I3	0.58	1.24E-03	2.03E-02
CYP2S1	0.58	1.73E-03	2.52E-02
GALNT7	0.57	1.79E-04	5.08E-03
KLHL2	0.57	3.79E-07	5.47E-05
GPR87	0.57	8.26E-04	1.51E-02
SLC4A4	0.57	6.56E-05	2.52E-03
SRD5A3	0.57	3.45E-03	3.86E-02
KRT17	0.56	2.57E-03	3.21E-02
FAM164C	0.56	2.73E-03	3.33E-02
CREB3L4	0.56	3.63E-03	4.01E-02
TMEM154	0.56	3.37E-04	8.03E-03
TNNI3	0.56	1.88E-03	2.65E-02
GLTP	0.56	2.23E-04	6.09E-03
TXNDC17	0.55	4.58E-04	9.97E-03
ZNF57	0.55	6.08E-04	1.23E-02
SDC1	0.55	5.08E-04	1.08E-02
WEE1	0.55	7.55E-04	1.41E-02
JUP	0.55	2.01E-04	5.59E-03
MT1E	0.55	1.38E-05	7.83E-04
ITGB6	0.55	1.43E-03	2.21E-02
C1orf106	0.55	4.60E-05	1.99E-03
SLC25A25	0.55	6.37E-04	1.26E-02
BAG3	0.55	4.39E-04	9.78E-03
FUNDC1	0.55	2.07E-04	5.73E-03
PGD	0.55	1.61E-04	4.64E-03
DENND2C	0.55	2.12E-03	2.85E-02
CDH26	0.55	1.78E-05	9.49E-04
RAB25	0.54	4.42E-04	9.82E-03
TNFRSF21	0.54	4.00E-03	4.28E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
MRPL15	0.54	1.02E-04	3.41E-03
SERINC2	0.54	6.20E-04	1.24E-02
PCNA	0.54	4.16E-03	4.40E-02
MCM4	0.54	5.51E-04	1.15E-02
TTLL9	0.53	1.57E-03	2.35E-02
SEMA3A	0.53	5.33E-04	1.13E-02
SIX2	0.53	1.33E-05	7.62E-04
GSR	0.53	2.34E-06	2.16E-04
SLC44A4	0.53	1.19E-03	1.98E-02
SNX7	0.52	5.83E-07	7.76E-05
SLC25A5	0.52	1.04E-04	3.45E-03
YIF1B	0.52	5.40E-05	2.19E-03
FAM83A	0.52	3.38E-03	3.81E-02
TSPAN8	0.52	1.99E-03	2.73E-02
SLC26A2	0.52	1.72E-03	2.52E-02
MRPL13	0.52	1.58E-04	4.59E-03
DHRS9	0.52	1.43E-03	2.21E-02
IFFO2	0.52	2.46E-04	6.52E-03
RNF145	0.52	3.29E-06	2.78E-04
SLC16A3	0.52	1.44E-04	4.29E-03
PAQR6	0.52	3.28E-03	3.73E-02
MUC5AC	0.51	2.18E-03	2.90E-02
RUVBL2	0.51	1.37E-04	4.19E-03
AHCY	0.51	9.23E-05	3.16E-03
EPN3	0.51	3.45E-04	8.18E-03
CDKN1A	0.51	2.42E-03	3.07E-02
STYK1	0.51	5.54E-04	1.16E-02
HEATR2	0.51	9.35E-07	1.06E-04
ADORA2B	0.51	7.54E-04	1.41E-02
GLRX2	0.51	2.06E-03	2.81E-02
H2AFX	0.50	4.28E-04	9.61E-03
CMTM4	0.50	8.00E-05	2.87E-03
OAT	0.50	2.56E-04	6.71E-03
DSP	0.50	5.41E-04	1.13E-02
TTLL12	0.50	4.86E-04	1.05E-02
TRIM29	0.50	3.19E-04	7.81E-03
C6orf115	0.50	3.29E-04	7.91E-03
MYB	0.50	1.65E-03	2.45E-02
ESRP1	0.50	3.42E-06	2.86E-04
IRAK3	0.50	1.89E-03	2.66E-02
FDXR	0.49	1.53E-04	4.48E-03
CDC42BPG	0.49	6.87E-04	1.32E-02
PRRG4	0.49	3.66E-04	8.58E-03
ABHD2	0.49	3.33E-05	1.54E-03
CISD1	0.49	2.11E-05	1.09E-03
IRF6	0.49	4.01E-04	9.13E-03
FXYD3	0.49	2.71E-06	2.46E-04
DDIT4	0.49	2.48E-03	3.13E-02
FUT6	0.48	1.27E-03	2.05E-02
SCNN1A	0.48	7.47E-06	5.26E-04
MPZL2	0.48	2.35E-04	6.31E-03
RCC1	0.48	1.17E-04	3.74E-03
PRDX1	0.48	2.55E-05	1.26E-03
WFDC2	0.48	1.00E-03	1.75E-02
TUBG1	0.47	1.78E-03	2.57E-02
GCLC	0.47	3.73E-04	8.71E-03
CHN2	0.47	2.30E-03	2.98E-02
ERMP1	0.47	7.48E-04	1.41E-02
ASS1	0.47	4.38E-03	4.55E-02
LOC203274	0.47	2.09E-03	2.83E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
B3GNT5	0.47	6.44E-04	1.27E-02
DLAT	0.47	1.34E-04	4.12E-03
LOC113230	0.47	3.06E-03	3.56E-02
ST14	0.47	3.76E-03	4.09E-02
TM7SF2	0.46	4.68E-05	2.00E-03
SLC35A2	0.46	1.36E-05	7.77E-04
CNIH	0.46	1.28E-03	2.07E-02
OCIAD2	0.46	3.83E-03	4.15E-02
ACOX3	0.46	7.20E-06	5.13E-04
MYO5B	0.46	8.66E-05	3.03E-03
SGPP2	0.46	2.61E-03	3.23E-02
MT1P2	0.46	2.45E-04	6.52E-03
ALCAM	0.45	1.03E-04	3.44E-03
PKP2	0.45	1.29E-03	2.08E-02
ARHGEF5	0.45	2.16E-03	2.88E-02
CD24	0.45	2.70E-03	3.31E-02
VAMP8	0.45	7.45E-05	2.73E-03
MFSD4	0.45	4.10E-05	1.81E-03
CTSC	0.45	2.45E-03	3.09E-02
RAB11FIP1	0.45	1.17E-05	6.99E-04
SLC6A8	0.45	3.17E-05	1.49E-03
CD9	0.45	7.71E-04	1.43E-02
AP1M2	0.45	2.09E-03	2.83E-02
BCL2L1	0.45	2.55E-04	6.71E-03
PGM2L1	0.45	4.76E-03	4.80E-02
CCDC64B	0.44	1.72E-03	2.52E-02
TALDO1	0.44	8.22E-05	2.93E-03
IKZF2	0.44	1.42E-03	2.21E-02
CDH1	0.44	1.11E-04	3.58E-03
ATP6V1D	0.44	8.00E-05	2.87E-03
RBM47	0.44	1.06E-04	3.48E-03
FAIM	0.44	3.20E-03	3.68E-02
TP53INP2	0.43	4.52E-04	9.93E-03
LAMC2	0.43	3.47E-03	3.87E-02
PKM2	0.43	1.14E-04	3.66E-03
C10orf81	0.43	1.84E-03	2.64E-02
BAG1	0.43	2.01E-05	1.04E-03
MARVELD2	0.43	1.01E-04	3.40E-03
TIMM13	0.43	7.79E-04	1.44E-02
AQP3	0.43	2.84E-05	1.38E-03
PTPN3	0.43	8.48E-07	9.76E-05
CCT5	0.43	8.56E-05	3.01E-03
EYA1	0.43	2.89E-03	3.42E-02
EZR	0.42	1.44E-03	2.22E-02
GGCT	0.42	1.20E-03	1.99E-02
ABCD3	0.42	6.73E-05	2.57E-03
SBK1	0.42	1.02E-03	1.77E-02
WDR34	0.42	6.39E-04	1.27E-02
LOC387723	0.42	1.80E-03	2.59E-02
ERLIN1	0.42	1.52E-06	1.64E-04
MICALL1	0.42	1.91E-03	2.67E-02
SYTL2	0.42	3.27E-03	3.72E-02
FAHD1	0.42	1.09E-03	1.85E-02
ATP5G3	0.42	1.42E-04	4.26E-03
GNAI1	0.42	4.08E-06	3.23E-04
MT2A	0.42	1.85E-03	2.64E-02
ORMDL2	0.42	5.42E-05	2.19E-03
MFSD6	0.42	3.67E-05	1.64E-03
SAT1	0.42	4.26E-04	9.60E-03
CHCHD3	0.42	6.66E-04	1.30E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
EYA2	0.42	8.58E-06	5.80E-04
DHCR24	0.42	1.79E-04	5.08E-03
HS3ST1	0.42	4.83E-03	4.85E-02
SH3RF2	0.41	4.24E-03	4.46E-02
FAM83H	0.41	1.63E-03	2.42E-02
BMPR1B	0.41	1.66E-03	2.45E-02
C14orf156	0.41	3.54E-04	8.35E-03
HMGB3P1	0.41	2.57E-04	6.71E-03
MYH14	0.41	7.40E-05	2.72E-03
S100A11	0.41	9.28E-06	6.12E-04
IDE	0.41	5.92E-06	4.41E-04
ANXA2	0.41	4.58E-05	1.99E-03
LRP11	0.41	2.60E-03	3.22E-02
KCTD11	0.41	2.91E-03	3.45E-02
COX5A	0.41	1.61E-05	8.75E-04
ANXA2P2	0.40	3.39E-05	1.56E-03
KIAA1522	0.40	1.06E-04	3.49E-03
TPI1	0.40	1.38E-04	4.20E-03
CAPN5	0.40	1.52E-03	2.31E-02
RNF6	0.40	1.00E-05	6.44E-04
FAM84A	0.40	2.42E-04	6.48E-03
SLC9A3R1	0.40	1.51E-03	2.30E-02
C6orf226	0.40	5.35E-04	1.13E-02
LIPH	0.40	3.17E-04	7.80E-03
SERINC5	0.40	2.28E-03	2.97E-02
HTATIP2	0.40	5.37E-04	1.13E-02
SPINT1	0.40	1.31E-03	2.09E-02
CREB3L1	0.40	3.61E-03	4.00E-02
TPPP	0.40	3.67E-03	4.02E-02
CSTB	0.40	4.98E-04	1.06E-02
IPO4	0.40	2.41E-03	3.06E-02
TNS4	0.40	1.90E-03	2.67E-02
IRAK1	0.40	4.38E-03	4.55E-02
MUC4	0.39	7.21E-05	2.69E-03
EXPH5	0.39	3.92E-04	9.00E-03
ATP2C2	0.39	5.46E-05	2.19E-03
GOT2	0.39	1.85E-03	2.64E-02
BZW1	0.39	1.26E-03	2.05E-02
MT1X	0.39	4.37E-03	4.55E-02
STYXL1	0.39	2.33E-03	3.01E-02
COMTD1	0.39	1.02E-03	1.77E-02
CCDC109A	0.39	4.74E-04	1.03E-02
SLC6A9	0.39	4.28E-03	4.49E-02
MFSD2A	0.39	1.48E-03	2.27E-02
BSCL2	0.39	6.49E-04	1.28E-02
IPMK	0.39	4.94E-03	4.93E-02
ATP1A1	0.39	3.60E-09	1.35E-06
RPS6KA1	0.38	2.56E-04	6.71E-03
EPHA2	0.38	2.22E-03	2.92E-02
TMEM189	0.38	6.38E-04	1.26E-02
ZBTB7C	0.38	1.52E-03	2.31E-02
NUDT1	0.38	6.13E-04	1.24E-02
IRX3	0.38	2.68E-03	3.29E-02
F11R	0.38	1.42E-06	1.55E-04
C7orf55	0.38	4.81E-04	1.04E-02
UNC119B	0.38	3.27E-03	3.73E-02
GRHL2	0.38	7.68E-04	1.43E-02
TUBA1C	0.38	7.69E-05	2.81E-03
NPAS2	0.38	1.71E-03	2.50E-02
ANKRD54	0.38	3.90E-03	4.20E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
RHOV	0.38	2.11E-03	2.85E-02
CAPN1	0.37	1.30E-03	2.08E-02
PTS	0.37	2.11E-04	5.81E-03
F3	0.37	2.85E-03	3.40E-02
KCTD1	0.37	4.54E-04	9.94E-03
DOLK	0.37	2.19E-03	2.90E-02
PERP	0.37	1.47E-04	4.34E-03
REEP3	0.37	2.97E-04	7.54E-03
SYBU	0.37	2.74E-03	3.33E-02
NEBL	0.37	3.09E-04	7.67E-03
TBC1D2	0.37	2.13E-04	5.87E-03
H2AFZ	0.37	4.06E-03	4.33E-02
KLF5	0.37	1.26E-03	2.05E-02
SLC35C1	0.37	2.73E-03	3.33E-02
CCDC104	0.37	4.18E-03	4.41E-02
C1orf31	0.37	3.08E-03	3.57E-02
C14orf45	0.37	3.94E-03	4.23E-02
EIF6	0.37	1.42E-03	2.21E-02
HSPBP1	0.37	1.20E-03	1.99E-02
RIPK4	0.36	2.12E-03	2.86E-02
GPX1	0.36	4.80E-04	1.04E-02
MYO5C	0.36	3.24E-04	7.84E-03
B4GALNT3	0.36	1.99E-03	2.73E-02
MRPS33	0.36	3.29E-03	3.74E-02
SGPL1	0.36	1.07E-04	3.52E-03
HNRNPAB	0.36	9.19E-04	1.63E-02
EXOC3	0.36	5.85E-04	1.19E-02
KDM1B	0.36	4.10E-03	4.36E-02
ASCC3	0.36	3.21E-03	3.68E-02
SPINT2	0.36	1.58E-04	4.60E-03
ENDOG	0.36	4.32E-03	4.52E-02
DGKH	0.36	5.05E-03	5.00E-02
TP53TG1	0.36	2.23E-03	2.92E-02
TMEM93	0.36	1.06E-03	1.82E-02
FAM57A	0.36	2.85E-03	3.40E-02
MESDC1	0.36	4.38E-03	4.55E-02
RPL26L1	0.35	3.00E-04	7.57E-03
SNRNP40	0.35	2.51E-03	3.15E-02
C11orf51	0.35	1.55E-04	4.53E-03
TPBG	0.35	3.53E-04	8.34E-03
SLC9A8	0.35	2.19E-04	6.01E-03
NDUFA8	0.35	8.14E-04	1.49E-02
SNRPG	0.35	7.98E-05	2.87E-03
PDLIM1	0.35	3.61E-04	8.49E-03
VDAC1	0.35	5.36E-05	2.18E-03
CEBPB	0.35	1.65E-03	2.45E-02
SMC6	0.35	3.54E-03	3.94E-02
BLVRB	0.35	8.35E-04	1.52E-02
FLAD1	0.35	3.86E-03	4.17E-02
ITGA2	0.35	2.54E-03	3.18E-02
MYO1B	0.35	1.21E-03	2.01E-02
C16orf61	0.35	2.85E-03	3.40E-02
MAP7	0.35	6.64E-04	1.30E-02
GPR56	0.34	3.95E-06	3.19E-04
ALDH1A1	0.34	2.29E-03	2.97E-02
MARCH3	0.34	3.97E-03	4.25E-02
ARCN1	0.34	2.66E-04	6.89E-03
TMEM99	0.34	3.18E-03	3.67E-02
CNOT6	0.34	5.01E-04	1.07E-02
CPT1A	0.34	1.30E-03	2.08E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
TAGLN2	0.34	4.99E-04	1.07E-02
TFAP2C	0.34	4.50E-03	4.64E-02
SMPD2	0.34	1.87E-03	2.65E-02
HSPE1	0.34	1.59E-03	2.37E-02
ZDHHC20	0.34	8.08E-04	1.49E-02
RNF43	0.34	2.00E-03	2.74E-02
CXADR	0.34	8.68E-04	1.57E-02
UNC13B	0.34	1.27E-03	2.05E-02
BSPRY	0.34	3.59E-03	3.99E-02
CLDN12	0.34	1.88E-03	2.65E-02
PDCD10	0.34	1.42E-03	2.21E-02
COX7A2	0.34	5.95E-04	1.21E-02
B3GNT2	0.34	1.38E-03	2.16E-02
STAP2	0.34	2.31E-03	2.99E-02
SLMO2	0.33	1.37E-03	2.16E-02
FOXA1	0.33	1.44E-03	2.22E-02
NDUFV2	0.33	2.27E-04	6.15E-03
MRPL3	0.33	1.16E-03	1.96E-02
MRPL47	0.33	8.60E-05	3.02E-03
KIF13B	0.33	5.04E-03	5.00E-02
HBXIP	0.33	2.09E-03	2.83E-02
CCT3	0.33	2.82E-03	3.39E-02
EPCAM	0.33	2.85E-03	3.40E-02
VILL	0.33	1.40E-03	2.20E-02
CTNNA1	0.33	1.95E-06	1.95E-04
FIBP	0.33	3.42E-05	1.57E-03
PSMA5	0.33	1.98E-03	2.73E-02
MPDU1	0.33	1.40E-03	2.20E-02
PLIN3	0.33	2.35E-03	3.02E-02
ATP5B	0.32	2.99E-04	7.56E-03
UGDH	0.32	3.29E-03	3.74E-02
POLDIP2	0.32	1.17E-03	1.97E-02
COX6A1	0.32	1.01E-04	3.40E-03
ANXA11	0.32	1.43E-04	4.27E-03
KPNA3	0.32	2.28E-03	2.97E-02
CEBPD	0.32	1.87E-03	2.65E-02
ALDOA	0.32	9.27E-04	1.64E-02
CBARA1	0.32	1.01E-03	1.76E-02
EPT1	0.32	1.98E-03	2.73E-02
PPP4C	0.32	3.46E-03	3.87E-02
RHBDL2	0.32	4.05E-03	4.32E-02
GDE1	0.32	5.14E-05	2.11E-03
HIGD1A	0.32	1.51E-03	2.30E-02
MSH3	0.32	4.21E-03	4.44E-02
MIF	0.31	4.98E-03	4.95E-02
NSDHL	0.31	2.99E-03	3.51E-02
CAPN2	0.31	2.23E-04	6.09E-03
GCNT1	0.31	3.64E-03	4.02E-02
CDK16	0.31	1.88E-03	2.65E-02
MTIF2	0.31	4.40E-03	4.56E-02
COX7B	0.31	4.64E-03	4.73E-02
CYTH2	0.31	3.40E-04	8.11E-03
COX6C	0.31	2.58E-03	3.21E-02
AP2S1	0.31	2.82E-03	3.39E-02
PSMB6	0.31	1.91E-03	2.67E-02
CLINT1	0.31	3.17E-04	7.80E-03
TWF1	0.30	2.00E-04	5.57E-03
RILPL2	0.30	3.40E-03	3.82E-02
PON2	0.30	1.61E-03	2.40E-02
DHRS3	0.30	1.70E-03	2.50E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
PSMB3	0.30	1.02E-03	1.77E-02
CTSD	0.30	2.43E-03	3.08E-02
CNNM4	0.30	2.13E-03	2.86E-02
COQ9	0.30	4.88E-03	4.89E-02
NDFIP2	0.30	2.03E-04	5.65E-03
CTAGE5	0.30	3.17E-03	3.66E-02
NDUFA6	0.30	4.37E-03	4.55E-02
TSPAN3	0.30	2.03E-03	2.78E-02
PRSS23	0.30	2.25E-03	2.95E-02
CAMSAP1	0.29	4.55E-03	4.67E-02
PRPS2	0.29	2.74E-03	3.33E-02
SYTL1	0.29	4.30E-03	4.50E-02
PACSIN2	0.29	9.73E-04	1.72E-02
MFSD1	0.29	3.00E-03	3.52E-02
RNF144B	0.29	1.02E-03	1.77E-02
ERBB3	0.29	2.83E-03	3.39E-02
PPP1R7	0.29	6.58E-04	1.29E-02
ROD1	0.29	1.15E-03	1.94E-02
LASS6	0.29	2.45E-03	3.10E-02
VANGL1	0.28	3.01E-03	3.53E-02
DDR1	0.28	5.79E-05	2.29E-03
KCNQ1	0.28	4.73E-03	4.80E-02
GPD2	0.28	4.24E-03	4.46E-02
ICT1	0.28	1.30E-03	2.08E-02
SOX2	0.27	4.38E-03	4.55E-02
NUDT5	0.27	3.07E-03	3.57E-02
TRAF4	0.27	4.73E-03	4.80E-02
BRP44	0.27	2.77E-03	3.36E-02
UQCR10	0.27	1.48E-03	2.27E-02
SRP68	0.27	2.77E-03	3.36E-02
HEBP2	0.27	4.91E-03	4.90E-02
MAGEF1	0.27	1.42E-03	2.21E-02
PMVK	0.27	2.25E-03	2.95E-02
NDUFAB1	0.27	8.52E-04	1.54E-02
PEF1	0.27	3.32E-04	7.95E-03
CTBP2	0.27	4.19E-05	1.83E-03
CCT6A	0.27	1.97E-03	2.73E-02
MYO6	0.26	3.44E-03	3.86E-02
CCT2	0.26	3.61E-03	4.00E-02
FAM60A	0.26	3.34E-03	3.78E-02
TRIM2	0.26	5.28E-04	1.12E-02
MOSPD3	0.26	4.29E-03	4.50E-02
ATP5I	0.25	1.67E-03	2.46E-02
KIAA0146	0.25	4.96E-04	1.06E-02
HSPA8	0.25	2.65E-04	6.88E-03
CTSB	0.25	4.43E-03	4.58E-02
CSDA	0.25	1.24E-03	2.03E-02
HSBP1	0.24	7.26E-04	1.37E-02
GLUL	0.24	2.40E-03	3.06E-02
UBE2H	0.24	4.59E-03	4.70E-02
COX5B	0.24	4.16E-03	4.40E-02
ATP6V1G1	0.24	3.26E-03	3.72E-02
NDUFB2	0.24	4.94E-04	1.06E-02
STK24	0.24	1.57E-03	2.35E-02
TRAF7	0.24	1.36E-03	2.16E-02
PNPO	0.24	2.35E-03	3.02E-02
RDH13	0.24	2.68E-03	3.29E-02
KPNA6	0.24	1.82E-03	2.61E-02
SMARCC1	0.24	6.37E-04	1.26E-02
NDUFS3	0.24	2.83E-04	7.23E-03

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
TUBA1B	0.23	3.49E-03	3.90E-02
HDGF	0.23	1.07E-03	1.82E-02
RAB11A	0.23	2.23E-03	2.92E-02
UBE2N	0.23	2.40E-03	3.05E-02
APTX	0.23	2.19E-03	2.90E-02
HNRNPF	0.23	4.67E-03	4.75E-02
CHP	0.23	9.64E-04	1.71E-02
TMBIM6	0.23	2.09E-03	2.83E-02
FAM103A1	0.23	2.42E-03	3.07E-02
PFKP	0.22	2.87E-03	3.41E-02
NDUFB1	0.22	2.50E-03	3.15E-02
PSMD10	0.22	3.35E-03	3.78E-02
NDUFS6	0.22	8.23E-04	1.50E-02
CSNK1A1	0.22	1.20E-03	1.99E-02
HSPD1	0.22	3.66E-03	4.02E-02
ATP5L	0.22	4.56E-03	4.68E-02
NFE2L2	0.22	3.40E-03	3.82E-02
UBE2J2	0.21	3.04E-03	3.54E-02
ENSA	0.21	2.60E-03	3.23E-02
NDUFA2	0.21	2.18E-03	2.90E-02
VPS35	0.21	1.73E-03	2.52E-02
LOC100507029	0.21	3.05E-03	3.55E-02
MDM2	0.21	1.48E-03	2.27E-02
MYL12B	0.21	3.26E-03	3.72E-02
ATP5A1	0.20	1.70E-03	2.49E-02
PSMD4	0.19	4.72E-03	4.80E-02
TMEM9B	0.19	4.25E-03	4.47E-02
ATP6V0C	0.19	2.89E-03	3.42E-02
TMCO1	0.18	2.33E-03	3.01E-02
AIMP2	0.16	4.15E-03	4.40E-02
SRRM2	-0.16	2.33E-03	3.01E-02
CNBP	-0.17	4.50E-03	4.64E-02
BRD4	-0.17	9.93E-04	1.74E-02
SNRNP70	-0.18	2.09E-03	2.83E-02
GDI1	-0.18	5.38E-04	1.13E-02
RBM39	-0.18	2.13E-03	2.86E-02
SF3B1	-0.18	3.59E-03	3.99E-02
HNRNPA3	-0.18	2.60E-03	3.23E-02
SMARCA2	-0.18	7.63E-04	1.42E-02
FBXL3	-0.19	2.73E-03	3.33E-02
HNRNPH1	-0.20	2.62E-03	3.24E-02
USP48	-0.20	2.28E-03	2.97E-02
RBM25	-0.20	2.31E-03	2.99E-02
SRSF2IP	-0.20	1.42E-03	2.21E-02
MLL3	-0.20	2.03E-03	2.78E-02
FGFR1	-0.21	3.64E-03	4.02E-02
JMJD1C	-0.21	3.66E-03	4.02E-02
MGEA5	-0.21	4.79E-03	4.82E-02
PSAP	-0.21	3.02E-04	7.57E-03
SPTAN1	-0.21	1.64E-03	2.43E-02
PCMTD1	-0.22	3.34E-03	3.78E-02
ZNF135	-0.22	5.00E-03	4.96E-02
EMP3	-0.22	4.06E-03	4.32E-02
ARGLU1	-0.22	1.91E-03	2.67E-02
SDCCAG1	-0.22	1.43E-03	2.21E-02
ARL6IP5	-0.22	2.39E-03	3.05E-02
ZBTB38	-0.22	4.61E-03	4.71E-02
ADD1	-0.22	3.28E-04	7.90E-03
NOTCH1	-0.23	5.40E-04	1.13E-02
GLG1	-0.23	3.98E-03	4.26E-02

Bronchial biopsies transcriptome							
Female severe asthma (n=23) v Female healthy controls (n=10)				Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			6	Number of upregulated genes			566
Number of downregulated genes			7	Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
				EPC1	-0.23	4.08E-03	4.34E-02
				SEC62	-0.23	4.76E-03	4.80E-02
				MGA	-0.23	1.14E-03	1.92E-02
				ZNF43	-0.23	2.28E-03	2.97E-02
				LUC7L3	-0.23	1.20E-03	2.00E-02
				MACF1	-0.23	4.03E-03	4.31E-02
				DDX6	-0.23	6.09E-05	2.38E-03
				LOC100190939	-0.23	8.75E-04	1.57E-02
				SRSF7	-0.23	1.03E-03	1.78E-02
				C16orf58	-0.23	3.51E-03	3.92E-02
				NBPF12	-0.24	2.38E-03	3.04E-02
				HEXA	-0.24	2.82E-03	3.39E-02
				ATF7	-0.24	1.55E-03	2.33E-02
				RNF146	-0.24	1.30E-03	2.08E-02
				SOS1	-0.24	2.02E-03	2.77E-02
				SEL1L	-0.24	1.11E-03	1.87E-02
				GOPC	-0.24	2.72E-03	3.33E-02
				MEF2D	-0.24	2.80E-03	3.38E-02
				FLCN	-0.24	4.19E-03	4.42E-02
				POLR2J2	-0.24	2.86E-03	3.40E-02
				LOC100132832	-0.24	4.89E-03	4.89E-02
				PHC3	-0.25	4.56E-03	4.68E-02
				GAS6	-0.25	2.34E-03	3.01E-02
				NCOA1	-0.25	3.73E-03	4.07E-02
				STAG3L1	-0.25	3.06E-03	3.56E-02
				MEF2A	-0.25	1.98E-03	2.73E-02
				FBRSL1	-0.25	4.45E-04	9.83E-03
				NCOA2	-0.25	5.89E-04	1.20E-02
				ADAM33	-0.25	6.85E-04	1.32E-02
				SH3BP5	-0.26	2.87E-03	3.41E-02
				N4BP2L2	-0.26	1.54E-03	2.32E-02
				ATXN1	-0.26	8.29E-04	1.51E-02
				ANKRD12	-0.26	8.15E-04	1.49E-02
				PIAS1	-0.26	1.87E-03	2.65E-02
				COL16A1	-0.26	4.36E-03	4.55E-02
				HYI	-0.26	1.25E-03	2.04E-02
				OGT	-0.26	5.66E-04	1.17E-02
				KCTD7	-0.26	2.00E-03	2.75E-02
				CTDSPL2	-0.27	1.37E-03	2.16E-02
				TOR1AIP1	-0.27	3.66E-03	4.02E-02
				KIAA1109	-0.27	3.13E-03	3.64E-02
				CDC42EP3	-0.27	2.97E-03	3.50E-02
				CHST14	-0.27	4.03E-03	4.31E-02
				ANKRD10	-0.27	6.33E-04	1.26E-02
				NUMA1	-0.27	6.40E-05	2.47E-03
				RBM12B	-0.27	4.09E-03	4.34E-02
				PRR5	-0.27	2.93E-03	3.46E-02
				RBL2	-0.27	7.39E-04	1.40E-02
				NOTCH2	-0.27	2.33E-03	3.01E-02
				LOC389634	-0.28	4.38E-03	4.55E-02
				CDKN1C	-0.28	4.76E-03	4.80E-02
				PAPLN	-0.28	4.74E-03	4.80E-02
				GATA2	-0.28	4.75E-03	4.80E-02
				SELK	-0.28	1.80E-03	2.59E-02
				RBPJ	-0.28	2.18E-03	2.90E-02
				DEF6	-0.28	2.37E-03	3.03E-02
				S100PBP	-0.28	3.01E-03	3.53E-02
				SEC63	-0.29	4.51E-03	4.65E-02
				POLR3GL	-0.29	2.13E-03	2.86E-02
				UBASH3A	-0.29	4.74E-03	4.80E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
SNRK	-0.29	2.94E-03	3.47E-02
ARHGAP17	-0.29	4.55E-03	4.67E-02
EPM2AIP1	-0.29	2.26E-03	2.95E-02
CBFA2T3	-0.30	4.14E-03	4.39E-02
MYLIP	-0.30	4.54E-04	9.94E-03
SLC10A3	-0.30	3.73E-03	4.07E-02
USP6	-0.30	3.33E-03	3.78E-02
EDEM1	-0.30	1.30E-03	2.08E-02
GOLGA8H	-0.30	2.50E-03	3.14E-02
ARHGAP31	-0.30	3.40E-03	3.82E-02
C9orf95	-0.30	2.14E-03	2.87E-02
MAN2B1	-0.30	1.50E-04	4.42E-03
ANKH	-0.30	1.03E-03	1.77E-02
LPIN1	-0.30	3.21E-03	3.68E-02
AR	-0.30	2.34E-03	3.01E-02
FOLR2	-0.31	4.42E-03	4.57E-02
CWF19L2	-0.31	1.24E-03	2.03E-02
MPHOSPH8	-0.31	5.97E-04	1.21E-02
FLJ10357	-0.31	2.06E-03	2.80E-02
SNX22	-0.31	2.54E-03	3.18E-02
NFIB	-0.31	5.06E-03	5.00E-02
CBX7	-0.31	3.36E-03	3.80E-02
SYT15	-0.31	1.59E-03	2.37E-02
TMEM98	-0.31	4.97E-03	4.94E-02
NFIX	-0.31	5.94E-05	2.33E-03
LDB1	-0.31	1.93E-03	2.68E-02
EVC	-0.32	4.57E-03	4.68E-02
CD47	-0.32	1.18E-05	7.00E-04
VPS13B	-0.32	3.45E-04	8.18E-03
CMTM3	-0.32	3.69E-03	4.03E-02
IL11RA	-0.32	2.39E-03	3.05E-02
ZNF503	-0.32	2.05E-03	2.80E-02
SFRS18	-0.32	2.47E-04	6.53E-03
RNF123	-0.32	3.74E-03	4.07E-02
AKT3	-0.32	3.15E-03	3.64E-02
PKP4	-0.32	1.51E-03	2.30E-02
ZBTB4	-0.33	2.35E-03	3.02E-02
EZH1	-0.33	1.29E-04	4.02E-03
MFAP2	-0.33	3.86E-03	4.17E-02
FRMD4A	-0.33	2.28E-04	6.15E-03
FAM127A	-0.33	1.07E-03	1.82E-02
SLC27A3	-0.33	4.29E-03	4.50E-02
KPNA5	-0.33	3.02E-03	3.53E-02
PNCK	-0.34	2.21E-03	2.91E-02
ZBTB20	-0.34	6.78E-04	1.31E-02
BDH2	-0.34	2.19E-03	2.90E-02
ZNF322A	-0.34	1.87E-03	2.65E-02
PDGFRB	-0.34	3.19E-03	3.67E-02
RCN3	-0.34	3.25E-03	3.72E-02
DNM1	-0.34	4.27E-04	9.60E-03
GLS	-0.34	3.21E-04	7.81E-03
TBX2	-0.34	2.73E-03	3.33E-02
ZNF827	-0.34	1.37E-03	2.16E-02
INSIG1	-0.34	2.20E-03	2.91E-02
NEK3	-0.34	2.21E-03	2.91E-02
SEMA4D	-0.34	3.24E-03	3.71E-02
TEAD2	-0.34	2.15E-05	1.10E-03
HBP1	-0.34	7.36E-05	2.71E-03
HOXB5	-0.34	1.02E-03	1.77E-02
RORA	-0.34	6.16E-04	1.24E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
POLR3D	-0.35	4.70E-03	4.78E-02
CSAD	-0.35	7.19E-05	2.69E-03
CALHM2	-0.35	2.69E-03	3.30E-02
CTSF	-0.35	1.61E-04	4.64E-03
CLK4	-0.35	9.91E-05	3.36E-03
NCRNA00201	-0.35	2.16E-03	2.88E-02
CYorf15B	-0.35	1.53E-03	2.31E-02
LOC100510649	-0.35	1.06E-03	1.82E-02
AK4	-0.35	3.90E-03	4.20E-02
OAF	-0.35	8.78E-04	1.58E-02
MTMR11	-0.35	2.83E-03	3.39E-02
TMEM39A	-0.35	1.80E-04	5.09E-03
ZNF275	-0.35	4.54E-03	4.67E-02
SEPT8	-0.35	2.10E-04	5.81E-03
MBNL1	-0.35	1.42E-04	4.26E-03
CDKN1B	-0.35	4.94E-05	2.06E-03
FLJ38717	-0.36	4.16E-03	4.40E-02
ZNF704	-0.36	2.74E-03	3.33E-02
GPRC5B	-0.36	1.10E-03	1.87E-02
IPW	-0.36	1.95E-03	2.71E-02
PBXIP1	-0.36	3.57E-05	1.61E-03
ATP8B2	-0.36	3.76E-04	8.77E-03
ETS1	-0.36	1.41E-03	2.20E-02
PRCP	-0.36	1.06E-03	1.81E-02
STXBP1	-0.36	1.69E-03	2.49E-02
HERC1	-0.37	4.23E-04	9.55E-03
PDGFC	-0.37	3.18E-03	3.67E-02
C11orf68	-0.37	2.33E-04	6.26E-03
NTAN1	-0.37	1.10E-03	1.87E-02
SENP7	-0.37	6.84E-04	1.32E-02
ITGA9	-0.37	8.11E-04	1.49E-02
HERPUD1	-0.37	9.76E-04	1.72E-02
PLEKHO2	-0.37	6.87E-04	1.32E-02
BOC	-0.37	1.88E-03	2.65E-02
KCNN3	-0.38	5.04E-03	5.00E-02
MDM4	-0.38	1.04E-05	6.49E-04
ZRANB2	-0.38	1.04E-05	6.49E-04
SH3BGRL	-0.38	1.39E-04	4.21E-03
PJA1	-0.38	3.65E-03	4.02E-02
EVL	-0.38	2.26E-06	2.13E-04
CYTH3	-0.38	6.46E-04	1.28E-02
CTSO	-0.38	7.04E-04	1.34E-02
HTRA3	-0.38	6.68E-06	4.85E-04
NDN	-0.38	2.82E-03	3.39E-02
MLL	-0.38	9.02E-06	6.00E-04
GLIPR1	-0.38	1.91E-03	2.67E-02
SPTBN1	-0.38	4.87E-05	2.04E-03
PODN	-0.38	9.13E-04	1.63E-02
ATM	-0.39	9.99E-07	1.12E-04
DDAH2	-0.39	2.24E-06	2.13E-04
ZNF780B	-0.39	9.13E-04	1.63E-02
TMEM57	-0.39	7.63E-04	1.42E-02
HCST	-0.39	3.12E-04	7.71E-03
VAMP2	-0.39	9.58E-06	6.24E-04
C1orf115	-0.39	1.12E-03	1.90E-02
SETBP1	-0.39	3.95E-03	4.24E-02
ARHGEF40	-0.39	5.58E-04	1.16E-02
PXK	-0.39	5.65E-04	1.17E-02
GNB4	-0.40	1.06E-04	3.49E-03
ORAI1	-0.40	4.84E-03	4.86E-02

Bronchial biopsies transcriptome			
Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes	6		
Number of downregulated genes	7		
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes	566		
Number of downregulated genes	646		
Gene	**Log2 fold change**	***P* value**	**FDR**
BCL6B	-0.40	1.27E-04	3.97E-03
KIRREL	-0.40	2.91E-03	3.44E-02
TSPYL2	-0.40	4.67E-05	2.00E-03
COL8A2	-0.40	2.28E-03	2.97E-02
PPP1R12A	-0.40	1.53E-03	2.32E-02
PDE2A	-0.40	1.26E-03	2.05E-02
UBE2E2	-0.40	3.81E-03	4.13E-02
ZNF22	-0.40	1.83E-03	2.63E-02
C9orf47	-0.41	3.15E-03	3.64E-02
RNASET2	-0.41	2.61E-03	3.23E-02
SELM	-0.41	4.95E-03	4.93E-02
GOLIM4	-0.41	2.82E-04	7.23E-03
LOC100505679	-0.41	2.65E-03	3.26E-02
LOC100422781	-0.41	1.66E-03	2.45E-02
CCDC7	-0.41	2.87E-03	3.41E-02
C5orf56	-0.41	1.17E-04	3.74E-03
FAM46A	-0.41	2.19E-03	2.90E-02
NCRNA00182	-0.41	1.50E-03	2.30E-02
NES	-0.41	2.55E-03	3.19E-02
RUNX1T1	-0.41	1.86E-03	2.65E-02
MARVELD1	-0.41	2.19E-04	6.01E-03
ZNF266	-0.42	3.24E-03	3.71E-02
TPST2	-0.42	1.74E-03	2.53E-02
SLC22A17	-0.42	2.18E-03	2.90E-02
ATP11C	-0.42	4.67E-05	2.00E-03
GALM	-0.42	8.44E-05	2.98E-03
GIMAP5	-0.42	3.59E-03	3.99E-02
RAB11FIP3	-0.42	1.94E-03	2.69E-02
MXRA8	-0.42	3.20E-04	7.81E-03
TCF12	-0.42	7.67E-04	1.43E-02
TNFSF12	-0.42	4.96E-04	1.06E-02
TTC28	-0.42	4.90E-03	4.90E-02
RASGRP2	-0.42	1.86E-03	2.65E-02
ITGB7	-0.42	4.01E-04	9.13E-03
ZNF483	-0.42	9.04E-04	1.62E-02
ERN1	-0.42	1.32E-04	4.08E-03
DENND5A	-0.43	1.04E-03	1.79E-02
LOC100507237	-0.43	2.59E-03	3.22E-02
NR1D2	-0.43	2.10E-03	2.84E-02
C22orf25	-0.43	4.55E-04	9.95E-03
FN3K	-0.43	2.64E-04	6.87E-03
ENO2	-0.43	4.91E-04	1.06E-02
ARMCX2	-0.43	4.43E-03	4.58E-02
ZNF37BP	-0.43	1.34E-03	2.13E-02
SKAP1	-0.43	1.69E-03	2.49E-02
RFTN1	-0.43	1.35E-03	2.15E-02
ZDBF2	-0.43	3.87E-03	4.17E-02
GPSM1	-0.44	1.56E-03	2.34E-02
APOE	-0.44	4.53E-03	4.67E-02
SPOCK2	-0.44	1.25E-04	3.92E-03
AXL	-0.44	1.33E-03	2.11E-02
PHTF2	-0.44	2.95E-05	1.42E-03
CAPRIN2	-0.44	2.86E-05	1.39E-03
KIF26A	-0.44	7.17E-04	1.36E-02
IGSF8	-0.45	3.06E-04	7.67E-03
AGAP11	-0.45	8.00E-05	2.87E-03
BHLHE41	-0.45	4.97E-03	4.94E-02
TNXB	-0.45	2.90E-06	2.53E-04
LAMA4	-0.45	5.64E-04	1.17E-02
LDOC1	-0.45	4.58E-03	4.69E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
RAB8B	-0.45	1.79E-04	5.08E-03
PECAM1	-0.45	2.15E-03	2.88E-02
FAM65A	-0.45	5.62E-06	4.23E-04
ITM2C	-0.45	1.42E-05	7.96E-04
CCDC88A	-0.46	3.98E-04	9.09E-03
ZBTB46	-0.46	2.83E-06	2.52E-04
FBLN5	-0.46	4.30E-03	4.50E-02
ANO6	-0.46	3.18E-05	1.49E-03
PPP1R3B	-0.46	1.02E-03	1.77E-02
MAGEH1	-0.46	1.03E-04	3.43E-03
C3orf42	-0.46	1.02E-03	1.77E-02
PLXND1	-0.46	7.11E-05	2.68E-03
SEPT6	-0.46	4.85E-05	2.04E-03
MS4A6A	-0.46	2.58E-03	3.21E-02
SHE	-0.46	1.19E-03	1.98E-02
LOC286437	-0.46	2.45E-04	6.52E-03
ATP8A1	-0.47	3.13E-05	1.49E-03
LOC400464	-0.47	3.19E-03	3.67E-02
VIM	-0.47	4.79E-03	4.82E-02
CXorf36	-0.47	1.21E-03	2.00E-02
AIF1L	-0.47	8.64E-04	1.56E-02
CNOT6L	-0.47	3.99E-03	4.27E-02
STMN3	-0.47	5.85E-05	2.31E-03
DNAJB9	-0.47	1.04E-03	1.79E-02
ASAM	-0.47	1.54E-03	2.32E-02
MAN1A1	-0.47	3.85E-03	4.17E-02
ANKRD44	-0.47	4.74E-04	1.03E-02
DAB2	-0.47	1.35E-03	2.15E-02
LOC100130468	-0.47	1.19E-04	3.78E-03
DPYSL2	-0.47	2.82E-03	3.39E-02
PTCH1	-0.48	3.80E-03	4.12E-02
GAL3ST4	-0.48	2.83E-04	7.23E-03
PECI	-0.48	2.47E-03	3.11E-02
ESAM	-0.48	1.11E-03	1.88E-02
ROBO3	-0.48	1.22E-03	2.01E-02
CREB3L2	-0.48	1.03E-05	6.49E-04
IL16	-0.48	5.07E-07	6.83E-05
RPL23AP32	-0.48	6.35E-04	1.26E-02
LOC400043	-0.48	3.21E-03	3.68E-02
MEOX1	-0.49	5.29E-05	2.15E-03
TRAF1	-0.49	4.06E-04	9.21E-03
ENTPD1	-0.49	9.28E-05	3.17E-03
LIMCH1	-0.49	2.59E-03	3.22E-02
ITPR2	-0.49	1.24E-04	3.92E-03
MRAS	-0.49	4.18E-05	1.83E-03
INPP5D	-0.49	8.11E-05	2.90E-03
IFFO1	-0.49	1.67E-05	8.99E-04
INMT	-0.49	8.14E-04	1.49E-02
SIT1	-0.49	3.20E-04	7.81E-03
KBTBD3	-0.50	8.87E-05	3.07E-03
FBLN2	-0.50	3.38E-03	3.81E-02
LIX1L	-0.50	1.97E-05	1.03E-03
TGFBI	-0.50	8.79E-05	3.06E-03
HMHA1	-0.50	7.07E-04	1.34E-02
LOC96610	-0.50	1.69E-04	4.85E-03
CTSW	-0.50	5.05E-03	5.00E-02
TBC1D4	-0.51	1.92E-03	2.68E-02
TSPAN33	-0.51	1.32E-04	4.08E-03
CSF1	-0.51	8.43E-08	1.51E-05
C1QTNF2	-0.51	5.03E-04	1.07E-02

Bronchial biopsies transcriptome			
Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
TBX3	-0.51	1.73E-04	4.96E-03
CHST11	-0.51	4.58E-04	9.97E-03
EFEMP2	-0.51	1.34E-07	2.17E-05
RBMS3	-0.51	1.82E-03	2.61E-02
CD74	-0.52	7.55E-04	1.41E-02
PTPRCAP	-0.52	4.45E-04	9.83E-03
GIMAP6	-0.52	1.75E-03	2.54E-02
TCF21	-0.52	3.69E-03	4.03E-02
NR3C1	-0.52	1.22E-08	3.52E-06
LOC400550	-0.52	5.77E-04	1.18E-02
S1PR1	-0.53	1.08E-03	1.83E-02
CNRIP1	-0.53	1.98E-03	2.73E-02
LIFR	-0.53	1.54E-03	2.32E-02
ZAK	-0.53	7.90E-05	2.87E-03
LOC389834	-0.53	6.98E-04	1.33E-02
RBPMS2	-0.53	5.89E-04	1.20E-02
DAPK1	-0.53	8.82E-05	3.06E-03
IL17RD	-0.53	6.66E-08	1.26E-05
PRDM6	-0.53	1.22E-03	2.02E-02
ECSCR	-0.54	1.35E-03	2.15E-02
PDK1	-0.54	9.51E-04	1.69E-02
FNBP1	-0.54	1.15E-09	5.99E-07
COL6A2	-0.54	6.62E-07	8.43E-05
ZNF385D	-0.54	1.39E-03	2.19E-02
TSPAN11	-0.54	2.57E-03	3.21E-02
CERCAM	-0.54	3.83E-04	8.85E-03
SCARNA17	-0.54	3.03E-05	1.44E-03
P2RY8	-0.54	9.77E-04	1.72E-02
ANKRD36BP2	-0.54	1.26E-03	2.05E-02
LOC100128252	-0.54	2.46E-04	6.52E-03
HSPA13	-0.54	5.44E-05	2.19E-03
LOC144571	-0.54	2.69E-04	6.92E-03
SLC39A10	-0.54	2.37E-03	3.03E-02
LOC283663	-0.55	2.54E-03	3.18E-02
IGHA1	-0.55	1.27E-07	2.08E-05
DIO2	-0.55	7.61E-04	1.42E-02
ANTXR1	-0.55	2.16E-05	1.10E-03
SERPING1	-0.56	2.29E-03	2.97E-02
ANXA6	-0.56	8.52E-04	1.54E-02
EVI2A	-0.56	6.99E-04	1.33E-02
MTMR9LP	-0.56	1.29E-04	4.01E-03
SLIT3	-0.56	1.22E-05	7.18E-04
SIGLEC6	-0.56	3.56E-05	1.61E-03
DEGS1	-0.56	1.88E-05	9.98E-04
PPM1M	-0.56	1.07E-05	6.58E-04
GIMAP4	-0.56	4.40E-03	4.56E-02
CD4	-0.56	9.15E-05	3.15E-03
C14orf139	-0.57	7.57E-04	1.42E-02
ZNF331	-0.57	3.69E-04	8.63E-03
MEG3	-0.57	1.49E-04	4.40E-03
FYN	-0.57	4.32E-04	9.66E-03
RARRES2	-0.57	3.10E-04	7.68E-03
CCND2	-0.57	6.03E-06	4.46E-04
THSD4	-0.57	1.85E-03	2.64E-02
LTBP4	-0.57	3.24E-06	2.76E-04
CHN1	-0.57	2.54E-04	6.68E-03
HLA-DQB1	-0.58	4.61E-03	4.71E-02
ID4	-0.58	1.92E-04	5.37E-03
PRKG1	-0.58	1.31E-05	7.53E-04
SGCE	-0.58	2.19E-03	2.90E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
HLA-DRB6	-0.58	2.26E-04	6.12E-03
FXYD5	-0.58	1.91E-05	1.01E-03
NRP1	-0.58	5.53E-06	4.19E-04
DCHS1	-0.58	1.01E-08	3.02E-06
NBL1	-0.59	1.09E-04	3.56E-03
SSPN	-0.59	2.38E-05	1.19E-03
HEG1	-0.59	4.07E-05	1.80E-03
CD248	-0.59	3.55E-12	3.87E-09
HOXA3	-0.59	1.97E-06	1.95E-04
PLXDC1	-0.59	9.67E-06	6.26E-04
BTN3A2	-0.59	3.53E-03	3.93E-02
FBN1	-0.60	1.85E-03	2.64E-02
PPP1R12B	-0.60	1.77E-03	2.57E-02
CGNL1	-0.60	5.63E-05	2.25E-03
LOC100505881	-0.60	4.30E-04	9.65E-03
HTRA1	-0.60	1.39E-03	2.18E-02
ID2	-0.60	6.54E-06	4.78E-04
TSPAN7	-0.60	9.04E-04	1.62E-02
HLF	-0.61	6.18E-04	1.24E-02
SEPP1	-0.61	3.06E-06	2.64E-04
SPARCL1	-0.61	4.63E-03	4.72E-02
ITGA8	-0.61	2.81E-03	3.39E-02
ZEB1	-0.61	3.87E-04	8.92E-03
IL10RA	-0.61	2.41E-03	3.06E-02
ARHGEF6	-0.61	5.85E-05	2.31E-03
A2M	-0.61	3.08E-04	7.67E-03
ST3GAL5	-0.61	3.78E-04	8.79E-03
DDR2	-0.62	2.68E-04	6.91E-03
MEF2C	-0.62	7.99E-07	9.46E-05
RNASE6	-0.62	5.73E-04	1.18E-02
CCR5	-0.62	2.05E-03	2.80E-02
COL15A1	-0.62	6.75E-04	1.31E-02
ABCA8	-0.62	1.17E-05	6.99E-04
GIMAP7	-0.62	9.86E-04	1.73E-02
CD79A	-0.62	1.81E-04	5.10E-03
DERL3	-0.63	1.72E-06	1.78E-04
EDIL3	-0.63	5.84E-04	1.19E-02
KANK2	-0.63	6.21E-04	1.24E-02
RASAL3	-0.63	2.45E-05	1.22E-03
TRAF5	-0.63	5.83E-06	4.36E-04
PEG3-AS	-0.63	3.43E-05	1.57E-03
CD27	-0.64	7.63E-06	5.31E-04
MAP4K1	-0.64	1.26E-05	7.31E-04
MRC2	-0.64	1.25E-07	2.07E-05
PCDH18	-0.64	1.98E-05	1.03E-03
KLRK1	-0.64	7.10E-05	2.68E-03
RASA4	-0.64	3.89E-04	8.93E-03
CD34	-0.64	8.68E-05	3.03E-03
POU2AF1	-0.64	1.14E-03	1.92E-02
CD48	-0.64	1.79E-04	5.08E-03
MMP2	-0.64	3.67E-03	4.02E-02
RAB30	-0.65	9.37E-09	2.88E-06
C1orf38	-0.65	3.80E-04	8.81E-03
ZKSCAN3	-0.65	4.06E-05	1.80E-03
TSPAN18	-0.65	8.26E-06	5.65E-04
CCDC3	-0.65	2.91E-04	7.40E-03
HSPA12B	-0.65	8.46E-07	9.76E-05
ADRA2A	-0.65	3.76E-03	4.09E-02
JAM3	-0.66	3.15E-04	7.78E-03
GEM	-0.66	1.92E-03	2.68E-02

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
ARMCX1	-0.66	1.42E-04	4.26E-03
CYBRD1	-0.66	6.71E-04	1.31E-02
PER3	-0.66	1.16E-05	6.99E-04
RAMP2	-0.66	1.10E-04	3.56E-03
HOXA4	-0.67	2.17E-07	3.37E-05
NR2F1	-0.67	1.39E-06	1.53E-04
PRKACB	-0.67	9.80E-08	1.70E-05
GATM	-0.67	3.17E-03	3.66E-02
TRIM78P	-0.67	8.28E-05	2.94E-03
FMNL3	-0.67	1.29E-08	3.61E-06
ZNF423	-0.67	4.22E-04	9.55E-03
PARVG	-0.67	3.01E-04	7.57E-03
WIPF1	-0.68	6.47E-06	4.76E-04
CPVL	-0.68	3.94E-03	4.23E-02
AQP1	-0.68	5.18E-05	2.12E-03
TIMP3	-0.68	5.77E-04	1.18E-02
CYP21A2	-0.68	1.07E-05	6.58E-04
TMC8	-0.68	8.76E-06	5.86E-04
CYS1	-0.69	1.23E-03	2.03E-02
LOC100506948	-0.69	3.25E-04	7.84E-03
PCOLCE	-0.69	7.30E-07	8.74E-05
C1S	-0.69	3.07E-04	7.67E-03
CIITA	-0.69	7.80E-04	1.44E-02
SMAD9	-0.69	5.18E-05	2.12E-03
C10orf72	-0.69	7.07E-07	8.55E-05
NLRC5	-0.69	1.61E-03	2.40E-02
RHOJ	-0.70	2.96E-05	1.43E-03
SACS	-0.70	6.82E-08	1.26E-05
SLIT2	-0.70	5.70E-05	2.27E-03
SPON1	-0.70	7.76E-04	1.44E-02
CD2	-0.70	7.19E-05	2.69E-03
TBX5	-0.70	4.30E-05	1.87E-03
C1orf54	-0.71	3.73E-07	5.45E-05
EPB41L2	-0.71	6.92E-05	2.62E-03
FOXF1	-0.71	3.07E-04	7.67E-03
HLA-DRA	-0.72	5.14E-05	2.11E-03
GUCY1A3	-0.72	4.07E-07	5.73E-05
GPR155	-0.72	3.04E-07	4.60E-05
MYOM1	-0.72	8.57E-04	1.55E-02
MS4A7	-0.72	1.60E-05	8.74E-04
PDE5A	-0.72	7.34E-05	2.71E-03
RASD2	-0.72	1.01E-05	6.46E-04
ENPP2	-0.72	6.66E-05	2.55E-03
PRO0471	-0.73	1.26E-03	2.05E-02
OSR2	-0.74	3.54E-05	1.60E-03
HLA-DRB1	-0.74	1.47E-05	8.16E-04
OLFML3	-0.74	8.10E-04	1.49E-02
COL6A1	-0.74	4.27E-08	9.64E-06
MAF	-0.74	9.29E-06	6.12E-04
AOC3	-0.74	1.24E-03	2.03E-02
PEAR1	-0.75	8.98E-05	3.10E-03
MPO	-0.75	3.77E-03	4.10E-02
SASH3	-0.75	6.75E-04	1.31E-02
IL7R	-0.76	8.46E-06	5.76E-04
CD3G	-0.76	1.57E-03	2.35E-02
KCNMA1	-0.76	5.98E-05	2.34E-03
HLA-DMA	-0.76	1.19E-04	3.78E-03
CD3E	-0.77	9.82E-04	1.73E-02
CFH	-0.77	1.13E-04	3.65E-03
LDB2	-0.77	6.30E-05	2.45E-03

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
CDR1	-0.77	3.40E-03	3.82E-02
COLEC12	-0.77	3.45E-03	3.86E-02
MATN2	-0.77	4.79E-08	9.89E-06
IFIT3	-0.77	1.37E-03	2.16E-02
IKZF3	-0.77	8.25E-06	5.65E-04
SEPT6	-0.78	3.13E-10	2.08E-07
KIAA1274	-0.78	4.77E-08	9.89E-06
COL1A1	-0.78	8.36E-10	5.01E-07
GPR124	-0.78	1.08E-05	6.58E-04
PKDCC	-0.78	1.51E-03	2.30E-02
ZEB2	-0.79	5.68E-10	3.58E-07
TIMP2	-0.79	1.10E-04	3.57E-03
MOXD1	-0.79	3.21E-05	1.50E-03
MGP	-0.80	1.18E-03	1.97E-02
GVINP1	-0.80	1.52E-04	4.46E-03
PENK	-0.80	1.43E-04	4.27E-03
AKAP12	-0.81	2.43E-04	6.50E-03
ITGAL	-0.81	1.25E-04	3.92E-03
SPARC	-0.81	1.93E-05	1.02E-03
ISLR	-0.81	2.45E-06	2.24E-04
LHFP	-0.82	2.34E-05	1.17E-03
ELF5	-0.82	3.81E-04	8.83E-03
FAT4	-0.83	4.52E-04	9.93E-03
MFAP4	-0.83	3.67E-03	4.02E-02
TRBC2	-0.83	1.99E-06	1.95E-04
SEMA6D	-0.83	3.44E-07	5.08E-05
CXCL10	-0.84	1.93E-03	2.69E-02
MPEG1	-0.84	3.92E-03	4.22E-02
CD37	-0.84	1.32E-04	4.08E-03
SLC40A1	-0.84	2.68E-07	4.11E-05
CCDC80	-0.84	3.03E-06	2.63E-04
SCARA5	-0.84	9.57E-06	6.24E-04
HLA-DPB1	-0.85	9.99E-05	3.37E-03
GPR126	-0.85	3.85E-07	5.50E-05
COL5A1	-0.85	4.14E-08	9.53E-06
CD3D	-0.85	4.05E-06	3.23E-04
FAM171B	-0.85	9.17E-05	3.15E-03
CD52	-0.86	3.67E-03	4.02E-02
RGN	-0.87	4.82E-05	2.03E-03
CHST1	-0.87	8.94E-04	1.60E-02
AEBP1	-0.87	1.20E-05	7.09E-04
BTN3A1	-0.87	2.21E-08	5.76E-06
LTBP2	-0.88	1.25E-04	3.92E-03
FLRT2	-0.88	1.36E-09	6.64E-07
FILIP1L	-0.88	2.83E-03	3.39E-02
KIT	-0.88	7.27E-05	2.70E-03
GZMA	-0.89	1.88E-03	2.65E-02
LOC399959	-0.90	8.71E-06	5.86E-04
IL2RG	-0.90	3.02E-04	7.57E-03
JAM2	-0.91	1.13E-07	1.93E-05
CPE	-0.91	8.04E-06	5.57E-04
GPNMB	-0.92	5.13E-05	2.11E-03
DCN	-0.92	4.77E-05	2.03E-03
LTBP1	-0.93	2.32E-06	2.16E-04
SMOC2	-0.94	6.39E-05	2.47E-03
CTSK	-0.94	1.96E-05	1.03E-03
PPP1R1B	-0.95	4.78E-03	4.81E-02
SEC14L3	-0.95	1.83E-03	2.63E-02
HSPB6	-0.96	1.47E-05	8.16E-04
PPAP2B	-0.96	3.75E-06	3.10E-04

Bronchial biopsies transcriptome

Female severe asthma (n=23) v Female healthy controls (n=10)			
Number of upregulated genes			6
Number of downregulated genes			7
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			566
Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**
GFRA1	-0.96	2.84E-06	2.52E-04
HLA-DPA1	-0.97	2.89E-06	2.53E-04
EDNRB	-0.98	7.31E-05	2.71E-03
LOC91316	-0.98	4.45E-08	9.67E-06
CRISPLD1	-0.98	6.73E-04	1.31E-02
IGLV6-57	-0.99	1.24E-05	7.26E-04
CCL5	-0.99	4.47E-04	9.86E-03
LOC100506718	-1.00	3.02E-10	2.08E-07
C11orf96	-1.00	2.14E-05	1.10E-03
ADH1B	-1.00	4.73E-06	3.68E-04
VCAM1	-1.00	4.70E-05	2.00E-03
BGN	-1.01	6.94E-06	4.98E-04
OMD	-1.01	4.80E-05	2.03E-03
CCL21	-1.01	3.11E-06	2.66E-04
ITM2A	-1.01	6.41E-08	1.24E-05
FGL2	-1.01	1.93E-09	8.57E-07
S1PR3	-1.02	2.25E-04	6.12E-03
CXCL12	-1.03	7.24E-11	5.80E-08
TGFB3	-1.04	3.88E-16	2.32E-12
TRAC	-1.05	2.01E-08	5.36E-06
MYOC	-1.05	1.87E-04	5.25E-03
LRRN4CL	-1.05	1.19E-03	1.99E-02
SOD3	-1.06	1.91E-08	5.19E-06
C5orf13	-1.07	4.86E-12	4.85E-09
FBLN1	-1.07	6.93E-07	8.46E-05
MGC29506	-1.09	6.84E-07	8.46E-05
FXYD6	-1.10	9.15E-10	5.22E-07
PI16	-1.11	7.26E-11	5.80E-08
COL1A2	-1.11	3.17E-08	7.75E-06
COL3A1	-1.13	6.75E-07	8.46E-05
SFRP4	-1.14	6.33E-04	1.26E-02
SLAMF7	-1.15	1.54E-06	1.65E-04
IGLV4-60	-1.15	2.23E-06	2.13E-04
TPSAB1	-1.17	6.83E-05	2.60E-03
PRRX1	-1.19	1.34E-12	1.61E-09
SFRP2	-1.19	1.82E-06	1.85E-04
APOC1	-1.20	5.11E-06	3.92E-04
COL14A1	-1.22	3.05E-09	1.26E-06
LOC100506621	-1.23	2.56E-08	6.53E-06
MXRA5	-1.24	6.01E-08	1.20E-05
CCL19	-1.24	1.42E-04	4.26E-03
IGL@	-1.25	9.06E-07	1.03E-04
CPA3	-1.26	4.43E-04	9.83E-03
APLNR	-1.27	1.04E-09	5.66E-07
TRBC1	-1.27	3.79E-05	1.69E-03
LOC100287927	-1.28	1.28E-05	7.38E-04
FRZB	-1.29	3.03E-03	3.54E-02
PTGDS	-1.32	5.06E-07	6.83E-05
LUM	-1.32	3.88E-06	3.16E-04
IGLV3-19	-1.33	4.68E-09	1.64E-06
CXCL14	-1.36	1.61E-11	1.48E-08
IGLV2-11	-1.38	6.80E-06	4.91E-04
LPO	-1.40	2.27E-03	2.96E-02
DPT	-1.40	1.42E-13	2.42E-10
CHRDL1	-1.43	2.71E-08	6.77E-06
IGLV2-23	-1.43	2.82E-05	1.38E-03
COL9A3	-1.44	1.30E-03	2.08E-02
IGLJ3	-1.45	6.27E-08	1.23E-05
TCEAL2	-1.46	3.68E-14	8.80E-11
IGHM	-1.51	4.07E-06	3.23E-04

Bronchial biopsies transcriptome							
Female severe asthma (n=23) v Female healthy controls (n=10)				Male severe asthma (n=23) v Male healthy controls (n=16)			
Number of upregulated genes			6	Number of upregulated genes			566
Number of downregulated genes			7	Number of downregulated genes			646
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
				IGKC	-1.66	4.35E-08	9.64E-06
				LOC100508797	-1.67	3.65E-17	4.37E-13
				IGLV1-40	-1.69	6.13E-07	7.98E-05
				CXCL9	-1.75	1.05E-05	6.53E-04
				MATN1	-1.76	1.37E-04	4.19E-03
				IGKV3-20	-1.83	1.14E-15	4.54E-12
				IGHG1	-1.89	6.90E-09	2.29E-06
				CHAD	-1.92	1.03E-06	1.15E-04
				OGN	-1.92	1.23E-08	3.52E-06
				IGLV1-36	-1.95	5.24E-08	1.06E-05
				IGKV4-1	-2.00	3.57E-09	1.35E-06
				IGHD	-2.07	6.53E-13	9.77E-10
				IGJ	-2.41	1.23E-12	1.61E-09
				SCGB3A2	-2.44	8.05E-07	9.46E-05
				COL2A1	-3.76	6.18E-14	1.23E-10
				CYTL1	-4.15	2.03E-15	6.09E-12

Table S4.9 All differentially expressed genes between mild to moderate asthma and healthy controls in females and males with blood transcriptomes.

Blood transcriptome							
Female mild/moderate asthma (n=37) v Female healthy controls (n=34)				Male mild/moderate asthma (n=40) v Male healthy controls (n=53)			
Number of upregulated genes			0	Number of upregulated genes			27
Number of downregulated genes			2	Number of downregulated genes			10
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
FLJ36848	-0.36	1.67E-06	1.69E-02	CCL23	1.03	9.05E-06	1.33E-02
KRT77	-1.41	2.45E-07	4.96E-03	PRSS33	0.90	7.46E-07	6.60E-03
				ALOX15	0.86	1.62E-05	1.64E-02
				OLIG2	0.72	2.62E-06	1.12E-02
				IDO1	0.70	3.15E-05	2.19E-02
				SIGLEC8	0.66	3.14E-05	2.19E-02
				ADORA3	0.64	1.00E-06	6.60E-03
				IL5RA	0.64	2.38E-05	1.84E-02
				VSTM1	0.59	8.66E-06	1.33E-02
				CCR3	0.54	3.41E-06	1.12E-02
				SLC29A1	0.52	1.80E-05	1.68E-02
				SMPD3	0.50	1.91E-05	1.68E-02
				CEBPE	0.48	1.40E-05	1.54E-02
				C10orf128	0.39	1.16E-04	4.25E-02
				MYCT1	0.36	7.05E-05	3.69E-02
				STXBP5	0.35	9.18E-05	3.88E-02
				SORD	0.35	1.28E-05	1.54E-02
				CAT	0.34	5.18E-05	3.10E-02
				COCH	-0.31	9.43E-05	3.88E-02
				NXT1	-0.33	7.49E-05	3.69E-02
				RAB11FIP3	-0.36	8.11E-05	3.69E-02
				LOC100509749	-0.39	6.91E-06	1.33E-02

Table S4.10 All differentially expressed genes between severe asthma and healthy controls in females and males with blood transcriptomes.

Blood transcriptome

Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**
DAAM2	1.48	9.96E-06	8.47E-03
OLFM4	1.32	1.24E-05	9.58E-03
CCL23	1.24	6.83E-06	7.70E-03
PRSS33	0.93	4.00E-05	1.63E-02
ARG1	0.89	3.55E-04	4.56E-02
BPI	0.86	2.89E-04	4.36E-02
RNASE3	0.84	3.16E-04	4.43E-02
ADORA3	0.81	9.75E-07	3.02E-03
IDO1	0.75	1.98E-04	3.76E-02
SIGLEC8	0.74	1.22E-04	3.13E-02
IL5RA	0.72	5.68E-05	1.85E-02
SLPI	0.70	8.71E-06	8.47E-03
RNASE2	0.64	1.67E-04	3.49E-02
GRB10	0.64	4.08E-04	4.56E-02
CEBPE	0.63	1.54E-05	1.12E-02
C2orf58	0.62	4.78E-04	4.63E-02
LOC100288781	0.61	1.83E-06	4.41E-03
INSC	0.60	1.53E-04	3.38E-02
ABP1	0.58	1.41E-04	3.35E-02
CLC	0.57	2.13E-04	3.88E-02
ZNF321	0.51	3.34E-05	1.63E-02
SLC5A9	0.50	3.02E-04	4.40E-02
SMPD3	0.48	3.84E-04	4.56E-02
LOC203274	0.47	1.96E-04	3.76E-02
CARD16	0.44	4.31E-04	4.56E-02
LOC100505956	0.42	5.59E-05	1.85E-02
C10orf128	0.42	8.51E-05	2.34E-02
GAPT	0.42	3.96E-05	1.63E-02
KCTD21	0.40	2.49E-06	4.41E-03
C3AR1	0.40	3.57E-05	1.63E-02
CYP4F12	0.39	2.73E-04	4.23E-02
NFIL3	0.34	2.96E-04	4.36E-02
GPR109B	0.33	2.93E-04	4.36E-02
ADAM8	0.31	2.21E-04	3.92E-02
HSPC157	0.31	3.45E-04	4.55E-02
UCK2	-0.32	1.83E-05	1.20E-02
ITM2C	-0.32	1.66E-04	3.49E-02
RAB11FIP3	-0.33	4.40E-04	4.56E-02
TNFRSF21	-0.34	1.90E-04	3.73E-02
SLC3A1	-0.35	1.30E-04	3.23E-02
CD38	-0.36	3.83E-04	4.56E-02
EPPK1	-0.41	2.61E-04	4.10E-02
LILRA4	-0.45	5.39E-05	1.85E-02
IGLJ3	-0.46	2.53E-04	4.10E-02
IGKV4-1	-0.50	2.62E-04	4.10E-02
IGLV2-11	-0.52	5.28E-04	4.92E-02
LOC100287927	-0.53	1.82E-04	3.65E-02
IGKV3-20	-0.54	1.66E-05	1.14E-02
IGLV1-40	-0.55	4.37E-04	4.56E-02
IGLV2-23	-0.55	7.75E-05	2.28E-02
CUX2	-0.62	9.46E-07	3.02E-03
IGLV1-36	-0.65	4.03E-04	4.56E-02
MGC29506	-0.85	8.08E-08	5.01E-04

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
OLFM4	2.42	2.94E-17	9.98E-14
MMP8	2.03	2.77E-17	9.98E-14
CRISP3	1.94	3.41E-17	9.98E-14
CEACAM6	1.86	2.09E-16	4.16E-13
DEFA4	1.84	3.83E-15	5.54E-12
CTSG	1.82	3.76E-17	9.98E-14
BPI	1.75	6.03E-17	1.37E-13
ARG1	1.63	4.71E-16	8.33E-13
LTF	1.62	3.26E-13	2.47E-10
DAAM2	1.58	9.04E-11	3.06E-08
OLAH	1.55	6.01E-12	3.09E-09
RNASE3	1.54	1.70E-14	2.08E-11
CEACAM8	1.54	6.61E-12	3.29E-09
LCN2	1.52	2.01E-13	1.69E-10
CD177	1.49	4.59E-10	1.03E-07
MS4A3	1.47	1.62E-12	1.03E-09
HP	1.43	2.79E-18	4.44E-14
OLR1	1.34	3.11E-11	1.18E-08
ELANE	1.31	4.52E-12	2.40E-09
CAMP	1.23	5.83E-18	4.64E-14
ABCA13	1.20	1.20E-09	2.35E-07
ANXA3	1.19	5.59E-14	5.23E-11
ORM1	1.19	4.01E-12	2.36E-09
MPO	1.18	6.89E-13	4.99E-10
CCL23	1.17	6.83E-08	5.20E-06
RNF182	1.13	1.73E-05	3.79E-04
TCN1	1.13	1.89E-12	1.15E-09
CLEC4D	1.04	3.24E-09	5.20E-07
TPST1	1.00	3.06E-09	4.96E-07
SH3GL3	0.96	2.13E-05	4.40E-04
SLPI	0.95	2.73E-13	2.17E-10
ACSM2A	0.93	9.50E-05	1.37E-03
BCL2A1	0.92	4.59E-08	3.85E-06
LECT2	0.89	1.46E-05	3.36E-04
ECHDC3	0.89	1.03E-08	1.29E-06
RETN	0.88	8.55E-12	4.00E-09
RNASE2	0.87	2.49E-11	9.76E-09
LY96	0.84	4.39E-07	2.21E-05
S100A12	0.82	4.83E-14	4.80E-11
C19orf59	0.82	2.60E-14	2.95E-11
IL1R2	0.81	9.95E-09	1.25E-06
INHBA	0.81	6.27E-08	4.92E-06
CLEC5A	0.80	1.50E-11	6.10E-09
LOC203274	0.79	1.79E-10	5.01E-08
NFE4	0.78	9.96E-11	3.30E-08
CEBPE	0.78	6.59E-11	2.33E-08
C12orf59	0.77	1.09E-06	4.64E-05
ASPH	0.77	7.95E-07	3.59E-05
SLC26A8	0.75	3.47E-07	1.79E-05
MMP9	0.75	8.15E-08	5.87E-06
TIMM8A	0.74	8.74E-04	7.38E-03
EVI2A	0.74	1.35E-05	3.19E-04
POM121L9P	0.73	5.90E-03	3.17E-02
GRB10	0.73	5.55E-07	2.68E-05
BMX	0.73	2.65E-09	4.39E-07
AZU1	0.73	1.62E-08	1.77E-06

Blood transcriptome

Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
DSC2	0.73	1.68E-08	1.80E-06
CD24	0.73	2.96E-08	2.76E-06
PFKFB2	0.73	1.56E-07	9.72E-06
UTS2	0.72	4.06E-04	4.09E-03
C2orf58	0.72	8.50E-09	1.13E-06
MOCS1	0.72	3.87E-06	1.22E-04
LOC643792	0.71	3.76E-04	3.86E-03
INSC	0.71	1.67E-07	1.02E-05
ANKRD22	0.71	4.98E-05	8.48E-04
F5	0.70	6.76E-16	1.08E-12
SULT1B1	0.69	2.23E-08	2.18E-06
IL18R1	0.68	4.06E-05	7.22E-04
SAMSN1	0.67	1.34E-07	8.65E-06
COX7B	0.67	1.63E-04	2.02E-03
GPR84	0.67	1.41E-09	2.70E-07
PRSS33	0.67	3.14E-04	3.34E-03
PGLYRP1	0.67	7.15E-08	5.37E-06
ADORA3	0.67	9.22E-08	6.50E-06
FGF13	0.66	7.71E-04	6.68E-03
CARD16	0.66	8.56E-10	1.77E-07
VSTM1	0.66	6.58E-09	9.43E-07
CHIT1	0.66	1.47E-07	9.35E-06
RPL22L1	0.65	5.13E-04	4.87E-03
COMMD8	0.64	3.23E-04	3.43E-03
RAB13	0.64	4.47E-12	2.40E-09
LOC283027	0.63	2.97E-03	1.89E-02
BEX1	0.63	3.08E-06	1.05E-04
NRG1	0.62	2.63E-07	1.45E-05
PRUNE2	0.61	5.22E-06	1.54E-04
LIN7A	0.60	3.32E-11	1.20E-08
ST6GALNAC3	0.60	7.13E-06	1.95E-04
COX6C	0.59	5.22E-04	4.92E-03
LOC100288781	0.59	1.54E-06	6.06E-05
VNN1	0.59	6.36E-06	1.78E-04
ALDOAP2	0.58	4.39E-04	4.34E-03
PPBP	0.58	5.19E-08	4.22E-06
C18orf32	0.57	4.07E-06	1.27E-04
OLIG2	0.57	2.14E-04	2.49E-03
LRRN1	0.57	7.44E-05	1.15E-03
ZNF267	0.57	7.63E-04	6.63E-03
SLC28A3	0.56	2.84E-05	5.44E-04
RPS27L	0.56	7.72E-05	1.17E-03
TLR5	0.56	1.33E-10	3.99E-08
FCGR1A	0.56	6.25E-05	1.00E-03
FOLR3	0.56	9.53E-03	4.49E-02
S100P	0.56	1.02E-04	1.44E-03
NDUFB3	0.55	2.60E-08	2.48E-06
LOC151438	0.55	8.95E-06	2.34E-04
SLC37A3	0.55	1.45E-05	3.35E-04
CLC	0.55	2.37E-05	4.78E-04
CSTA	0.55	1.84E-08	1.89E-06
LOC284751	0.55	2.16E-04	2.50E-03
ATP8B4	0.55	2.59E-05	5.12E-04
IL1R1	0.55	2.63E-06	9.30E-05
HECW2	0.54	2.79E-06	9.66E-05
ZNF354A	0.54	1.77E-04	2.15E-03
GGH	0.54	3.29E-06	1.09E-04
TMEM45A	0.54	1.00E-04	1.43E-03
C11orf74	0.53	2.88E-05	5.50E-04
CCRL1	0.53	2.75E-03	1.79E-02

Blood transcriptome			
Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
ACSL1	0.53	6.23E-09	9.26E-07
PMP22	0.53	3.79E-03	2.29E-02
PADI4	0.53	1.05E-07	7.22E-06
SLC22A15	0.52	8.21E-06	2.19E-04
DACH1	0.52	1.73E-07	1.05E-05
VSIG4	0.52	2.09E-06	7.66E-05
ZNF788	0.52	1.11E-05	2.72E-04
C5orf30	0.52	2.59E-04	2.89E-03
LOC100505956	0.52	5.67E-08	4.51E-06
CYP4F12	0.52	1.67E-08	1.80E-06
MAOA	0.52	2.15E-04	2.50E-03
THBS1	0.52	3.31E-07	1.72E-05
IL5RA	0.52	2.84E-04	3.10E-03
CDO1	0.52	1.11E-05	2.72E-04
SYCP1	0.51	8.18E-05	1.23E-03
SLC22A4	0.51	3.35E-08	3.04E-06
POLR2K	0.51	1.37E-03	1.05E-02
PROS1	0.51	1.30E-03	1.00E-02
PTX3	0.51	1.29E-04	1.72E-03
MYL9	0.51	1.44E-04	1.84E-03
DAPK2	0.51	6.47E-08	5.01E-06
PCOLCE2	0.51	2.74E-06	9.56E-05
FCGR1B	0.51	7.68E-05	1.17E-03
CD163	0.50	3.80E-08	3.33E-06
RBPMS2	0.50	5.09E-03	2.85E-02
RPL36A	0.50	2.15E-03	1.48E-02
SPTLC3	0.50	4.93E-03	2.77E-02
CYP4F2	0.50	9.89E-06	2.51E-04
GPR34	0.50	8.34E-05	1.24E-03
APOB48R	0.50	1.46E-05	3.36E-04
NUDT16P1	0.49	8.49E-09	1.13E-06
C5orf32	0.49	4.91E-08	4.05E-06
GAPT	0.49	1.11E-09	2.22E-07
MOSC1	0.49	2.72E-06	9.48E-05
PAQR6	0.49	5.57E-06	1.62E-04
CLTCL1	0.49	1.16E-07	7.76E-06
RPH3A	0.49	2.36E-03	1.60E-02
IRAK3	0.49	4.29E-06	1.31E-04
TXN	0.49	1.25E-10	3.87E-08
FLJ36644	0.49	1.36E-05	3.20E-04
IL18	0.49	1.36E-08	1.57E-06
DPY19L1P1	0.49	5.58E-05	9.21E-04
NEIL3	0.48	9.24E-05	1.34E-03
RSL24D1	0.48	1.10E-02	4.98E-02
IPMK	0.48	9.24E-04	7.68E-03
FCHO2	0.48	5.10E-04	4.84E-03
BCAT1	0.48	7.53E-05	1.16E-03
RNF175	0.48	1.27E-07	8.33E-06
SLCO4C1	0.48	5.53E-05	9.18E-04
SLC5A9	0.48	3.09E-05	5.83E-04
LOC100509635	0.48	3.00E-07	1.58E-05
NAT1	0.48	8.67E-04	7.35E-03
GPR160	0.47	4.07E-04	4.09E-03
ALPL	0.47	1.25E-05	3.01E-04
ANLN	0.47	3.74E-05	6.75E-04
WDFY3	0.47	6.64E-07	3.12E-05
NFIL3	0.47	7.51E-08	5.53E-06
CNTNAP3	0.47	4.31E-03	2.51E-02
C2orf76	0.47	6.45E-05	1.02E-03
STXBP5	0.47	2.29E-07	1.31E-05

Blood transcriptome

Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
S100A8	0.47	4.40E-12	2.40E-09
ABCA1	0.46	2.92E-05	5.58E-04
CA1	0.46	3.34E-03	2.07E-02
C8orf83	0.46	9.81E-07	4.29E-05
MGC70870	0.46	9.35E-08	6.52E-06
PYGL	0.46	3.75E-10	9.19E-08
KIAA0101	0.46	5.98E-06	1.71E-04
LOC100128737	0.46	1.78E-07	1.06E-05
TMTC1	0.46	4.96E-03	2.79E-02
FLJ39051	0.46	2.84E-06	9.81E-05
NCRNA00255	0.46	5.74E-03	3.11E-02
DHRS9	0.45	4.51E-04	4.43E-03
CA4	0.45	9.82E-06	2.50E-04
CYP1B1	0.45	5.93E-05	9.67E-04
LOC344595	0.45	1.07E-02	4.89E-02
FKBP9	0.45	6.32E-06	1.78E-04
FAS	0.45	5.43E-04	5.08E-03
TRPM6	0.45	1.71E-07	1.04E-05
RPS7	0.45	4.54E-03	2.61E-02
GPR97	0.45	2.43E-05	4.88E-04
SIPA1L2	0.45	4.26E-04	4.23E-03
MRPL13	0.45	2.83E-03	1.83E-02
SIGLEC8	0.45	3.24E-03	2.03E-02
HK3	0.45	3.29E-09	5.23E-07
TACSTD2	0.44	1.41E-04	1.82E-03
RPL34	0.44	4.54E-03	2.61E-02
ZNF608	0.44	3.61E-05	6.58E-04
CEACAM4	0.44	1.41E-04	1.82E-03
TLR2	0.44	1.19E-07	7.94E-06
QPCT	0.44	4.25E-07	2.16E-05
DYSF	0.44	3.44E-06	1.12E-04
CR1	0.44	7.42E-10	1.57E-07
RAB33B	0.44	1.36E-03	1.04E-02
SNORD89	0.44	3.07E-06	1.04E-04
NQO2	0.44	5.69E-06	1.64E-04
ROPN1L	0.43	1.92E-06	7.20E-05
PIWIL4	0.43	6.64E-07	3.12E-05
MRVI1	0.43	1.35E-06	5.50E-05
TMEM88	0.43	2.57E-05	5.09E-04
HIST1H3E	0.43	1.93E-04	2.30E-03
RPL26L1	0.43	4.30E-05	7.55E-04
ST3GAL6	0.43	1.71E-05	3.76E-04
RRAGD	0.43	2.12E-08	2.12E-06
ZNF117	0.43	1.84E-03	1.32E-02
LGALS12	0.43	2.57E-04	2.88E-03
ITGA2B	0.43	5.15E-04	4.87E-03
CXCL6	0.43	3.90E-04	3.96E-03
IDO1	0.43	7.68E-03	3.85E-02
TP53I3	0.43	3.60E-08	3.22E-06
CYBRD1	0.43	1.70E-05	3.74E-04
HIST1H2BC	0.43	2.85E-05	5.45E-04
KAZ	0.43	1.94E-05	4.13E-04
PDCD10	0.43	1.20E-03	9.45E-03
EXOC8	0.42	1.51E-03	1.13E-02
FAM133A	0.42	7.61E-04	6.63E-03
LOC100271840	0.42	5.59E-04	5.20E-03
COL9A2	0.42	3.65E-05	6.63E-04
GPR109B	0.42	7.50E-07	3.45E-05
MFSD9	0.42	7.86E-05	1.19E-03
ABHD13	0.42	9.36E-03	4.44E-02

Blood transcriptome

Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
ZNF321	0.42	1.06E-04	1.47E-03
ERG	0.42	9.69E-06	2.48E-04
SLC8A1	0.42	8.71E-07	3.87E-05
FAM198B	0.42	1.54E-04	1.94E-03
MMP25	0.42	1.53E-05	3.48E-04
NAIP	0.42	3.28E-07	1.71E-05
ASGR2	0.41	3.43E-05	6.35E-04
BEND7	0.41	2.56E-04	2.87E-03
HAUS4	0.41	1.81E-06	6.89E-05
LOC100134822	0.41	6.27E-05	1.00E-03
EAF2	0.41	4.53E-03	2.61E-02
KREMEN1	0.41	4.74E-05	8.19E-04
LILRA5	0.41	1.65E-04	2.04E-03
C14orf2	0.41	1.84E-04	2.22E-03
GALNT14	0.41	6.55E-06	1.83E-04
SLC29A1	0.41	7.95E-04	6.87E-03
LRG1	0.41	1.56E-04	1.96E-03
SGMS1	0.41	3.43E-03	2.11E-02
RAB32	0.41	7.94E-14	7.02E-11
C10orf128	0.41	3.09E-06	1.05E-04
CES1	0.41	3.99E-03	2.37E-02
LTB4R	0.41	7.86E-07	3.56E-05
C21orf130	0.41	5.42E-03	2.99E-02
SPP1	0.41	1.10E-02	4.99E-02
JAG1	0.40	1.17E-05	2.85E-04
SUCNR1	0.40	5.24E-06	1.54E-04
C14orf129	0.40	1.04E-02	4.80E-02
SIGLEC5	0.40	1.34E-04	1.76E-03
ENTPD1	0.40	5.99E-09	8.99E-07
FAR2	0.40	4.71E-06	1.42E-04
HNMT	0.40	2.10E-05	4.38E-04
EMR2	0.40	1.68E-05	3.71E-04
KCTD21	0.40	2.83E-08	2.68E-06
SLC22A1	0.40	1.01E-04	1.43E-03
ARAP3	0.40	9.71E-06	2.49E-04
NDC80	0.40	6.32E-04	5.73E-03
MEGF9	0.40	2.79E-07	1.52E-05
GALNT4	0.39	2.02E-03	1.42E-02
DUSP1	0.39	1.03E-05	2.59E-04
FPR2	0.39	9.50E-05	1.37E-03
REEP4	0.39	4.21E-04	4.19E-03
LIG4	0.39	8.36E-03	4.11E-02
LRRC4	0.39	2.37E-05	4.77E-04
RGL4	0.39	7.82E-07	3.56E-05
SORT1	0.39	1.25E-06	5.16E-05
HEBP2	0.39	4.17E-10	9.62E-08
BEST1	0.39	5.05E-07	2.49E-05
ANKRD28	0.39	2.05E-03	1.44E-02
COX7C	0.39	4.27E-03	2.49E-02
AQP9	0.39	1.62E-09	3.00E-07
UQCRQ	0.39	6.68E-05	1.05E-03
MSRB3	0.39	1.23E-07	8.09E-06
CASP5	0.39	2.90E-03	1.86E-02
HAT1	0.39	1.63E-03	1.20E-02
SMPD3	0.39	2.88E-04	3.12E-03
SLC22A16	0.39	5.97E-04	5.47E-03
CARD6	0.38	5.76E-09	8.73E-07
KLHL2	0.38	2.02E-03	1.42E-02
LOC100131801	0.38	1.75E-05	3.81E-04
CACNA1E	0.38	5.36E-06	1.57E-04

Blood transcriptome			
Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
HMGB2	0.38	1.42E-03	1.08E-02
GADD45A	0.38	3.53E-05	6.49E-04
LSM3	0.38	1.26E-03	9.82E-03
DPH3	0.38	2.19E-08	2.15E-06
PIK3R6	0.38	1.78E-04	2.16E-03
SRPK1	0.38	2.95E-06	1.01E-04
NDUFA4	0.38	1.31E-03	1.01E-02
B3GNT5	0.38	7.46E-04	6.52E-03
KCNE1	0.38	1.40E-05	3.27E-04
LOC401233	0.38	4.48E-03	2.59E-02
S100A9	0.38	1.08E-07	7.33E-06
psiTPTE22	0.38	8.28E-07	3.72E-05
PLBD1	0.38	1.20E-08	1.43E-06
LOC100131607	0.38	1.05E-06	4.53E-05
PLOD2	0.38	8.25E-05	1.23E-03
FAM124B	0.38	2.53E-04	2.84E-03
ITGA9	0.38	1.28E-05	3.07E-04
LPAR4	0.37	5.12E-03	2.86E-02
NDUFA1	0.37	1.73E-07	1.05E-05
NCF4	0.37	2.44E-07	1.37E-05
KCNJ15	0.37	1.65E-04	2.05E-03
JAK2	0.37	1.60E-04	1.99E-03
GNG10	0.37	2.21E-04	2.55E-03
ACSL4	0.37	1.16E-04	1.58E-03
LOC285771	0.37	7.81E-05	1.18E-03
CCDC126	0.37	1.56E-03	1.16E-02
FAM19A2	0.37	6.31E-03	3.34E-02
CD9	0.37	1.06E-03	8.57E-03
TMEM167A	0.37	5.09E-05	8.60E-04
DHRS13	0.37	2.27E-04	2.59E-03
LPAR6	0.37	8.78E-03	4.25E-02
FAM106A	0.37	4.80E-04	4.64E-03
ADAMDEC1	0.37	2.26E-03	1.54E-02
CKS2	0.37	6.10E-03	3.26E-02
CEP55	0.37	1.09E-02	4.98E-02
FAM160B1	0.36	1.31E-03	1.01E-02
PI3	0.36	1.14E-04	1.56E-03
RPS3A	0.36	4.73E-03	2.68E-02
SF3B14	0.36	1.92E-06	7.20E-05
LOC388210	0.36	2.69E-04	2.97E-03
FGD4	0.36	7.71E-08	5.63E-06
LOC116437	0.36	3.08E-04	3.29E-03
APOBEC3A	0.36	1.01E-04	1.44E-03
MGST1	0.36	2.26E-07	1.31E-05
ADCY4	0.36	2.23E-05	4.55E-04
IL18RAP	0.36	4.20E-03	2.47E-02
CCNE2	0.36	6.44E-05	1.02E-03
CECR6	0.36	5.02E-05	8.51E-04
ANKRD12	0.36	7.66E-05	1.17E-03
ZC3H12C	0.36	2.67E-03	1.75E-02
MME	0.36	3.54E-05	6.51E-04
CSF2RA	0.36	4.13E-08	3.53E-06
NSMCE2	0.36	1.67E-03	1.22E-02
GNG11	0.36	8.95E-04	7.49E-03
ADAM8	0.36	3.29E-06	1.09E-04
UBL5	0.36	6.94E-08	5.24E-06
FADD	0.36	1.25E-08	1.48E-06
POTEKP	0.35	6.24E-06	1.76E-04
CDKN2B	0.35	1.36E-03	1.04E-02
C3AR1	0.35	5.20E-06	1.54E-04

Blood transcriptome

Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
OSCAR	0.35	4.40E-06	1.34E-04
KRCC1	0.35	1.95E-04	2.31E-03
RBP7	0.35	1.37E-06	5.57E-05
PDK4	0.35	5.96E-03	3.20E-02
BPGM	0.35	9.84E-03	4.61E-02
RNASE4	0.35	1.79E-04	2.17E-03
SERPINB2	0.35	5.10E-03	2.85E-02
PLIN4	0.35	2.59E-05	5.12E-04
CD58	0.35	8.23E-05	1.23E-03
CYB5R4	0.35	4.80E-05	8.26E-04
FBXL13	0.35	4.28E-05	7.53E-04
MCTP2	0.35	7.72E-07	3.52E-05
LOC731424	0.35	6.33E-04	5.74E-03
UQCRB	0.35	1.51E-03	1.13E-02
C11orf82	0.35	2.83E-05	5.44E-04
KIAA1466	0.35	3.40E-03	2.10E-02
SAP30	0.35	1.65E-05	3.67E-04
BCL2L15	0.34	2.44E-04	2.77E-03
YOD1	0.34	3.96E-03	2.36E-02
ZNF467	0.34	5.14E-04	4.87E-03
NLRC4	0.34	1.12E-06	4.75E-05
HIST2H2AA3	0.34	4.25E-04	4.23E-03
AGTPBP1	0.34	2.09E-05	4.35E-04
LPCAT2	0.34	1.29E-04	1.72E-03
TMEM45B	0.34	5.05E-04	4.81E-03
GMNN	0.34	2.10E-03	1.46E-02
DDIT3	0.34	1.67E-07	1.02E-05
FRAT1	0.34	1.84E-06	6.99E-05
CKLF	0.34	4.02E-10	9.40E-08
IFNG	0.34	2.67E-03	1.75E-02
CREB5	0.34	3.28E-06	1.09E-04
TGFA	0.34	2.87E-04	3.11E-03
TLR8	0.34	5.65E-06	1.64E-04
TAF7	0.34	6.94E-04	6.15E-03
SLC11A1	0.34	2.00E-06	7.43E-05
G6PD	0.34	2.78E-04	3.05E-03
CD68	0.34	1.78E-04	2.16E-03
ADAM9	0.34	8.87E-04	7.44E-03
HRASLS5	0.34	2.96E-05	5.63E-04
OSBPL1A	0.34	2.04E-05	4.28E-04
SPINLW1	0.33	2.61E-04	2.90E-03
PGM2	0.33	3.47E-05	6.39E-04
RRM2B	0.33	2.65E-03	1.74E-02
GYG1	0.33	5.81E-07	2.78E-05
FPGT	0.33	1.06E-02	4.87E-02
XRCC4	0.33	9.37E-04	7.76E-03
ALOX5	0.33	2.33E-08	2.25E-06
DGAT2	0.33	1.38E-05	3.25E-04
RNF141	0.33	5.94E-06	1.70E-04
PLXNC1	0.33	2.97E-07	1.58E-05
NRBF2	0.33	2.01E-04	2.38E-03
FAM63A	0.33	1.68E-05	3.71E-04
IDI1	0.33	1.29E-04	1.72E-03
DKFZp667F0711	0.33	2.98E-03	1.90E-02
JDP2	0.33	3.72E-06	1.18E-04
CLU	0.33	8.69E-03	4.22E-02
C7orf53	0.33	1.34E-04	1.76E-03
PPP1R3D	0.33	1.24E-05	2.99E-04
TM6SF1	0.33	4.80E-04	4.64E-03
DENND3	0.33	1.25E-06	5.16E-05

Blood transcriptome			
Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
FBXO30	0.33	4.33E-03	2.52E-02
EPAS1	0.33	9.11E-05	1.33E-03
SGOL2	0.33	4.59E-04	4.50E-03
CYP4F3	0.33	3.73E-05	6.74E-04
ZFYVE16	0.33	4.16E-03	2.45E-02
CDA	0.33	1.53E-03	1.14E-02
SELT	0.33	4.11E-04	4.12E-03
MBOAT2	0.33	6.96E-05	1.08E-03
HK2	0.33	2.60E-06	9.27E-05
LOC644538	0.33	1.29E-04	1.72E-03
SLC2A5	0.33	2.58E-04	2.88E-03
CLEC4E	0.33	1.05E-03	8.50E-03
KCNE3	0.33	2.80E-07	1.52E-05
PTGES	0.32	4.08E-04	4.10E-03
LOC100288618	0.32	6.37E-05	1.01E-03
KIF27	0.32	7.10E-04	6.26E-03
EIF2C3	0.32	8.16E-05	1.23E-03
NSUN7	0.32	6.60E-03	3.45E-02
TMEM212	0.32	2.65E-03	1.74E-02
BAZ2B	0.32	1.92E-03	1.36E-02
DOCK4	0.32	1.50E-03	1.12E-02
HBXIP	0.32	1.06E-10	3.44E-08
LOC401397	0.32	3.04E-03	1.93E-02
CASC5	0.32	2.84E-03	1.83E-02
OBFC2A	0.32	2.93E-03	1.87E-02
NT5C2	0.32	2.94E-10	7.80E-08
FLJ36031	0.32	4.12E-06	1.28E-04
KIF1B	0.32	9.19E-06	2.39E-04
GALNT7	0.32	2.05E-04	2.42E-03
ATP5I	0.32	4.72E-04	4.59E-03
TOP2A	0.32	4.16E-03	2.45E-02
PFDN5	0.32	6.44E-03	3.39E-02
STEAP4	0.32	3.14E-05	5.91E-04
EEF1E1	0.32	6.35E-03	3.35E-02
PLXDC2	0.32	2.00E-05	4.22E-04
SGMS2	0.32	1.14E-03	9.10E-03
TP53I11	0.32	3.83E-05	6.87E-04
TMEM92	0.32	9.30E-04	7.72E-03
CENPW	0.32	4.43E-04	4.37E-03
ARSB	0.32	3.58E-06	1.16E-04
SHKBP1	0.32	1.32E-03	1.01E-02
ZNF230	0.32	5.94E-04	5.45E-03
BST1	0.32	1.78E-05	3.87E-04
ZNF578	0.32	1.13E-04	1.55E-03
MTMR6	0.32	1.93E-03	1.37E-02
CTSD	0.32	8.70E-04	7.37E-03
C9orf84	0.32	1.61E-03	1.18E-02
NUDT16	0.31	7.59E-05	1.16E-03
CCR3	0.31	9.18E-03	4.38E-02
LOC100507345	0.31	7.15E-03	3.65E-02
SH3GLB1	0.31	4.42E-07	2.21E-05
ANXA1	0.31	4.91E-04	4.71E-03
SLC24A3	0.31	1.09E-03	8.78E-03
AIF1	0.31	3.82E-08	3.33E-06
IMPA2	0.31	5.91E-05	9.64E-04
KLF5	0.31	3.47E-04	3.62E-03
ERLIN1	0.31	8.16E-05	1.23E-03
CDK1	0.31	2.37E-04	2.70E-03
FHDC1	0.31	1.17E-03	9.31E-03
TXNDC3	0.31	2.59E-03	1.71E-02

Blood transcriptome

Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
AREG	0.31	3.45E-03	2.13E-02
FAR1	0.31	8.03E-03	3.98E-02
SLC17A6	0.31	3.68E-04	3.79E-03
NTNG2	0.31	1.92E-04	2.29E-03
SEMA4A	0.31	3.34E-04	3.52E-03
LOC285696	0.31	6.40E-04	5.79E-03
MGC16075	0.31	3.67E-05	6.65E-04
CCDC17	0.31	2.85E-04	3.10E-03
TTC35	0.31	5.10E-03	2.85E-02
C9orf72	0.31	5.06E-03	2.84E-02
LILRB2	0.31	2.16E-06	7.87E-05
MYB	0.31	2.33E-03	1.58E-02
TBCA	0.31	3.19E-06	1.07E-04
ABHD3	0.31	1.01E-02	4.68E-02
HIP1R	-0.31	1.23E-04	1.65E-03
ATP8B2	-0.31	9.94E-06	2.52E-04
FBXO21	-0.31	2.09E-08	2.11E-06
CD5	-0.31	8.45E-06	2.24E-04
NCRNA00171	-0.31	1.44E-03	1.09E-02
UTP20	-0.31	1.44E-05	3.33E-04
LOC643529	-0.31	1.87E-03	1.34E-02
LOC440104	-0.31	1.95E-06	7.24E-05
LOC572558	-0.31	4.87E-06	1.46E-04
NFIX	-0.31	5.12E-03	2.86E-02
LBH	-0.31	8.55E-08	6.06E-06
LDLRAP1	-0.31	5.67E-05	9.34E-04
MSI2	-0.31	5.41E-09	8.28E-07
IKZF3	-0.31	7.20E-08	5.38E-06
EGOT	-0.31	1.98E-04	2.34E-03
TSPYL5	-0.31	5.81E-03	3.14E-02
PAPD7	-0.31	1.19E-06	4.97E-05
CBFA2T2	-0.31	1.40E-11	5.85E-09
USP36	-0.31	5.18E-10	1.13E-07
LOC100507266	-0.31	2.43E-03	1.63E-02
CD79B	-0.31	2.04E-04	2.40E-03
TGIF2	-0.31	7.29E-12	3.52E-09
KIAA0355	-0.31	1.19E-06	4.99E-05
FCGBP	-0.31	1.78E-03	1.28E-02
PPAT	-0.31	5.96E-04	5.46E-03
PELP1	-0.31	3.09E-07	1.63E-05
LOC100131564	-0.31	1.74E-05	3.79E-04
PATZ1	-0.31	2.51E-11	9.76E-09
LOC100130097	-0.31	1.28E-03	9.96E-03
SLC7A6	-0.31	6.71E-08	5.13E-06
LOC100133130	-0.31	3.26E-05	6.07E-04
PTPLAD1	-0.31	1.66E-05	3.67E-04
DFFB	-0.31	1.90E-04	2.27E-03
PASK	-0.31	1.64E-03	1.20E-02
SEL1L3	-0.32	2.35E-05	4.75E-04
WDR4	-0.32	3.38E-08	3.05E-06
NAA40	-0.32	9.33E-08	6.52E-06
LY9	-0.32	3.72E-07	1.90E-05
CD72	-0.32	7.51E-03	3.79E-02
ZNF202	-0.32	5.29E-07	2.58E-05
FOXK1	-0.32	2.41E-09	4.07E-07
NSUN6	-0.32	4.35E-08	3.68E-06
LOC100507557	-0.32	1.05E-02	4.85E-02
AGAP4	-0.32	1.72E-06	6.62E-05
GNE	-0.32	1.39E-04	1.81E-03
ZNF37A	-0.32	6.24E-08	4.92E-06

Blood transcriptome

Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
LOC100129637	-0.32	9.67E-07	4.25E-05
HIST1H2AG	-0.32	5.30E-03	2.93E-02
TSR2	-0.32	3.88E-10	9.28E-08
C19orf2	-0.32	9.62E-05	1.39E-03
GPR18	-0.32	6.02E-05	9.79E-04
CPA5	-0.32	2.51E-03	1.67E-02
SUPV3L1	-0.32	6.80E-09	9.60E-07
C2CD2	-0.32	1.23E-05	2.97E-04
TARP	-0.32	3.77E-03	2.28E-02
ZNF764	-0.32	2.60E-06	9.27E-05
NT5E	-0.32	5.61E-04	5.21E-03
PMEPA1	-0.32	4.16E-05	7.36E-04
NXT1	-0.32	2.67E-05	5.23E-04
LOC100289019	-0.32	3.59E-06	1.16E-04
MDC1	-0.33	6.44E-09	9.42E-07
LOC100506469	-0.33	4.27E-08	3.63E-06
NPIPL3	-0.33	2.18E-03	1.50E-02
LOC100509088	-0.33	9.30E-09	1.19E-06
C2orf40	-0.33	3.09E-03	1.95E-02
QSOX2	-0.33	3.62E-08	3.22E-06
METT11D1	-0.33	1.76E-10	5.01E-08
LOC339988	-0.33	8.28E-05	1.23E-03
LARGE	-0.33	2.42E-04	2.74E-03
BOLA2	-0.33	2.87E-14	3.04E-11
CYorf15B	-0.33	5.28E-07	2.58E-05
ZCCHC2	-0.33	3.96E-04	4.00E-03
PPM1K	-0.33	2.47E-05	4.93E-04
NAPEPLD	-0.33	3.16E-10	8.01E-08
LOC100506501	-0.33	2.51E-03	1.67E-02
AFAP1L2	-0.33	5.10E-05	8.61E-04
TBC1D4	-0.33	3.19E-03	2.00E-02
SNHG12	-0.33	5.45E-08	4.39E-06
ZNF785	-0.33	3.19E-11	1.18E-08
DDHD2	-0.33	2.75E-04	3.02E-03
ZNHIT6	-0.33	1.65E-06	6.37E-05
LOC100509749	-0.33	1.15E-06	4.84E-05
TEX10	-0.33	1.78E-10	5.01E-08
ZNF418	-0.33	2.56E-04	2.87E-03
STAP1	-0.33	4.88E-03	2.75E-02
SERPINF1	-0.33	1.78E-05	3.87E-04
NMT2	-0.34	1.56E-05	3.53E-04
PCSK7	-0.34	3.17E-10	8.01E-08
HCRP1	-0.34	9.83E-07	4.29E-05
OSBPL10	-0.34	5.71E-05	9.39E-04
CXXC5	-0.34	1.96E-07	1.17E-05
C10orf58	-0.34	2.92E-08	2.73E-06
LMLN	-0.34	9.42E-09	1.20E-06
TSPYL2	-0.34	2.14E-07	1.25E-05
HSP90AB1	-0.34	7.93E-08	5.76E-06
ZNF831	-0.34	1.66E-07	1.02E-05
LOC100506360	-0.34	1.59E-04	1.98E-03
RGP1	-0.34	1.33E-08	1.54E-06
BCL7A	-0.34	2.92E-06	1.00E-04
OAS2	-0.34	2.15E-05	4.43E-04
MAP3K9	-0.34	9.13E-09	1.18E-06
PLCG1	-0.34	3.33E-06	1.10E-04
AMIGO1	-0.34	2.61E-05	5.15E-04
BCL11B	-0.34	1.39E-07	8.90E-06
LOC284749	-0.34	9.08E-04	7.58E-03
BIN1	-0.34	1.37E-07	8.80E-06

Blood transcriptome

Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
NOL9	-0.34	6.51E-09	9.42E-07
FOLR1	-0.34	4.24E-04	4.22E-03
HTATSF1	-0.35	6.57E-04	5.92E-03
MMP19	-0.35	6.49E-07	3.07E-05
CD22	-0.35	1.97E-03	1.39E-02
EPHX2	-0.35	9.66E-05	1.39E-03
C4orf10	-0.35	7.38E-09	1.02E-06
FMNL3	-0.35	1.28E-07	8.36E-06
HLA-DOA	-0.35	7.73E-06	2.07E-04
GAL3ST4	-0.35	5.10E-06	1.51E-04
IFI6	-0.35	4.86E-03	2.75E-02
NCRNA00185	-0.36	2.86E-04	3.11E-03
KCNA3	-0.36	7.00E-05	1.09E-03
LOC144571	-0.36	2.90E-03	1.86E-02
CLIC3	-0.36	2.37E-04	2.70E-03
CXCR7	-0.36	2.00E-07	1.18E-05
ANO7L1	-0.36	1.90E-04	2.27E-03
GPAT2	-0.36	4.36E-03	2.54E-02
CELSR3	-0.36	3.30E-05	6.13E-04
SPIB	-0.36	2.76E-04	3.03E-03
CCDC50	-0.36	1.05E-05	2.63E-04
TRAF5	-0.37	3.10E-05	5.85E-04
LILRA4	-0.37	1.21E-05	2.94E-04
P2RX5	-0.37	4.63E-04	4.52E-03
ABCB4	-0.37	4.10E-04	4.12E-03
ZNF141	-0.37	1.50E-09	2.83E-07
ZNF275	-0.37	6.49E-09	9.42E-07
RAB30	-0.37	1.14E-05	2.79E-04
HIST1H3H	-0.37	2.54E-04	2.85E-03
LOC100132288	-0.37	8.20E-04	7.02E-03
TSPAN3	-0.37	7.45E-07	3.44E-05
AQP3	-0.37	3.72E-08	3.29E-06
METAP1D	-0.37	1.80E-08	1.86E-06
C11orf80	-0.37	2.43E-07	1.37E-05
ZBTB4	-0.37	1.32E-11	5.69E-09
KLHL3	-0.37	1.43E-05	3.31E-04
PRKXP1	-0.37	1.43E-03	1.08E-02
PAX5	-0.38	4.76E-04	4.62E-03
ARL10	-0.38	4.75E-07	2.35E-05
LAX1	-0.38	3.73E-05	6.75E-04
EPPK1	-0.38	1.92E-04	2.29E-03
UNG	-0.38	1.72E-09	3.14E-07
FAM102A	-0.38	1.32E-07	8.58E-06
IL28RA	-0.38	1.58E-05	3.56E-04
CNTNAP2	-0.38	2.03E-04	2.40E-03
GAR1	-0.38	1.27E-10	3.87E-08
MS4A1	-0.39	2.82E-03	1.82E-02
TRIB2	-0.39	1.21E-06	5.03E-05
NOP14	-0.39	3.39E-08	3.05E-06
MORC4	-0.39	2.90E-07	1.55E-05
TNFRSF21	-0.39	7.98E-08	5.77E-06
SEMA4C	-0.39	4.64E-07	2.31E-05
STRBP	-0.39	1.03E-04	1.45E-03
GPM6B	-0.39	1.63E-14	2.08E-11
ANKRD36BP2	-0.40	6.88E-03	3.57E-02
RUNDC2A	-0.40	6.92E-05	1.08E-03
BTLA	-0.40	1.38E-04	1.79E-03
TCF4	-0.40	1.38E-07	8.86E-06
ZNF662	-0.40	2.08E-04	2.44E-03
LOC100287616	-0.40	1.45E-04	1.86E-03

Blood transcriptome			
Female severe asthma (n=182) v Female healthy controls (n=34)			
Number of upregulated genes			71
Number of downregulated genes			62
Gene	**Log2 fold change**	***P* value**	**FDR**

Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			490
Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**
LOC400464	-0.40	5.22E-06	1.54E-04
HAUS5	-0.40	1.25E-10	3.87E-08
TTC21B	-0.40	2.36E-04	2.69E-03
NCRNA00246B	-0.40	1.17E-04	1.59E-03
TARSL2	-0.41	1.22E-05	2.95E-04
SHISA4	-0.41	1.81E-04	2.20E-03
ZNF512B	-0.41	2.03E-09	3.62E-07
C14orf64	-0.41	8.28E-08	5.94E-06
YIPF2	-0.41	2.91E-06	1.00E-04
BCL11A	-0.41	6.95E-08	5.24E-06
LOC100131541	-0.41	6.09E-06	1.73E-04
CD27	-0.41	6.29E-06	1.77E-04
FCRL1	-0.41	9.09E-03	4.35E-02
ATAD5	-0.41	2.68E-10	7.22E-08
AFF3	-0.41	2.11E-04	2.47E-03
NELL2	-0.42	2.27E-04	2.59E-03
ATF7IP2	-0.42	4.64E-08	3.87E-06
PDE9A	-0.42	4.34E-07	2.19E-05
MUM1	-0.42	1.02E-12	7.04E-10
FCRLA	-0.42	3.75E-04	3.84E-03
CR2	-0.42	2.98E-05	5.66E-04
ABLIM1	-0.42	8.64E-09	1.13E-06
PIK3C2B	-0.43	1.55E-06	6.08E-05
LOC100507192	-0.43	5.99E-05	9.76E-04
C2orf89	-0.43	1.89E-05	4.05E-04
FAIM3	-0.43	4.58E-08	3.85E-06
PIGL	-0.43	1.53E-06	6.06E-05
LOC641518	-0.43	6.33E-04	5.74E-03
POU6F1	-0.43	2.73E-09	4.49E-07
APBA2	-0.43	7.54E-08	5.53E-06
PLEKHG1	-0.44	2.09E-03	1.45E-02
LOC100190986	-0.44	3.89E-08	3.36E-06
LRIG2	-0.44	7.93E-09	1.08E-06
LOC283713	-0.45	4.47E-04	4.40E-03
CD79A	-0.45	5.80E-04	5.35E-03
POU2AF1	-0.45	1.71E-05	3.75E-04
FLJ33630	-0.45	1.12E-08	1.36E-06
LRRN3	-0.45	6.72E-03	3.50E-02
LOC100288152	-0.46	1.36E-04	1.78E-03
CARM1	-0.46	1.88E-05	4.05E-04
CAND2	-0.46	6.46E-08	5.01E-06
TRAJ17	-0.46	1.32E-05	3.14E-04
LOC150759	-0.47	4.41E-05	7.71E-04
LOC100288282	-0.47	1.11E-05	2.72E-04
BLNK	-0.47	1.34E-04	1.76E-03
HLA-DOB	-0.47	2.79E-05	5.39E-04
HBZ	-0.47	1.06E-03	8.62E-03
GCOM1	-0.47	7.65E-05	1.17E-03
HIST1H1D	-0.48	4.62E-05	8.00E-04
LOC283663	-0.48	2.41E-04	2.74E-03
BACH2	-0.48	1.14E-06	4.83E-05
MAN1C1	-0.48	1.71E-08	1.81E-06
FOSB	-0.48	1.49E-08	1.67E-06
FAM171A1	-0.49	2.28E-07	1.31E-05
LOC389834	-0.49	9.64E-05	1.39E-03
FAM129C	-0.49	3.60E-06	1.16E-04
ZMAT4	-0.49	8.48E-09	1.13E-06
NAF1	-0.49	1.06E-11	4.84E-09
SLC16A10	-0.50	1.05E-06	4.53E-05
IGHM	-0.52	1.35E-04	1.76E-03

Blood transcriptome							
Female severe asthma (n=182) v Female healthy controls (n=34)				Male severe asthma (n=114) v Male healthy controls (n=53)			
Number of upregulated genes			71	Number of upregulated genes			490
Number of downregulated genes			62	Number of downregulated genes			240
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
				CCR6	-0.52	1.78E-08	1.85E-06
				PZP	-0.53	2.16E-08	2.14E-06
				EBF1	-0.55	4.39E-06	1.34E-04
				RAB11FIP3	-0.55	1.55E-12	1.03E-09
				LAMP3	-0.55	7.37E-04	6.45E-03
				S100B	-0.56	2.20E-03	1.51E-02
				ZCCHC18	-0.56	2.83E-07	1.53E-05
				USP18	-0.56	1.96E-05	4.17E-04
				TCL1A	-0.57	2.00E-03	1.40E-02
				LOC285972	-0.59	1.75E-09	3.16E-07
				CUX2	-0.60	3.35E-07	1.73E-05
				NOG	-0.68	7.60E-06	2.05E-04
				TSPAN13	-0.69	3.94E-06	1.23E-04
				KRT77	-0.80	1.86E-05	4.00E-04

Table S4.11 All differentially expressed proteins between mild to moderate asthma and healthy controls in females and males with blood proteomics.

Blood proteomic							
Female mild/moderate asthma (n=38) v Female healthy controls (n=35)				Male mild/moderate asthma (n=39) v Male healthy controls (n=57)			
Number of upregulated proteins			0	Number of upregulated proteins			27
Number of downregulated proteins			2	Number of downregulated proteins			10
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
FLJ36848	-0.36	1.67E-06	1.69E-02	CCL23	1.03	9.05E-06	1.33E-02
KRT77	-1.41	2.45E-07	4.96E-03	PRSS33	0.90	7.46E-07	6.60E-03
				ALOX15	0.86	1.62E-05	1.64E-02
				OLIG2	0.72	2.62E-06	1.12E-02
				IDO1	0.70	3.15E-05	2.19E-02
				SIGLEC8	0.66	3.14E-05	2.19E-02
				ADORA3	0.64	1.00E-06	6.60E-03
				IL5RA	0.64	2.38E-05	1.84E-02
				VSTM1	0.59	8.66E-06	1.33E-02
				CCR3	0.54	3.41E-06	1.12E-02
				SLC29A1	0.52	1.80E-05	1.68E-02
				SMPD3	0.50	1.91E-05	1.68E-02
				CEBPE	0.48	1.40E-05	1.54E-02
				C10orf128	0.39	1.16E-04	4.25E-02
				MYCT1	0.36	7.05E-05	3.69E-02
				STXBP5	0.35	9.18E-05	3.88E-02
				SORD	0.35	1.28E-05	1.54E-02
				CAT	0.34	5.18E-05	3.10E-02
				HK2	0.26	2.22E-05	1.83E-02
				SCPEP1	0.25	5.63E-05	3.15E-02
				ZW10	0.24	7.65E-05	3.69E-02
				MYD88	0.23	9.19E-05	3.88E-02
				EVI2B	0.21	7.23E-06	1.33E-02
				PLIN2	0.20	5.74E-05	3.15E-02
				CDC42SE1	0.13	5.47E-06	1.33E-02
				TRMT5	0.10	3.67E-05	2.42E-02
				CDC42	0.09	1.18E-04	4.25E-02
				TGIF2	-0.14	1.13E-04	4.25E-02
				FBXO31	-0.24	8.07E-05	3.69E-02
				TMEM99	-0.25	9.83E-05	3.92E-02
				ARL6IP4	-0.26	1.19E-04	4.25E-02
				ZNF785	-0.27	1.23E-05	1.54E-02
				ALKBH7	-0.27	4.15E-05	2.61E-02
				COCH	-0.31	9.43E-05	3.88E-02
				NXT1	-0.33	7.49E-05	3.69E-02
				RAB11FIP3	-0.36	8.11E-05	3.69E-02
				LOC100509749	-0.39	6.91E-06	1.33E-02

Table S4.12 All differentially expressed proteins between severe asthma and healthy controls in females and males with blood proteomics.

Blood proteomic							
Female severe asthma (n=203) v Female healthy controls (n=35)				Male severe asthma (n=124) v Male healthy controls (n=57)			
Number of upregulated proteins			28	Number of upregulated proteins			
Number of downregulated proteins			25	Number of downregulated proteins			
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
CX3CL1	0.34	2.86E-07	1.69E-05	IRF8	0.46	1.68E-05	1.15E-04
HPGDS	0.22	1.71E-04	1.51E-03	CX3CL1	0.34	4.53E-09	2.68E-07
TGFB3	0.21	2.29E-04	1.93E-03	HPGDS	0.29	5.80E-08	1.45E-06
SPRR3	0.21	2.20E-03	1.15E-02	CCL23	0.28	1.03E-07	2.03E-06
CCL23	0.18	2.33E-03	1.18E-02	CD163	0.26	4.12E-07	6.08E-06
CD163	0.17	2.79E-03	1.37E-02	TNF	0.26	3.21E-09	2.68E-07
C8G	0.17	8.30E-05	9.79E-04	ISG15	0.25	8.32E-04	3.59E-03
TNF	0.17	9.73E-04	6.15E-03	C8G	0.23	1.54E-08	5.72E-07
CSF2	0.17	9.77E-05	1.07E-03	SPRR3	0.23	7.55E-05	4.16E-04
SCGB1A1	0.17	1.34E-03	8.20E-03	TGFB3	0.23	4.55E-06	3.50E-05
PTGS1	0.15	4.41E-03	2.00E-02	PSORS1C2	0.23	2.19E-06	2.04E-05
CERS2	0.15	5.03E-03	2.14E-02	RASD2	0.22	2.72E-06	2.26E-05
MS4A15	0.14	2.15E-03	1.15E-02	CHIT1	0.22	3.25E-04	1.64E-03
PSORS1C2	0.13	1.06E-02	3.70E-02	SCGB1A1	0.20	6.43E-07	8.13E-06
C9	0.13	2.70E-04	2.08E-03	C9	0.19	2.96E-09	2.68E-07
IL1R2	0.13	1.89E-03	1.04E-02	IL1R2	0.19	6.54E-08	1.45E-06
IL2RA	0.13	6.42E-03	2.47E-02	CERS2	0.19	9.01E-06	6.38E-05
ARFGAP1	0.13	1.46E-03	8.60E-03	CSF2	0.19	1.90E-06	1.87E-05
FGL2	0.12	3.10E-03	1.48E-02	PTGS1	0.18	1.74E-07	3.08E-06
MAGI1	0.11	5.96E-03	2.40E-02	FGL2	0.18	4.22E-08	1.24E-06
MMP1	0.11	1.18E-02	4.01E-02	MS4A15	0.17	7.12E-07	8.41E-06
CPA3	0.10	4.87E-03	2.14E-02	ARFGAP1	0.17	5.99E-07	8.13E-06
PPAP2A	0.10	5.10E-03	2.14E-02	IL2RA	0.17	3.42E-07	5.51E-06
TRIM33	0.09	9.22E-03	3.27E-02	IL10	0.16	4.97E-05	3.07E-04
LEP	0.08	4.84E-05	7.15E-04	MAGI1	0.15	2.81E-06	2.26E-05
CFI	0.08	5.20E-03	2.14E-02	IL17RB	0.15	4.57E-03	1.50E-02
PDGFB	0.08	4.19E-03	1.95E-02	CERS4	0.14	1.15E-06	1.20E-05
IL4	0.06	6.12E-03	2.41E-02	CPA3	0.13	7.65E-06	5.64E-05
MOCOS	-0.05	8.62E-03	3.11E-02	MRPL43	0.12	9.23E-07	1.02E-05
MPO	-0.08	8.60E-03	3.11E-02	PPAP2A	0.10	3.55E-04	1.70E-03
CRISP3	-0.10	7.83E-03	2.95E-02	TBX21	0.10	3.33E-05	2.19E-04
IL12B	-0.10	1.26E-02	4.20E-02	TRIM33	0.09	3.33E-04	1.64E-03
UGT8	-0.14	1.32E-05	2.59E-04	SPARCL1	0.08	2.05E-03	7.42E-03
SLC11A1	-0.16	1.75E-03	1.00E-02	PDGFB	0.08	5.08E-04	2.25E-03
TAS2R10	-0.17	9.26E-04	6.07E-03	VCAM1	-0.04	2.00E-03	7.42E-03
ROS1	-0.17	7.02E-07	2.49E-05	IL10RA	-0.05	6.48E-03	1.90E-02
ADCY2	-0.18	1.35E-04	1.26E-03	CCR6	-0.06	1.34E-03	5.38E-03
MMP7	-0.18	9.18E-04	6.07E-03	ELANE	-0.06	1.85E-02	4.75E-02
RETNLB	-0.19	2.74E-07	1.69E-05	CERS3	-0.07	1.73E-03	6.64E-03
IL33	-0.21	2.54E-04	2.04E-03	FETUB	-0.07	2.54E-03	8.83E-03
RGS18	-0.21	5.16E-07	2.28E-05	SPTLC3	-0.08	1.90E-02	4.81E-02
IL26	-0.21	6.27E-05	7.93E-04	TTR	-0.08	1.37E-02	3.68E-02
HLA-DQA1	-0.21	1.03E-04	1.07E-03	TLR2	-0.08	5.03E-05	3.07E-04
SGMS1	-0.22	9.51E-07	2.81E-05	RETNLB	-0.08	1.15E-02	3.15E-02
IL1RL1	-0.23	6.13E-05	7.93E-04	CRISP3	-0.09	4.57E-03	1.50E-02
ACER2	-0.24	3.03E-05	4.88E-04	IL1B	-0.09	7.75E-05	4.16E-04
APOC3	-0.24	2.38E-06	5.27E-05	A2M	-0.09	5.65E-03	1.72E-02
NAPSA	-0.24	1.19E-04	1.17E-03	RGS18	-0.09	1.76E-02	4.65E-02
NOS2	-0.26	4.95E-04	3.65E-03	ORM1/2	-0.09	8.09E-03	2.27E-02
IL12A	-0.27	1.61E-06	4.06E-05	KIT	-0.10	6.53E-03	1.90E-02
SMPD1	-0.28	1.50E-05	2.66E-04	APOC3	-0.11	4.29E-03	1.46E-02
TNNI3	-0.29	7.10E-04	5.03E-03	IGF2	-0.11	2.01E-03	7.42E-03
CERS1	-0.78	4.53E-19	8.02E-17	IL3	-0.11	2.23E-03	7.88E-03
				IL5	-0.11	1.18E-03	4.86E-03
				IL33	-0.12	4.86E-03	1.51E-02
				NFKB2	-0.12	4.80E-03	1.51E-02

Blood proteomic							
Female severe asthma (n=203) v Female healthy controls (n=35)				Male severe asthma (n=124) v Male healthy controls (n=57)			
Number of upregulated proteins			28	Number of upregulated proteins			
Number of downregulated proteins			25	Number of downregulated proteins			
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
				SPHK2	-0.12	8.67E-03	2.40E-02
				SLC11A1	-0.12	4.08E-04	1.90E-03
				ECM1	-0.13	5.70E-05	3.36E-04
				PSORS1C1	-0.14	7.92E-03	2.26E-02
				DEGS1	-0.15	5.97E-03	1.79E-02
				CERS1	-0.15	1.84E-02	4.75E-02
				EPX	-0.16	4.62E-04	2.10E-03
				CCL11	-0.17	4.77E-03	1.51E-02
				IL12A	-0.18	2.46E-06	2.18E-05
				B4GALT5	-0.18	1.64E-03	6.44E-03
				HLA-DQA1	-0.21	1.62E-08	5.72E-07
				TSLP	-0.23	8.99E-04	3.79E-03
				S100A12	-0.24	8.90E-05	4.63E-04
				TNNI3	-0.28	6.90E-05	3.94E-04

Table S4.13 All differentially abundant metabolites between mild to moderate asthma and healthy controls in females and males with urine metabolomics.

Urine metabolomic							
Female mild/moderate asthma (n=43) v Female healthy controls (n=38)				Male mild/moderate asthma (n=44) v Male healthy controls (n=62)			
Number of upregulated metabolites			2	Number of upregulated metabolites			0
Number of downregulated metabolites			2	Number of downregulated metabolites			0
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
Tyrosine	0.42	1.00E-03	4.25E-02				
Octopamine	0.41	1.37E-03	4.25E-02				
Xylose	-0.71	1.42E-03	4.25E-02				
Pipecolic acid	-0.98	1.96E-03	4.40E-02				

Table S4.14 All differentially abundant metabolites between severe asthma and healthy controls in females and males with urine metabolomics.

Urine metabolomic							
Female severe asthma (n=236) v Female healthy controls (n=38)				Male severe asthma (n=140) v Male healthy controls (n=62)			
Number of upregulated metabolites			7	Number of upregulated metabolites			14
Number of downregulated metabolites			4	Number of downregulated metabolites			15
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
Maltose	1.11	1.36E-05	6.12E-04	Phenyllactic acid	1.02	5.29E-04	3.66E-03
Serotonin	0.67	4.55E-07	4.10E-05	Serotonin	0.70	1.20E-08	1.08E-06
Sucrose	0.50	3.92E-03	3.53E-02	1 Methyluric acid	0.67	1.71E-04	1.71E-03
Tyrosine	0.41	8.64E-05	2.59E-03	Sarcosine	0.61	2.00E-03	1.00E-02
S Adenosylhomocysteine	0.40	4.55E-04	8.19E-03	Maltose	0.60	3.70E-03	1.59E-02
Octopamine	0.38	2.19E-04	4.92E-03	Mannitol	0.54	1.18E-03	6.62E-03
Galacturonic acid	0.28	1.95E-03	2.50E-02	4 Pyridoxic acid	0.50	7.02E-04	4.21E-03
Serine	-0.26	6.03E-03	4.93E-02	1 3 7Trimethyluric acid	0.48	1.44E-02	4.48E-02
Tyramine	-0.44	2.35E-03	2.65E-02	1 7 Dimethyluric acid	0.44	1.25E-02	4.01E-02
Tryptamine	-0.56	2.68E-03	2.68E-02	Xylose	0.43	4.32E-03	1.77E-02
Caffeine	-0.89	1.01E-03	1.52E-02	3 Hydroxykynurenine	0.34	8.89E-03	3.20E-02
				Glutamic acid	0.29	5.85E-04	3.76E-03
				N Acetylputrescine	0.27	2.83E-04	2.31E-03
				S Adenosylhomocysteine	0.24	2.11E-03	1.00E-02
				Glutamine	-0.16	9.61E-03	3.33E-02
				Citrulline	-0.27	1.06E-02	3.52E-02
				Serine	-0.29	2.86E-06	6.43E-05
				Uracil	-0.30	6.28E-03	2.35E-02
				N Acetylcarnosine	-0.31	3.09E-04	2.32E-03
				O Acetylserine	-0.36	4.41E-05	6.62E-04
				Histidine	-0.39	5.45E-08	2.45E-06
				Carnitine	-0.42	1.09E-04	1.22E-03
				Prolylhydroxyproline	-0.44	8.60E-05	1.11E-03
				Lysine	-0.44	6.04E-03	2.35E-02
				Guanine	-0.50	1.66E-03	8.79E-03
				5 Aminolevulinic acid	-0.60	1.73E-06	5.20E-05
				Acetylcarnitine	-0.70	2.79E-03	1.26E-02
				Carnosine	-0.76	2.13E-04	1.92E-03
				Propionylcarnitine	-0.87	2.17E-05	3.90E-04

Table S4.15 All differentially abundant bacterial genera between mild to moderate asthma and healthy controls in females and males with sputum microbiomics.

Sputum microbiome							
Female mild/moderate asthma (n=11) v Female healthy controls (n=7)				Male mild/moderate asthma (n=13) v Male healthy controls (n=16)			
Number of upregulated genera			0	Number of upregulated genera			0
Number of downregulated genera			0	Number of downregulated genera			1
Gene	**Log2 fold change**	***P* value**	**FDR**	**Gene**	**Log2 fold change**	***P* value**	**FDR**
				Megasphaera	-2.36	2.67E-04	3.90E-02

Table S4.16 All differentially abundant bacterial genera between severe asthma and healthy controls in females and males with sputum microbiomics.

Sputum microbiome			
Female severe asthma (n=52) v Female healthy controls (n=7)			
Number of upregulated genera			5
Number of downregulated genera			0
Gene	**Log2 fold change**	***P* value**	**FDR**
Moraxella	9.97	8.08E-06	1.35E-03
Paracoccus	6.90	3.41E-04	2.85E-02
Methylobacterium	4.39	8.09E-04	3.38E-02
Blautia	4.00	7.98E-04	3.38E-02
Sediminibacterium	2.55	1.42E-03	4.74E-02

Male severe asthma (n=36) v Male healthy controls (n=16)			
Number of upregulated genera			5
Number of downregulated genera			6
Gene	**Log2 fold change**	***P* value**	**FDR**
Pseudomonas	4.60	5.32E-04	5.49E-03
Blautia	3.41	8.85E-05	1.37E-03
Acinetobacter	3.40	2.33E-03	1.61E-02
Sediminibacterium	2.64	5.37E-05	1.11E-03
Bradyrhizobium	2.47	9.90E-04	7.67E-03
Catonella	-1.83	8.47E-03	4.78E-02
Leptotrichia	-2.00	4.55E-04	5.49E-03
Atopobium	-2.34	8.29E-04	7.35E-03
Dialister	-2.47	3.18E-05	9.84E-04
Parvimonas	-2.52	3.66E-03	2.27E-02
Slackia	-4.08	1.04E-05	6.43E-04

Table S4.17 All differentially expressed genes on X and Y chromosomes between asthma and healthy controls in females and males with bronchial biopsies transcriptomes.

Bronchial biopsies transcriptome (X and Y chromosomes)			
Asthma (Severe: 23 female and 23 male, mild/moderate: 12 female and 12 male) v Healthy controls (10 female and 10 male)			
Number of upregulated genes			3
Number of downregulated genes			5
Gene	**Log2 fold change**	***P* value**	**FDR**
MAOA	0.67	1.03E-07	5.53E-05
TSC22D3	0.60	8.56E-05	1.15E-02
SLC6A8	0.32	5.41E-04	3.71E-02
ZNF275	-0.36	2.06E-04	2.13E-02
SLC25A43	-0.53	5.53E-04	3.71E-02
GPR174	-0.63	2.38E-04	2.13E-02
ITM2A	-0.69	2.78E-06	7.45E-04
TCEAL2	-0.76	4.23E-05	7.56E-03

Table S4.18 All differentially expressed genes on X and Y chromosomes between asthma and healthy controls in females and males with blood transcriptomes.

Blood transcriptome (X and Y chromosomes)			
Asthma (Severe: 114 female and 114 male, mild/moderate: 37 female and 37 male) v Female healthy controls (34 female and 34 male)			
Number of upregulated genes			33
Number of downregulated genes			37
Gene	**Log2 fold change**	***P* value**	**FDR**
COX7B	0.48	3.43E-04	7.44E-03
GPR34	0.46	2.51E-06	2.09E-04
GLOD5	0.40	1.81E-04	4.52E-03
BMX	0.39	6.50E-05	2.16E-03
LPAR4	0.35	2.27E-03	2.27E-02
VSIG4	0.34	1.93E-04	4.59E-03
BEX1	0.30	1.29E-03	1.43E-02
ACSL4	0.28	8.66E-05	2.54E-03
BEND2	0.27	1.04E-03	1.30E-02
ZNF630	0.24	6.84E-04	1.11E-02
GPR82	0.24	4.65E-03	3.91E-02
LAMP2	0.21	1.00E-06	1.66E-04
TLR8	0.21	1.28E-04	3.53E-03
F8	0.21	1.67E-03	1.71E-02
G6PD	0.20	5.36E-03	4.28E-02
NDUFA1	0.19	8.79E-04	1.22E-02
PRRG1	0.19	5.46E-03	4.28E-02
CSF2RA	0.19	4.66E-04	8.81E-03
STS	0.19	5.64E-04	1.00E-02
ZNF185	0.18	7.60E-04	1.15E-02
BTK	0.17	1.17E-03	1.38E-02
MOSPD2	0.17	2.34E-04	5.30E-03
SLITRK4	0.14	4.01E-03	3.58E-02
BRCC3	0.14	8.13E-04	1.19E-02
SAT1	0.13	2.09E-05	1.04E-03
PDK3	0.12	6.70E-04	1.11E-02
SH3BGRL	0.12	8.29E-05	2.54E-03
PHKA2	0.12	2.55E-03	2.44E-02
PDZD11	0.11	5.13E-03	4.20E-02
MSL3	0.11	5.73E-03	4.40E-02
TFE3	0.11	5.94E-03	4.48E-02
CXorf38	0.09	9.07E-04	1.22E-02
IGBP1	0.08	3.34E-03	3.08E-02
RPL10	-0.07	2.35E-03	2.30E-02
ELK1	-0.08	8.67E-04	1.22E-02
FAM120C	-0.09	6.02E-03	4.48E-02
SMC1A	-0.09	6.10E-03	4.48E-02
P2RY8	-0.10	6.89E-04	1.11E-02
SLC35A2	-0.11	1.13E-03	1.38E-02
MID1IP1	-0.11	6.95E-03	4.96E-02
GLUD2	-0.11	1.25E-03	1.42E-02
NONO	-0.11	2.94E-06	2.10E-04
PRPS1	-0.11	1.25E-03	1.42E-02
THOC2	-0.11	1.44E-04	3.77E-03
ARMCX6	-0.12	7.61E-04	1.15E-02
CD99	-0.12	1.00E-03	1.28E-02
SLC25A6	-0.12	1.64E-03	1.71E-02
MTMR1	-0.13	1.12E-05	7.01E-04
LAS1L	-0.13	4.27E-03	3.74E-02
APOOL	-0.14	3.59E-04	7.46E-03
SLC25A14	-0.14	3.72E-05	1.43E-03
CTPS2	-0.14	4.50E-04	8.81E-03
ZMYM3	-0.15	4.77E-04	8.81E-03
GPRASP2	-0.15	5.49E-03	4.28E-02

Blood transcriptome (X and Y chromosomes)			
Asthma (Severe: 114 female and 114 male, mild/moderate: 37 female and 37 male) v Female healthy controls (34 female and 34 male)			
Number of upregulated genes			33
Number of downregulated genes			37
Gene	**Log2 fold change**	***P* value**	**FDR**
CSTF2	-0.15	1.59E-05	8.82E-04
RBMX2	-0.16	4.41E-05	1.57E-03
P2RY10	-0.16	4.66E-03	3.91E-02
UPRT	-0.17	2.90E-03	2.73E-02
MAGEE1	-0.17	1.68E-03	1.71E-02
ZC3H12B	-0.17	4.70E-03	3.91E-02
BEX4	-0.18	9.41E-04	1.24E-02
DKC1	-0.20	1.92E-06	1.92E-04
TSPYL2	-0.22	2.56E-05	1.16E-03
HTATSF1	-0.23	3.59E-03	3.25E-02
MORC4	-0.23	3.08E-05	1.28E-03
ZNF275	-0.24	1.57E-06	1.92E-04
TSR2	-0.25	1.31E-08	3.26E-06
ZCCHC18	-0.26	1.52E-03	1.65E-02
BCORP1	-0.32	6.95E-03	4.96E-02
GPM6B	-0.32	2.04E-14	1.02E-11

Table S4.19 Sex differences in clinical characteristics of patients with severe asthma in RASP-UK.

Characteristics	Female severe asthma (n=26)	Male severe asthma (n=28)	p-value
Demographics			
Age (years)	53 [43, 61]	55 [40, 60]	0.3
Ethnicity, Caucasian (%)	22 (85%)	28 (100%)	0.1
BMI (kg/m^2)	34.6 ± 8.3	29.1 ± 5.4	0.006
Ex-smoker (%)	6 (23%)	8 (29%)	0.9
Asthma history			
Age onset (years)	18 [2, 29]	34 [14, 47]	0.03
Asthma duration (years)	32 [19, 42]	18 [11, 32]	0.048
Annual severe exacerbations	2.0 [1.0, 3.0]	1.0 [0.0, 2.3]	0.2
Annual A&E attendances	0.0 [0.0, 0.8]	0.0 [0.0, 0.0]	0.4
Lung function & symptom control			
FEV1 (% pred)	77.9 [64.0, 88.4]	75.1 [66.7, 91.4]	0.8
FEV1/FVC (%)	69.9 [57.6, 76.8]	64.9 [59.9, 71.6]	0.8
ACQ-5	1.9 [1.1, 2.6]	1.2 [0.6, 1.9]	0.06
Biomarkers			
Total IgE (kU/L)	109.5 [31.0, 227.5]	169.0 [78.5, 473.5]	0.07
FeNO (ppb)	27.5 [15.3, 45.0]	27.0 [16.0, 57.3]	0.6
Blood eosinophils (×10^9/L)	0.2 [0.1, 0.3]	0.2 [0.1, 0.3]	0.7
Sputum eosinophils (%)	1.5 [0.3, 6.4]	3.2 [0.5, 11.7]	0.2
Sputum neutrophils (%)	57.8 [26.2, 74.8]	57.4 [24.1, 76.9]	0.6
Type 2 phenotype			0.9
T2-high (%)	9 (35%)	9 (32%)	
T2-intermediate (%)	12 (46%)	12 (43%)	
T2-low (%)	5 (19%)	7 (25%)	
Medications			
ICS (%)	26 (100%)	28 (100%)	NA
ICS dose (mcg BDP eq)	1600 [1525, 2000]	2000 [1750, 2000]	0.01
Maintenance OCS (%)	9 (35%)	10 (36%)	1
OCS dose (mg)	5.0 [5.0, 10.0]	10.0 [8.1, 10.0]	0.1
SABA (%)	24 (96%)	27 (96%)	1
LABA (%)	25 (100%)	28 (100%)	NA
LAMA (%)	12 (48%)	13 (46%)	1
LTRA (%)	13 (52%)	10 (36%)	0.4
THEO (%)	5 (20%)	7 (25%)	0.9

Abbreviations: ACQ-5: Asthma Control Questionnaire - 5; BDP eq: beclometasone dipropionate equivalent; BMI: body mass index; FeNO: Fractional concentration of exhaled nitric oxide; FEV1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; ICS:

inhaled corticosteroids; Ig: immunoglobulin; IQR: interquartile range; LABA: long-acting Beta2 agonist; LAMA: long-acting muscarinic antagonist; LTRA: leukotriene receptor antagonist; OCS: oral corticosteroids; SD: standard deviation; THEO: theophylline.

Table S4.20 Sex differences in immunohistochemistry of patients with severe asthma in RASP-UK.

Characteristics	Female severe asthma (n=91)	Male severe asthma (n=103)	p-value
Total biopsy area, mm^2	1.5 [1.2, 2.2]	1.8 [1.2, 2.8]	0.3
Remodeling parameters			
Reticular basement membrane thickness (μm)	9.2 [7.1, 11.3]	10.2 [6.6, 12.8]	0.3
Epithelial area (%)	7.9 [4.8, 12.2]	8.9 [5.0, 15.3]	0.3
ASM area (mm^2)	0.22 [0.07, 0.41]	0.21 [0.05, 0.37]	0.5
ASM area (%)	14.5 [4.5, 23.8]	11.9 [2.9, 22.2]	0.3
Chalkley count	4.0 [3.3, 5.0]	4.3 [3.3, 5.5]	0.7
Eosinophils in lamina propria (cells/mm^2)	9.7 [2.5, 15.8]	8.9 [2.9, 22.1]	0.5
Neutrophils in lamina propria (cells/mm^2)	9.9 [3.8, 19.7]	9.8 [2.4, 15.9]	0.2
Mast cells in lamina propria (cells/mm^2)	16.3 [8.1, 27.1]	13.2 [6.4, 19.9]	0.2

Abbreviation: ASM: airways smooth muscle.

Table S4.21 All differentially expressed genes between severe asthma in females and males with bronchial biopsies transcriptomes in RASP-UK.

Differentially expressed genes are ranked by fold change. Genes which are also significantly differentially expressed between sexes in UBIOPRED are highlighted in bold, and the relevant contrasts which are congruent between studies are tabulated in green.

Biopsy Transcriptome (RASP-UK)				UBIOPRED					
Female severe asthma (n=26) v Male severe asthma (n=28)				Also significantly differentially expressed between sexes in UBIOPRED					
Number of upregulated genes			15						
Number of downregulated genes			14	Biopsy Transcriptome			Blood Transcriptome		
Gene	**Fold change (log2)**	***P* value**	**FDR**	**Health**	**Mild/Mod asthma**	**Severe asthma**	**Health**	**Mild/Mod asthma**	**Severe asthma**
MUC7	2.80	1.00E-05	1.44E-02						
SFTPA1	2.71	3.62E-06	8.48E-03						
PUDP	0.50	4.89E-07	1.80E-03						
PNPLA4	0.40	2.94E-07	1.52E-03				Yes	Yes	Yes
RPS4XP1	0.39	5.30E-06	1.05E-02						
RPS4X	0.34	3.18E-05	3.16E-02	Yes	Yes	Yes	Yes		Yes
KDM6A	0.31	2.48E-05	2.66E-02	Yes	Yes	Yes	Yes	Yes	Yes
ZFX	0.31	1.72E-06	4.43E-03		Yes	Yes	Yes	Yes	Yes
GEMIN8	0.31	9.72E-07	2.78E-03						Yes
ZRSR2	0.30	9.83E-06	1.44E-02				Yes	Yes	Yes
SYAP1	0.29	2.36E-05	2.66E-02				Yes		
EIF1AX	0.28	4.63E-07	1.80E-03	Yes	Yes		Yes	Yes	Yes
KDM5C	0.28	7.05E-07	2.27E-03		Yes	Yes	Yes	Yes	Yes
TXLNG	0.26	1.91E-05	2.46E-02				Yes	Yes	Yes
EIF2S3	0.17	5.20E-05	4.62E-02		Yes		Yes	Yes	Yes
FRG1DP	-0.29	1.56E-05	2.12E-02						
FRG1EP	-0.40	2.53E-05	2.66E-02						
FRG1FP	-0.47	3.97E-08	5.12E-04						
NLGN4X	-0.79	3.56E-05	3.40E-02						
LINC01597	-0.79	2.58E-05	2.66E-02						
ARSL	-0.81	2.31E-05	2.66E-02						
DUXAP9	-0.95	5.17E-05	4.62E-02						
TBL1Y	-1.22	8.18E-06	1.41E-02						
PRKY	-1.32	2.52E-09	6.51E-05	Yes	Yes	Yes	Yes	Yes	Yes
ZFY	-1.38	1.01E-05	1.44E-02				Yes	Yes	Yes
TTTY10	-1.49	1.00E-07	8.60E-04				Yes	Yes	Yes

NLGN4Y	-1.95	7.02E-06	1.29E-02
TTTY16	-2.04	4.92E-06	1.05E-02
CYP1A1	-3.80	1.44E-07	9.29E-04

Bibliography

Abdel-Aziz, M. I., Brinkman, P., Vijverberg, S. J. H., Neerincx, A. H., Riley, J. H., Bates, S., Hashimoto, S., Kermani, N. Z., Chung, K. F., Djukanovic, R., Dahlén, S. E., Adcock, I. M., Howarth, P. H., Sterk, P. J., Kraneveld, A. D., & Maitland-van der Zee, A. H. (2021). Sputum microbiome profiles identify severe asthma phenotypes of relative stability at 12 to 18 months. *J Allergy Clin Immunol*, *147*(1), 123-134. https://doi.org/10.1016/j.jaci.2020.04.018

Ahmed, B., Bush, A., & Davies, J. C. (2014). How to use: bacterial cultures in diagnosing lower respiratory tract infections in cystic fibrosis. *Archives of Disease in Childhood-Education and Practice*, *99*(5), 181-187.

Akenroye, A., Nopsopon, T., Hacker, J. J., & Laidlaw, T. M. (2024). Ratio of plasma IL-13/TNF- proportional, variant and CXCL10/CCL17 predicts mepolizumab and omalizumab response in asthma better than eosinophil count or immunoglobulin E level. *Sci Rep*, *14*(1), 10404. https://doi.org/10.1038/s41598-024-60864-3

Aldridge, S., & Teichmann, S. A. (2020). Single cell transcriptomics comes of age. *Nature Communications*, *11*(1), 4307.

Allen-Ramey, F. C., Gupta, S., & DiBonaventura, M. D. (2012). Patient characteristics, treatment patterns, and health outcomes among COPD phenotypes. *Int J Chron Obstruct Pulmon Dis*, *7*, 779-787. https://doi.org/10.2147/copd.S35501

Almond, M. H., & Chung, K. F. (2018). History and physical examination of asthma, COPD, and asthma-COPD overlap. In *Asthma, COPD, and Overlap* (pp. 145-158). CRC Press.

Anderson, G. P. (2008). Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet*, *372*(9643), 1107-1119. https://doi.org/10.1016/s0140-6736(08)61452-x

Arathimos, R., Suderman, M., Sharp, G. C., Burrows, K., Granell, R., Tilling, K., Gaunt, T. R., Henderson, J., Ring, S., Richmond, R. C., & Relton, C. L. (2017). Epigenome-wide association study of asthma and wheeze in childhood and adolescence. *Clin Epigenetics*, *9*, 112. https://doi.org/10.1186/s13148-017-0414-7

Asamoah, K., Chung, K. F., Kermani, N. Z., Bodinier, B., Dahlen, S.-E., Djukanovic, R., Bhavsar, P. K., Adcock, I. M., Vuckovic, D., & Chadeau-Hyam, M. (2024). Proteomic signatures of eosinophilic and neutrophilic asthma from serum and sputum. *EBioMedicine*, *99*.

Asamoah, K., Chung, K. F., Zounemat Kermani, N., Bodinier, B., Dahlen, S. E., Djukanovic, R., Bhavsar, P. K., Adcock, I. M., Vuckovic, D., & Chadeau-Hyam, M. (2024). Proteomic signatures of eosinophilic and neutrophilic asthma from serum and sputum. *EBioMedicine*, *99*, 104936. https://doi.org/10.1016/j.ebiom.2023.104936

Asher, M. I., Rutter, C. E., Bissell, K., Chiang, C.-Y., El Sony, A., Ellwood, E., Ellwood, P., García-Marcos, L., Marks, G. B., & Morales, E. (2021). Worldwide trends in the burden of asthma symptoms in school-aged children: Global Asthma Network Phase I cross-sectional study. *The Lancet*, *398*(10311), 1569-1580.

Ashiru-Oredope, D., Cunningham, N., Casale, E., Muller-Pebody, B., Hope, R., Brown, C. S., Hopkins, S., & on behalf of the, E. R. C. L. G. (2023). Reporting England's progress towards the ambitions in the UK action plan for antimicrobial resistance: the English surveillance programme for antimicrobial utilisation and resistance (ESPAUR). *Journal of Antimicrobial Chemotherapy*, *78*(10), 2387-2391. https://doi.org/10.1093/jac/dkad248

Asthma+LungUK. (2023). Investing in breath: reducing the economic cost of lung conditions through increased research and innovation. https://www.asthmaandlung.org.uk/investinginbreath-reducingeconomiccostoflungconditions-autumn-2023. Assessed on 28th July, 2025.

Atanasova, K. R., & Reznikov, L. R. (2018). Neuropeptides in asthma, chronic obstructive pulmonary disease and cystic fibrosis. *Respir Res*, *19*(1), 149. https://doi.org/10.1186/s12931-018-0846-4

Athey, B. D., Braxenthaler, M., Haas, M., & Guo, Y. (2013). tranSMART: An Open Source and Community-Driven Informatics and Data Sharing Platform for Clinical and Translational Research. *AMIA Jt Summits Transl Sci Proc*, *2013*, 6-8.

Baines, K. J., Simpson, J. L., Wood, L. G., Scott, R. J., & Gibson, P. G. (2011). Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. *J Allergy Clin Immunol*, *127*(1), 153-160, 160.e151-159. https://doi.org/10.1016/j.jaci.2010.10.024

Baltieri, L., Cazzo, E., Oliveira Modena, D. A., Gobato Rentel, R. C., Martins, L. C., & Chaim, E. A. (2022). Correlation between levels of adipokines and inflammatory mediators with spirometric parameters in individuals with obesity and symptoms of asthma: Cross-sectional study. *Pulmonology*, *28*(2), 105-112. https://doi.org/https://doi.org/10.1016/j.pulmoe.2020.04.003

Barnes, P. J., & Adcock, I. M. (2009). Glucocorticoid resistance in inflammatory diseases. *Lancet*, *373*(9678), 1905-1917. https://doi.org/10.1016/s0140-6736(09)60326-3

Beasley, R., Semprini, A., & Mitchell, E. A. (2015). Risk factors for asthma: is prevention possible? *The Lancet*, *386*(9998), 1075-1085.

Bel, E. H., Sousa, A., Fleming, L., Bush, A., Chung, K. F., Versnel, J., Wagener, A. H., Wagers, S. S., Sterk, P. J., & Compton, C. H. (2011). Diagnosis and definition of severe refractory asthma: an international consensus statement from the Innovative Medicine Initiative (IMI). *Thorax*, *66*(10), 910-917. https://doi.org/10.1136/thx.2010.153643

Benson, V. S., Hartl, S., Barnes, N., Galwey, N., Van Dyke, M. K., & Kwon, N. (2022). Blood eosinophil counts in the general population and airways disease: a comprehensive review and meta-analysis. *Eur Respir J*, *59*(1). https://doi.org/10.1183/13993003.04590-2020

Berry, M. A., Morgan, A., Shaw, D. E., Parker, D., Green, R. H., Brightling, C. E., Bradding, P., Wardlaw, A. J., & Pavord, I. D. (2007). Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax*. http://www.ncbi.nlm.nih.gov/pubmed/17356056 (Not in File)

Bhattacharya, S., Andorf, S., Gomes, L., Dunn, P., Schaefer, H., Pontius, J., Berger, P.,

Desborough, V., Smith, T., & Campbell, J. (2014). ImmPort: disseminating data to the public for the future of immunology. *Immunologic research*, *58*, 234-239.

Bigler, J., Boedigheimer, M., Schofield, J. P., Skipp, P. J., Corfield, J., Rowe, A., Sousa, A. R., Timour, M., Twehues, L., & Hu, X. (2017). A severe asthma disease signature from gene expression profiling of peripheral blood from U-BIOPRED cohorts. *American journal of respiratory and critical care medicine*, *195*(10), 1311-1320.

Bigler, J., Boedigheimer, M., Schofield, J. P. R., Skipp, P. J., Corfield, J., Rowe, A., Sousa, A. R., Timour, M., Twehues, L., Hu, X., Roberts, G., Welcher, A. A., Yu, W., Lefaudeux, D., Meulder, B., Auffray, C., Chung, K. F., Adcock, I. M., Sterk, P. J., & Djukanović, R. (2017). A Severe Asthma Disease Signature from Gene Expression Profiling of Peripheral Blood from U-BIOPRED Cohorts. *Am J Respir Crit Care Med*, *195*(10), 1311-1320. https://doi.org/10.1164/rccm.201604-0866OC

Bjornsdottir, U. S., Holgate, S. T., Reddy, P. S., Hill, A. A., McKee, C. M., Csimma, C. I., Weaver, A. A., Legault, H. M., Small, C. G., & Ramsey, R. C. (2011). Pathways activated during human asthma exacerbation as revealed by gene expression patterns in blood. *PLoS One*, *6*(7), e21902.

Boddy, C. E., Naveed, S., Craner, M., Murphy, A. C., Siddiqui, S., & Bradding, P. (2020). Clinical Outcomes in People with Difficult-to-Control Asthma Using Electronic Monitoring to Support Medication Adherence. *J Allergy Clin Immunol Pract*. https://doi.org/10.1016/j.jaip.2020.10.059

Bonilla, P. S., & de Guzman Strong, C. (2024). Nanopore long-read sequencing solves the conundrum of filaggrin genetics. *The Journal of investigative dermatology*, *144*(8), 1678.

Bonini, M., Di Paolo, M., Bagnasco, D., Baiardini, I., Braido, F., Caminati, M., Carpagnano, E., Contoli, M., Corsico, A., Del Giacco, S., Heffler, E., Lombardi, C., Menichini, I., Milanese, M., Scichilone, N., Senna, G., & Canonica, G. W. (2020). Minimal clinically important difference for asthma endpoints: an expert consensus report. *Eur Respir Rev*, *29*(156). https://doi.org/10.1183/16000617.0137-2019

Bonser, L. R., & Erle, D. J. (2017). Airway Mucus and Asthma: The Role of MUC5AC and MUC5B. *J Clin Med*, *6*(12). https://doi.org/10.3390/jcm6120112

Borrelli, R., Brussino, L., Lo Sardo, L., Quinternetto, A., Vitali, I., Bagnasco, D., Boem, M., Corradi, F., Badiu, I., Negrini, S., & Nicola, S. (2025). Sex-Based Differences in Asthma: Pathophysiology, Hormonal Influence, and Genetic Mechanisms. *Int J Mol Sci*, *26*(11). https://doi.org/10.3390/ijms26115288

Brigham, E. P., & West, N. E. (2015). Diagnosis of asthma: diagnostic testing. International forum of allergy & rhinology,

Brightling, C. E., Ammit, A. J., Kaur, D., Black, J. L., Wardlaw, A. J., Hughes, J. M., & Bradding, P. (2005). The CXCL10/CXCR3 axis mediates human lung mast cell migration to asthmatic airway smooth muscle. *Am J Respir Crit Care Med*, *171*(10), 1103-1108. https://doi.org/10.1164/rccm.200409-1220OC

Brown, M. A., Jabeen, M., Bharj, G., & Hinks, T. S. C. (2022). Non-typeable Haemophilus influenzae airways infection: the next treatable trait in asthma? *Eur Respir Rev*, *31*(165). https://doi.org/10.1183/16000617.0008-2022

Brusselle, G. G., VanderStichele, C., Jordens, P., Deman, R., Slabbynck, H., Ringoet, V., Verleden, G., Demedts, I. K., Verhamme, K., & Delporte, A. (2013). Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. *Thorax*, *68*(4), 322-329.

Buhl, R., Heaney, L. G., Loefroth, E., Larbig, M., Kostikas, K., Conti, V., & Cao, H. (2020). One-year follow up of asthmatic patients newly initiated on treatment with medium- or high-dose inhaled corticosteroid-long-acting β2-agonist in UK primary care settings. *Respiratory medicine*, *162*, 105859. https://doi.org/https://doi.org/10.1016/j.rmed.2019.105859

ulkhi, A. A., Shepard, K. V., 2nd, Casale, T. B., & Cardet, J. C. (2020). Elevated Testosterone Is Associated with Decreased Likelihood of Current Asthma Regardless of Sex. *J Allergy Clin Immunol Pract*, *8*(9), 3029-3035.e3024. https://doi.org/10.1016/j.jaip.2020.05.022

Bunyavanich, S., Becker, P. M., Altman, M. C., Lasky-Su, J., Ober, C., Zengler, K., Berdyshev, E., Bonneau, R., Chatila, T., Chatterjee, N., Chung, K. F., Cutcliffe, C., Davidson, W., Dong, G., Fang, G., Fulkerson, P., Himes, B. E., Liang, L., Mathias, R. A., . . . Hasegawa, K. (2024). Analytical challenges in omics research on asthma and allergy: A National Institute of Allergy and Infectious Diseases workshop. *J Allergy Clin Immunol*, *153*(4), 954-968. https://doi.org/10.1016/j.jaci.2024.01.014

Bush, A. (2019). Pathophysiological Mechanisms of Asthma. *Front Pediatr*, *7*, 68. https://doi.org/10.3389/fped.2019.00068

Busse, W. W. (2010). The relationship of airway hyperresponsiveness and airway inflammation: airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest*, *138*(2), 4S-10S.

Campbell, C. D., Gleeson, M., & Sulaiman, I. (2023). The role of the respiratory microbiome in asthma. *Front Allergy*, *4*, 1120999. https://doi.org/10.3389/falgy.2023.1120999

Cao, D. Y., Giani, J. F., Veiras, L. C., Bernstein, E. A., Okwan-Duodu, D., Ahmed, F., Bresee, C., Tourtellotte, W. G., Karumanchi, S. A., Bernstein, K. E., & Khan, Z. (2021). An ACE inhibitor reduces bactericidal activity of human neutrophils in vitro and impairs mouse neutrophil activity in vivo. *Sci Transl Med*, *13*(604). https://doi.org/10.1126/scitranslmed.abj2138

Carbajal-García, A., Reyes-García, J., Díaz-Hernández, V., Casas-Hernández, M. F., Flores-Murrieta, F. J., & Montaño, L. M. (2024). Testosterone Enhances K(V) Currents and Airway Smooth Muscle Relaxation Induced by ATP and UTP through P2Y(4) Receptors and Adenylyl Cyclase Pathway. *Int J Mol Sci*, *25*(9). https://doi.org/10.3390/ijms25094652

Carey, M. A., Card, J. W., Bradbury, J. A., Moorman, M. P., Haykal-Coates, N., Gavett, S. H., Graves, J. P., Walker, V. R., Flake, G. P., Voltz, J. W., Zhu, D., Jacobs, E. R., Dakhama, A., Larsen, G. L., Loader, J. E., Gelfand, E. W., Germolec, D. R., Korach, K. S., & Zeldin, D. C. (2007). Spontaneous airway hyperresponsiveness in estrogen receptor-alpha-deficient mice. *Am J Respir Crit Care Med*, *175*(2), 126-135. https://doi.org/10.1164/rccm.200509-1493OC

Carr, T. F., Zeki, A. A., & Kraft, M. T. (2018). Eosinophilic and Noneosinophilic Asthma.

American journal of respiratory and critical care medicine, *197*, 22–37.

Cazzola, M., Ora, J., Cavalli, F., Rogliani, P., & Matera, M. G. (2021). Treatable Mechanisms in Asthma. *Molecular Diagnosis & Therapy*, *25*(2), 111-121. https://doi.org/10.1007/s40291-021-00514-w

Cazzola, M., Rogliani, P., & Matera, M. G. (2020). The latest on the role of LAMAs in asthma. *Journal of Allergy and Clinical Immunology*, *146*(6), 1288-1291.

Chen, C.-Y., Wu, K.-H., Guo, B.-C., Lin, W.-Y., Chang, Y.-J., Wei, C.-W., Lin, M.-J., & Wu, H.-P. (2023). Personalized medicine in severe asthma: from biomarkers to biologics. *International journal of molecular sciences*, *25*(1), 182.

Chen, R., Wang, L., Koch, T., Curtis, V., Yin-DeClue, H., Handley, S. A., Shan, L., Holtzman, M. J., Castro, M., & Wang, L. (2020). Sex effects in the association between airway microbiome and asthma. *Ann Allergy Asthma Immunol*, *125*(6), 652-657.e653. https://doi.org/10.1016/j.anai.2020.09.007

Chen, X., Zhang, P., & Ma, Y. (2023). Prevalence of acute infection in adults with asthma exacerbation: A systematic review and meta-analysis. *Ann Thorac Med*, *18*(3), 132-151. https://doi.org/10.4103/atm.atm_422_22

Chin-See-Chong, T. C., Kappen, J. H., Palmer, E., Layhadi, J. A., Shamji, M. H., & van der Valk, J. P. M. (2025). Mechanisms and Treatment of Type 2 High and Low Asthma Endotypes. *Clin Exp Allergy*. https://doi.org/10.1111/cea.70084

Choi, J. H., Jeong, K. B., Park, Y. H., Yu, I., Lee, S. J., Lee, M. K., Kim, S. H., Lee, W. Y., Yong, S. J., & Lee, J. H. (2021). Comparison of Risk of Pneumonia Caused by Fluticasone Propionate versus Budesonide in Chronic Obstructive Pulmonary Disease: A Nationwide Retrospective Cohort Study. *Int J Chron Obstruct Pulmon Dis*, *16*, 3229-3237. https://doi.org/10.2147/copd.S332151

Chowdhury, N. U., Guntur, V. P., Newcomb, D. C., & Wechsler, M. E. (2021). Sex and gender in asthma. *Eur Respir Rev*, *30*(162). https://doi.org/10.1183/16000617.0067-2021

Choy, D. F., Hart, K. M., Borthwick, L. A., Shikotra, A., Nagarkar, D. R., Siddiqui, S., Jia, G., Ohri, C. M., Doran, E., Vannella, K. M., Butler, C. A., Hargadon, B., Sciurba, J. C., Gieseck, R. L., Thompson, R. W., White, S., Abbas, A. R., Jackman, J., Wu, L. C., . . . Bradding, P. (2015). TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. *Sci Transl Med*, *7*(301), 301ra129. https://doi.org/10.1126/scitranslmed.aab3142

Couillard, S., Shrimanker, R., Chaudhuri, R., Mansur, A. H., McGarvey, L. P., Heaney, L. G., Fowler, S. J., Bradding, P., Pavord, I. D., & Hinks, T. S. C. (2021). Fractional Exhaled Nitric Oxide Nonsuppression Identifies Corticosteroid-Resistant Type 2 Signaling in Severe Asthma. *Am J Respir Crit Care Med*, *204*(6), 731-734. https://doi.org/10.1164/rccm.202104-1040LE

Criner, G. J., Eberhardt, R., Fernandez-Bussy, S., Gompelmann, D., Maldonado, F., Patel, N., Shah, P. L., Slebos, D. J., Valipour, A., Wahidi, M. M., Weir, M., & Herth, F. J. (2020). Interventional Bronchoscopy. *Am J Respir Crit Care Med*, *202*(1), 29-50. https://doi.org/10.1164/rccm.201907-1292SO

Crossingham, I., Richardson, R., Hinks, T. S., Spencer, S., Couillard, S., Maynard-Paquette, A.-C., Thomassen, D., & Howell, I. (2022). Biologics for chronic severe asthma: a

network meta-analysis. *The Cochrane Database of Systematic Reviews*, *2022*(10), CD015411.

Crossman-Barnes, C.-J., Sach, T., Wilson, A., & Barton, G. (2019). Estimating loss in quality of life associated with asthma-related crisis events (ESQUARE): a cohort, observational study. *Health and Quality of Life Outcomes*, *17*(1), 58. https://doi.org/10.1186/s12955-019-1138-5

Deacon, K., & Knox, A. J. (2018). PINX1 and TERT Are Required for TNF-alpha-Induced Airway Smooth Muscle Chemokine Gene Expression. *J Immunol*, *200*(4), 1283-1294. https://doi.org/10.4049/jimmunol.1700414

DeBoer, M. D., Phillips, B. R., Mauger, D. T., Zein, J., Erzurum, S. C., Fitzpatrick, A. M., Gaston, B. M., Myers, R., Ross, K. R., Chmiel, J., Lee, M. J., Fahy, J. V., Peters, M., Ly, N. P., Wenzel, S. E., Fajt, M. L., Holguin, F., Moore, W. C., Peters, S. P., . . . Gerald Teague, W. (2018). Effects of endogenous sex hormones on lung function and symptom control in adolescents with asthma. *BMC pulmonary medicine*, *18*(1), 58. https://doi.org/10.1186/s12890-018-0612-x

DeLeon, S., Clinton, A., Fowler, H., Everett, J., Horswill, A. R., & Rumbaugh, K. P. (2014). Synergistic interactions of Pseudomonas aeruginosa and Staphylococcus aureus in an in vitro wound model. *Infect Immun*, *82*(11), 4718-4728. https://doi.org/10.1128/iai.02198-14

Delgado-Chaves, F. M., Jennings, M. J., Atalaia, A., Wolff, J., Horvath, R., Mamdouh, Z. M., Baumbach, J., & Baumbach, L. (2025). Transforming literature screening: The emerging role of large language models in systematic reviews. *Proc Natl Acad Sci U S A*, *122*(2), e2411962122. https://doi.org/10.1073/pnas.2411962122

Devilliers, M. A., Brisebarre, A., Petit, L. M. G., Polette, M., Deslee, G., Djukanovic, R., Dormoy, V., Perotin, J. M., & consortium, U. B. (2024). Airway epithelial cell cilia transcriptomic dysregulation is associated with the inflammatory phenotype in asthma. *Allergy*, *79*(7), 1982-1988. https://doi.org/10.1111/all.16063

Dimitropoulou, C., White, R. E., Ownby, D. R., & Catravas, J. D. (2005). Estrogen reduces carbachol-induced constriction of asthmatic airways by stimulating large-conductance voltage and calcium-dependent potassium channels. *Am J Respir Cell Mol Biol*, *32*(3), 239-247. https://doi.org/10.1165/rcmb.2004-0331OC

Diver, S., Sridhar, S., Khalfaoui, L. C., Russell, R. J., Emson, C., Griffiths, J. M., de Los Reyes, M., Yin, D., Colice, G., & Brightling, C. E. (2022). Feno differentiates epithelial gene expression clusters: Exploratory analysis from the MESOS randomized controlled trial. *J Allergy Clin Immunol*, *150*(4), 830-840. https://doi.org/10.1016/j.jaci.2022.04.024

Dominelli, P. B., & Molgat-Seon, Y. (2022). Sex, gender and the pulmonary physiology of exercise. *Eur Respir Rev*, *31*(163). https://doi.org/10.1183/16000617.0074-2021

Dragunas, G., Koster, C. S., de Souza Xavier Costa, N., Melgert, B. N., Munhoz, C. D., Gosens, R., & Mauad, T. (2025). Neuroplasticity and neuroimmune interactions in fatal asthma. *Allergy*, *80*(2), 462-473. https://doi.org/10.1111/all.16373

Dragunas, G., Woest, M. E., Nijboer, S., Bos, S. T., van Asselt, J., de Groot, A. P., Vohlidalova, E., Vermeulen, C. J., Ditz, B., Vonk, J. M., Koppelman, G. H., van den

Berge, M., Ten Hacken, N. H. T., Timens, W., Munhoz, C. D., Prakash, Y. S., Gosens, R., & Kistemaker, L. E. M. (2020). Cholinergic neuroplasticity in asthma driven by TrkB signaling. *FASEB J*, *34*(6), 7703-7717. https://doi.org/10.1096/fj.202000170R

Du Rand, I. A., Blaikley, J., Booton, R., Chaudhuri, N., Gupta, V., Khalid, S., Mandal, S., Martin, J., Mills, J., Navani, N., Rahman, N. M., Wrightson, J. M., & Munavvar, M. (2013). British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults: accredited by NICE. *Thorax*, *68 Suppl 1*, i1-i44. https://doi.org/thoraxjnl-2013-203618 [pii];10.1136/thoraxjnl-2013-203618 [doi] (Not in File)

Dunn, S. E., Perry, W. A., & Klein, S. L. (2024). Mechanisms and consequences of sex differences in immune responses. *Nature Reviews Nephrology*, *20*(1), 37-55. https://doi.org/10.1038/s41581-023-00787-w

Durack, J., Christian, L. S., Nariya, S., Gonzalez, J., Bhakta, N. R., Ansel, K. M., Beigelman, A., Castro, M., Dyer, A. M., Israel, E., Kraft, M., Martin, R. J., Mauger, D. T., Peters, S. P., Rosenberg, S. R., Sorkness, C. A., Wechsler, M. E., Wenzel, S. E., White, S. R., . . . Huang, Y. J. (2020). Distinct associations of sputum and oral microbiota with atopic, immunologic, and clinical features in mild asthma. *J Allergy Clin Immunol*, *146*(5), 1016-1026. https://doi.org/10.1016/j.jaci.2020.03.028

Eastwood, M. C., Busby, J., Jackson, D. J., Pavord, I. D., Hanratty, C. E., Djukanovic, R., Woodcock, A., Walker, S., Hardman, T. C., Arron, J. R., Choy, D. F., Bradding, P., Brightling, C. E., Chaudhuri, R., Cowan, D., Mansur, A. H., Fowler, S. J., Howarth, P., Lordan, J., . . . Heaney, L. G. (2023). A Randomized Trial of a Composite T2-Biomarker Strategy Adjusting Corticosteroid Treatment in Severe Asthma: A Post Hoc Analysis by Sex. *J Allergy Clin Immunol Pract*, *11*(4), 1233-1242.e1235. https://doi.org/10.1016/j.jaip.2022.12.019

Ejima, A., Abe, S., Shimba, A., Sato, S., Uehata, T., Tani-Ichi, S., Munakata, S., Cui, G., Takeuchi, O., Hirai, T., Kato, S., & Ikuta, K. (2022). Androgens Alleviate Allergic Airway Inflammation by Suppressing Cytokine Production in Th2 Cells. *J Immunol*, *209*(6), 1083-1094. https://doi.org/10.4049/jimmunol.2200294

El-Husseini, Z. W., Gosens, R., Dekker, F., & Koppelman, G. H. (2020). The genetics of asthma and the promise of genomics-guided drug target discovery. *Lancet Respir Med*, *8*(10), 1045-1056. https://doi.org/10.1016/s2213-2600(20)30363-5

England, H. S. f. (2021). *Health Survey for England*.

Essilfie, A. T., Simpson, J. L., Dunkley, M. L., Morgan, L. C., Oliver, B. G., Gibson, P. G., Foster, P. S., & Hansbro, P. M. (2012). Combined Haemophilus influenzae respiratory infection and allergic airways disease drives chronic infection and features of neutrophilic asthma. *Thorax*, *67*(7), 588-599. https://doi.org/10.1136/thoraxjnl-2011-200160

Fahy, J. V. (2015). Type 2 inflammation in asthma — present in most, absent in many. *Nature Reviews Immunology*, *15*(1), 57-65. https://doi.org/10.1038/nri3786

Finney, J. M., Walker, A. S., Peto, T. E., & Wyllie, D. H. (2011). An efficient record linkage scheme using graphical analysis for identifier error detection. *BMC Med Inform Decis Mak*, *11*, 7. https://doi.org/10.1186/1472-6947-11-7

Flood-Page, P., Menzies-Gow, A., Phipps, S., Ying, S., Wangoo, A., Ludwig, M. S., Barnes,

N., Robinson, D., & Kay, A. B. (2003). Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest*, *112*(7), 1029-1036. http://www.ncbi.nlm.nih.gov/pubmed/14523040 (Not in File)

Foundation, C. F. (2022). *Cystic Fibrosis Foundation Patient Registry 2021 Annual Data Report*.

Foundation, T. R. *The R Project for Statistical Computing*. Retrieved 23/05/2023 from http://www.r-project.org/index.html

Fraga-Silva, T. F. C., Boko, M. M. M., Martins, N. S., Cetlin, A. A., Russo, M., Vianna, E. O., & Bonato, V. L. D. (2023). Asthma-associated bacterial infections: Are they protective or deleterious? *J Allergy Clin Immunol Glob*, *2*(1), 14-22. https://doi.org/10.1016/j.jacig.2022.08.003

Fraga-Silva, T. F. d. C., Boko, M. M. M., Martins, N. S., Cetlin, A. A., Russo, M., Vianna, E. O., & Bonato, V. L. D. (2023). Asthma-associated bacterial infections: Are they protective or deleterious? *Journal of Allergy and Clinical Immunology: Global*, *2*(1), 14-22. https://doi.org/https://doi.org/10.1016/j.jacig.2022.08.003

Freitas, J., & Novais, F. (2025). Review on the relationship of asthma and mental disorders. *Personalized Medicine in Psychiatry*, *51-52*, 100157. https://doi.org/https://doi.org/10.1016/j.pmip.2025.100157

Froidure, A., Mouthuy, J., Durham, S. R., Chanez, P., Sibille, Y., & Pilette, C. (2015). Asthma phenotypes and IgE responses. *European Respiratory Journal*, *47*(1), 304-319.

Fujimura, K. E., & Lynch, S. V. (2015). Microbiota in allergy and asthma and the emerging relationship with the gut microbiome. *Cell Host Microbe*, *17*(5), 592-602. https://doi.org/10.1016/j.chom.2015.04.007

Fuseini, H., Cephus, J. Y., Wu, P., Davis, J. B., Contreras, D. C., Gandhi, V. D., Rathmell, J. C., & Newcomb, D. C. (2019). ERα Signaling Increased IL-17A Production in Th17 Cells by Upregulating IL-23R Expression, Mitochondrial Respiration, and Proliferation. *Front Immunol*, *10*, 2740. https://doi.org/10.3389/fimmu.2019.02740

Fuseini, H., Yung, J. A., Cephus, J. Y., Zhang, J., Goleniewska, K., Polosukhin, V. V., Peebles, R. S., & Newcomb, D. C. (2018). Testosterone decreases house dust mite–induced type 2 and IL-17a–mediated airway inflammation. *The Journal of Immunology*, *201*(7), 1843-1854.

Gadhvi, K., Kandeil, M., Raveendran, D., Choi, J., Davies, N., Nanchahal, S., Wing, O., Quint, J., & Whittaker, H. (2023). Inhaled Corticosteroids and Risk of Cardiovascular Disease in Chronic Obstructive Pulmonary Disease: A Systematic Review and Meta-Regression. *Chronic Obstr Pulm Dis*, *10*(3), 317-327. https://doi.org/10.15326/jcopdf.2022.0386

Garcia-Alonso, L., Holland, C. H., Ibrahim, M. M., Turei, D., & Saez-Rodriguez, J. (2019). Benchmark and integration of resources for the estimation of human transcription factor activities. *Genome Res*, *29*(8), 1363-1375. https://doi.org/10.1101/gr.240663.118

Garmendia, J., Viadas, C., Calatayud, L., Mell, J. C., Martí-Lliteras, P., Euba, B., Llobet, E., Gil, C., Bengoechea, J. A., Redfield, R. J., & Liñares, J. (2014). Characterization of

nontypable Haemophilus influenzae isolates recovered from adult patients with underlying chronic lung disease reveals genotypic and phenotypic traits associated with persistent infection. *PLoS One*, *9*(5), e97020. https://doi.org/10.1371/journal.pone.0097020

Gautam, Y., Afanador, Y., Abebe, T., López, J. E., & Mersha, T. B. (2019). Genome-wide analysis revealed sex-specific gene expression in asthmatics. *Hum Mol Genet*, *28*(15), 2600-2614. https://doi.org/10.1093/hmg/ddz074

Gautam, Y., Johansson, E., & Mersha, T. B. (2022). Multi-Omics Profiling Approach to Asthma: An Evolving Paradigm. *J Pers Med*, *12*(1). https://doi.org/10.3390/jpm12010066

Gauthier, M., Chakraborty, K., Oriss, T. B., Raundhal, M., Das, S., Chen, J., Huff, R., Sinha, A., Fajt, M., Ray, P., Wenzel, S. E., & Ray, A. (2017). Severe asthma in humans and mouse model suggests a CXCL10 signature underlies corticosteroid-resistant Th1 bias. *JCI insight*, *2*(13). https://doi.org/10.1172/jci.insight.94580

Gautier, C., & Charpin, D. (2017). Environmental triggers and avoidance in the management of asthma. *Journal of asthma and allergy*, 47-56.

George, L., Taylor, A. R., Esteve-Codina, A., Soler Artigas, M., Thun, G. A., Bates, S., Pavlidis, S., Wagers, S., Boland, A., Prasse, A., Boschetto, P., Parr, D. G., Nowinski, A., Barta, I., Hohlfeld, J., Greulich, T., van den Berge, M., Hiemstra, P. S., Timens, W., . . . Brightling, C. E. (2020). Blood eosinophil count and airway epithelial transcriptome relationships in COPD versus asthma. *Allergy*, *75*(2), 370-380. https://doi.org/10.1111/all.14016

Gharib, S. A., Nguyen, E. V., Lai, Y., Plampin, J. D., Goodlett, D. R., & Hallstrand, T. S. (2011). Induced sputum proteome in healthy subjects and asthmatic patients. *J Allergy Clin Immunol*, *128*(6), 1176-1184.e1176. https://doi.org/10.1016/j.jaci.2011.07.053

Gibson, P. G., Yang, I. A., Upham, J. W., Reynolds, P. N., Hodge, S., James, A. L., Jenkins, C., Peters, M. J., Marks, G. B., Baraket, M., Powell, H., Taylor, S. L., Leong, L. E. X., Rogers, G. B., & Simpson, J. L. (2017). Effect of azithromycin on asthma exacerbations and quality of life in adults with persistent uncontrolled asthma (AMAZES): a randomised, double-blind, placebo-controlled trial. *Lancet*, *390*(10095), 659-668. https://doi.org/10.1016/s0140-6736(17)31281-3

GINA. (2018). *GINA Difficult-to-treat & Severe Asthma in Adolescent and Adult Patients: Diagnosis and Management*. .

GINA. (2024). *2024 GINA Main Report*.

Goleva, E., Jackson, L. P., Harris, J. K., Robertson, C. E., Sutherland, E. R., Hall, C. F., Good, J. T., Jr., Gelfand, E. W., Martin, R. J., & Leung, D. Y. (2013). The effects of airway microbiome on corticosteroid responsiveness in asthma. *Am J Respir Crit Care Med*, *188*(10), 1193-1201. https://doi.org/10.1164/rccm.201304-0775OC

Gomez, J. L. (2019). Epigenetics in Asthma. *Curr Allergy Asthma Rep*, *19*(12), 56. https://doi.org/10.1007/s11882-019-0886-y

Gourley, T., Roys, S., Lukacs, N. W., Kunkel, S. L., Flavell, R. A., & Chang, C. H. (1999). A novel role for the major histocompatibility complex class II transactivator CIITA in the

repression of IL-4 production. *Immunity*, *10*(3), 377-386. https://doi.org/10.1016/s1074-7613(00)80037-0

Green, B. J., Wiriyachaiporn, S., Grainge, C., Rogers, G. B., Kehagia, V., Lau, L., Carroll, M. P., Bruce, K. D., & Howarth, P. H. (2014). Potentially pathogenic airway bacteria and neutrophilic inflammation in treatment resistant severe asthma. *PLoS One*, *9*(6), e100645. https://doi.org/10.1371/journal.pone.0100645

Gruffydd-Jones, K., Thomas, M., Roman-Rodríguez, M., Infantino, A., FitzGerald, J. M., Pavord, I., Haddon, J. M., Elsasser, U., & Vogelberg, C. (2019). Asthma impacts on workplace productivity in employed patients who are symptomatic despite background therapy: a multinational survey. *Journal of asthma and allergy*, 183-194.

Guitor, A. K., & Wright, G. D. (2018). Antimicrobial Resistance and Respiratory Infections. *Chest*, *154*(5), 1202-1212. https://doi.org/10.1016/j.chest.2018.06.019

Gulraiz, F., Bellinghausen, C., Bruggeman, C. A., & Stassen, F. R. (2015). Haemophilus influenzae increases the susceptibility and inflammatory response of airway epithelial cells to viral infections. *FASEB J*, *29*(3), 849-858. https://doi.org/10.1096/fj.14-254359

Haldar, P., Brightling, C. E., Hargadon, B., Gupta, S., Monteiro, W., Sousa, A., Marshall, R. P., Bradding, P., Green, R. H., Wardlaw, A. J., & Pavord, I. D. (2009). Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med*, *360*(10), 973-984. http://www.ncbi.nlm.nih.gov/pubmed/19264686 (Not in File)

Haley, K. J., Lasky-Su, J., Manoli, S. E., Smith, L. A., Shahsafaei, A., Weiss, S. T., & Tantisira, K. (2011). RUNX transcription factors: association with pediatric asthma and modulated by maternal smoking. *Am J Physiol Lung Cell Mol Physiol*, *301*(5), L693-701. https://doi.org/10.1152/ajplung.00348.2010

Han, Y.-Y., Yan, Q., Yang, G., Chen, W., Forno, E., & Celedon, J. C. (2020). Serum free testosterone and asthma, asthma hospitalisations and lung function in British adults. *Thorax*, *75*(10), 849-854.

Han, Y., Jia, Q., Jahani, P. S., Hurrell, B. P., Pan, C., Huang, P., Gukasyan, J., Woodward, N. C., Eskin, E., Gilliland, F. D., Akbari, O., Hartiala, J. A., & Allayee, H. (2020). Genome-wide analysis highlights contribution of immune system pathways to the genetic architecture of asthma. *Nature Communications*, *11*(1), 1776. https://doi.org/10.1038/s41467-020-15649-3

Han, Y. Y., Forno, E., & Celedón, J. C. (2020). Sex Steroid Hormones and Asthma in a Nationwide Study of U.S. Adults. *Am J Respir Crit Care Med*, *201*(2), 158-166. https://doi.org/10.1164/rccm.201905-0996OC

Harvey, B. J., & McElvaney, N. G. (2024). Sex differences in airway disease: estrogen and airway surface liquid dynamics. *Biol Sex Differ*, *15*(1), 56. https://doi.org/10.1186/s13293-024-00633-z

Heaney, L. G., Busby, J., Bradding, P., Chaudhuri, R., Mansur, A. H., Niven, R., Pavord, I. D., Lindsay, J. T., Costello, R. W., & Medical Research Council, U. K. R. A. S. P. (2019). Remotely Monitored Therapy and Nitric Oxide Suppression Identifies Nonadherence in Severe Asthma. *Am J Respir Crit Care Med*, *199*(4), 454-464. https://doi.org/10.1164/rccm.201806-1182OC

Heaney, L. G., Busby, J., Hanratty, C. E., Djukanovic, R., Woodcock, A., Walker, S. M.,

Hardman, T. C., Arron, J. R., Choy, D. F., Bradding, P., Brightling, C. E., Chaudhuri, R., Cowan, D. C., Mansur, A. H., Fowler, S. J., Niven, R. M., Howarth, P. H., Lordan, J. L., Menzies-Gow, A., . . . investigators for the, M. R. C. R. A. S. P. (2020). Composite type-2 biomarker strategy versus a symptom-risk-based algorithm to adjust corticosteroid dose in patients with severe asthma: a multicentre, single-blind, parallel group, randomised controlled trial. *Lancet Respir Med*. https://doi.org/10.1016/S2213-2600(20)30397-0

Heaney, L. G., Djukanovic, R., Woodcock, A., Walker, S., Matthews, J. G., Pavord, I. D., Bradding, P., Niven, R., Brightling, C. E., Chaudhuri, R., Arron, J. R., Choy, D. F., Cowan, D., Mansur, A., Menzies-Gow, A., Adcock, I., Chung, K. F., Corrigan, C., Coyle, P., . . . Hardman, T. (2016). Research in progress: Medical Research Council United Kingdom Refractory Asthma Stratification Programme (RASP-UK). *Thorax*, *71*(2), 187-189. https://doi.org/10.1136/thoraxjnl-2015-207326

Hekking, P. P., Loza, M. J., Pavlidis, S., de Meulder, B., Lefaudeux, D., Baribaud, F., Auffray, C., Wagener, A. H., Brinkman, P. I., Lutter, R. I., Bansal, A. T., Sousa, A. R., Bates, S. A., Pandis, Y., Fleming, L. J., Shaw, D. E., Fowler, S. J., Guo, Y., Meiser, A., . . . Sterk, P. J. (2018). Pathway discovery using transcriptomic profiles in adult-onset severe asthma. *J Allergy Clin Immunol*, *141*(4), 1280-1290. https://doi.org/10.1016/j.jaci.2017.06.037

Hekking, P. W., Wener, R. R., Amelink, M., Zwinderman, A. H., Bouvy, M. L., & Bel, E. H. (2015). The prevalence of severe refractory asthma. *J Allergy Clin Immunol*, *135*(4), 896-902. https://doi.org/10.1016/j.jaci.2014.08.042

Hernandez-Pacheco, N., Pino-Yanes, M., & Flores, C. (2019). Genomic Predictors of Asthma Phenotypes and Treatment Response. *Front Pediatr*, *7*, 6. https://doi.org/10.3389/fped.2019.00006

Hinks, T. S., Levine, S. J., & Brusselle, G. G. (2021). Treatment options in type-2 low asthma. *European Respiratory Journal*, *57*(1).

Hinks, T. S., Wallington, J. C., Williams, A. P., Djukanović, R., Staples, K. J., & Wilkinson, T. M. (2016). Steroid-induced Deficiency of Mucosal-associated Invariant T Cells in the Chronic Obstructive Pulmonary Disease Lung. Implications for Nontypeable Haemophilus influenzae Infection. *Am J Respir Crit Care Med*, *194*(10), 1208-1218. https://doi.org/10.1164/rccm.201601-0002OC

Hinks, T. S., Zhou, X., Staples, K. J., Dimitrov, B. D., Manta, A., Petrossian, T., Lum, P. Y., Smith, C. G., Ward, J. A., Howarth, P. H., Walls, A. F., Gadola, S. D., & Djukanović, R. (2015). Innate and adaptive T cells in asthmatic patients: Relationship to severity and disease mechanisms. *J Allergy Clin Immunol*, *136*(2), 323-333. https://doi.org/10.1016/j.jaci.2015.01.014

Hinks, T. S. C., Brown, T., Lau, L. C. K., Rupani, H., Barber, C., Elliott, S., Ward, J. A., Ono, J., Ohta, S., Izuhara, K., Djukanović, R., Kurukulaaratchy, R. J., Chauhan, A., & Howarth, P. H. (2016). Multidimensional endotyping in patients with severe asthma reveals inflammatory heterogeneity in matrix metalloproteinases and chitinase 3-like protein 1. *J Allergy Clin Immunol*, *138*(1), 61-75. https://doi.org/10.1016/j.jaci.2015.11.020

Hoffman, G. E., & Schadt, E. E. (2016). variancePartition: interpreting drivers of variation in complex gene expression studies. *BMC bioinformatics*, *17*(1), 483. https://doi.org/10.1186/s12859-016-1323-z

Holgate, S. T., Wenzel, S., Postma, D. S., Weiss, S. T., Renz, H., & Sly, P. D. (2015). Asthma. *Nature Reviews Disease Primers*, *1*(1), 15025. https://doi.org/10.1038/nrdp.2015.25

Hong, F., Breitling, R., McEntee, C. W., Wittner, B. S., Nemhauser, J. L., & Chory, J. (2006). RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis. *Bioinformatics*, *22*(22), 2825-2827.

Hough, K. P., Curtiss, M. L., Blain, T. J., Liu, R. M., Trevor, J., Deshane, J. S., & Thannickal, V. J. (2020). Airway Remodeling in Asthma. *Front Med (Lausanne)*, *7*, 191. https://doi.org/10.3389/fmed.2020.00191

Howell, I., Howell, A., & Pavord, I. D. (2023). Type 2 inflammation and biological therapies in asthma: Targeted medicine taking flight. *J Exp Med*, *220*(7). https://doi.org/10.1084/jem.20221212

Hsiao, H. P., Lin, M. C., Wu, C. C., Wang, C. C., & Wang, T. N. (2019). Sex-Specific Asthma Phenotypes, Inflammatory Patterns, and Asthma Control in a Cluster Analysis. *J Allergy Clin Immunol Pract*, *7*(2), 556-567.e515. https://doi.org/10.1016/j.jaip.2018.08.008

Hsu, J., Qin, X., Beavers, S. F., & Mirabelli, M. C. (2016). Asthma-related school absenteeism, morbidity, and modifiable factors. *American journal of preventive medicine*, *51*(1), 23-32.

Huang, Y. J., Nariya, S., Harris, J. M., Lynch, S. V., Choy, D. F., Arron, J. R., & Boushey, H. (2015). The airway microbiome in patients with severe asthma: Associations with disease features and severity. *J Allergy Clin Immunol*, *136*(4), 874-884. https://doi.org/10.1016/j.jaci.2015.05.044

Hudey, S. N., Ledford, D. K., & Cardet, J. C. (2020). Mechanisms of non-type 2 asthma. *Current opinion in immunology*, *66*, 123-128.

Idani, E., Raji, H., Madadizadeh, F., Cheraghian, B., Haddadzadeh Shoshtari, M., & Dastoorpoor, M. (2019). Prevalence of asthma and other allergic conditions in adults in Khuzestan, southwest Iran, 2018. *BMC Public Health*, *19*(1), 303. https://doi.org/10.1186/s12889-019-6491-0

Ingawale, D. K., Mandlik, S. K., & Patel, S. S. (2015). An emphasis on molecular mechanisms of anti-inflammatory effects and glucocorticoid resistance. *Journal of Complementary and Integrative Medicine*, *12*(1), 1-13.

Inoue, H., Hattori, T., Zhou, X., Etling, E. B., Modena, B. D., Trudeau, J. B., Holguin, F., & Wenzel, S. E. (2019). Dysfunctional ErbB2, an EGF receptor family member, hinders repair of airway epithelial cells from asthmatic patients. *J Allergy Clin Immunol*, *143*(6), 2075-2085 e2010. https://doi.org/10.1016/j.jaci.2018.11.046

Ioachimescu, O. C., & Desai, N. S. (2019). Nonallergic triggers and comorbidities in asthma exacerbations and disease severity. *Clinics in Chest Medicine*, *40*(1), 71-85.

Ionescu, C. M. (2013). The human respiratory system. In *The Human Respiratory System: An Analysis of the Interplay between Anatomy, Structure, Breathing and Fractal*

Dynamics (pp. 13-22). Springer.

Ivanova, O., Richards, L. B., Vijverberg, S. J., Neerincx, A. H., Sinha, A., Sterk, P. J., & Maitland-van der Zee, A. H. (2019). What did we learn from multiple omics studies in asthma? *Allergy*, *74*(11), 2129-2145. https://doi.org/10.1111/all.13833

Jabeen, M. F., Sanderson, N. D., Foster, D., Crook, D. W., Cane, J. L., Borg, C., Connolly, C., Thulborn, S., Pavord, I. D., Klenerman, P., Street, T. L., & Hinks, T. S. C. (2022). Identifying Bacterial Airways Infection in Stable Severe Asthma Using Oxford Nanopore Sequencing Technologies. *Microbiol Spectr*, *10*(2), e0227921. https://doi.org/10.1128/spectrum.02279-21

Jabeen, M. F., Sanderson, N. D., Tinè, M., Donachie, G., Barber, C., Azim, A., Lau, L. C. K., Brown, T., Pavord, I. D., Chauhan, A., Klenerman, P., Street, T. L., Marchi, E., Howarth, P. H., & Hinks, T. S. C. (2024). Species-level, metagenomic and proteomic analysis of microbe-immune interactions in severe asthma. *Allergy*, *79*(11), 2966-2980. https://doi.org/10.1111/all.16269

Jacobson, B. C., Moy, B., Colditz, G. A., & Fuchs, C. S. (2008). Postmenopausal hormone use and symptoms of gastroesophageal reflux. *Arch Intern Med*, *168*(16), 1798-1804. https://doi.org/10.1001/archinte.168.16.1798

Jain, R., Ray, J. M., Pan, J. H., & Brody, S. L. (2012). Sex hormone-dependent regulation of cilia beat frequency in airway epithelium. *Am J Respir Cell Mol Biol*, *46*(4), 446-453. https://doi.org/10.1165/rcmb.2011-0107OC

Jatakanon, A., Lim, S., & Barnes, P. J. (2000). Changes in sputum eosinophils predict loss of asthma control. *Am J Respir Crit Care Med*, *161*(1), 64-72. https://doi.org/10.1164/ajrccm.161.1.9809100

Johnsen, R. H., Heerfordt, C. K., Boel, J. B., Dessau, R. B., Ostergaard, C., Sivapalan, P., Eklöf, J., & Jensen, J.-U. S. (2023). Inhaled corticosteroids and risk of lower respiratory tract infection with Moraxella catarrhalis in patients with chronic obstructive pulmonary disease. *BMJ Open Respiratory Research*, *10*(1), e001726.

Johnson, W. E., Li, C., & Rabinovic, A. (2007). Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics*, *8*(1), 118-127.

Joulia, R., Patti, S., Traves, W. J., Loewenthal, L., Yates, L., Walker, S. A., Puttur, F., Al-Sahaf, M., Cahill, K. N., Lai, J., Siddiqui, S., Boyce, J. A., Israel, E., & Lloyd, C. M. (2025). A single-cell spatial chart of the airway wall reveals proinflammatory cellular ecosystems and their interactions in health and asthma. *Nature immunology*, *26*(6), 920-933. https://doi.org/10.1038/s41590-025-02161-3

Kaplan, A., Hardjojo, A., Yu, S., & Price, D. (2019). Asthma across age: insights from primary care. *Frontiers in Pediatrics*, *7*, 162.

Katoh, S. (2021). Critical Involvement of CD44 in T Helper Type 2 Cell-Mediated Eosinophilic Airway Inflammation in a Mouse Model of Acute Asthma. *Front Immunol*, *12*, 811600. https://doi.org/10.3389/fimmu.2021.811600

Kelly, R. S., Sordillo, J. E., Lasky-Su, J., Dahlin, A., Perng, W., Rifas-Shiman, S. L., Weiss, S. T., Gold, D. R., Litonjua, A. A., Hivert, M. F., Oken, E., & Wu, A. C. (2018). Plasma metabolite profiles in children with current asthma. *Clin Exp Allergy*, *48*(10), 1297-1304. https://doi.org/10.1111/cea.13183

Khalfaoui, L., Symon, F. A., Couillard, S., Hargadon, B., Chaudhuri, R., Bicknell, S., Mansur, A. H., Shrimanker, R., Hinks, T. S. C., Pavord, I. D., Fowler, S. J., Brown, V., McGarvey, L. P., Heaney, L. G., Austin, C. D., Howarth, P. H., Arron, J. R., Choy, D. F., & Bradding, P. (2022b). Airway remodelling rather than cellular infiltration characterizes both type2 cytokine biomarker-high and -low severe asthma. *Allergy*, *77*(10), 2974-2986. https://doi.org/10.1111/all.15376

Klaile, E., Klassert, T. E., Scheffrahn, I., Muller, M. M., Heinrich, A., Heyl, K. A., Dienemann, H., Grunewald, C., Bals, R., Singer, B. B., & Slevogt, H. (2013). Carcinoembryonic antigen (CEA)-related cell adhesion molecules are co-expressed in the human lung and their expression can be modulated in bronchial epithelial cells by non-typable Haemophilus influenzae, Moraxella catarrhalis, TLR3, and type I and II interferons. *Respir Res*, *14*(1), 85. https://doi.org/10.1186/1465-9921-14-85

Klein, S. L., & Flanagan, K. L. (2016). Sex differences in immune responses. *Nature Reviews Immunology*, *16*(10), 626-638. https://doi.org/10.1038/nri.2016.90

Kuo, C. S., Pavlidis, S., Loza, M., Baribaud, F., Rowe, A., Pandis, I., Hoda, U., Rossios, C., Sousa, A., Wilson, S. J., Howarth, P., Dahlen, B., Dahlen, S. E., Chanez, P., Shaw, D., Krug, N., Sandström, T., De Meulder, B., Lefaudeux, D., . . . Chung, K. F. (2017). A Transcriptome-driven Analysis of Epithelial Brushings and Bronchial Biopsies to Define Asthma Phenotypes in U-BIOPRED. *Am J Respir Crit Care Med*, *195*(4), 443-455. https://doi.org/10.1164/rccm.201512-2452OC

Kuo, C. S., Pavlidis, S., Loza, M., Baribaud, F., Rowe, A., Pandis, I., Sousa, A., Corfield, J., Djukanovic, R., Lutter, R., Sterk, P. J., Auffray, C., Guo, Y., Adcock, I. M., & Chung, K. F. (2017). T-helper cell type 2 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-BIOPRED. *Eur Respir J*, *49*(2). https://doi.org/10.1183/13993003.02135-2016

Kuruvilla, M. E., Lee, F. E., & Lee, G. B. (2019). Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease. *Clin Rev Allergy Immunol*, *56*(2), 219-233. https://doi.org/10.1007/s12016-018-8712-1

Kuruvilla, M. E., Vanijcharoenkarn, K., Shih, J. A., & Lee, F. E.-H. (2019). Epidemiology and risk factors for asthma. *Respiratory medicine*, *149*, 16-22.

Kwok, W. C., Pates, K., Shah, A., Jackson, L., & Frost, F. (2025). Antimicrobial resistance in chronic lung infection: the road to resistance. *Thorax*.

Kyriakopoulos, C., Gogali, A., Bartziokas, K., & Kostikas, K. (2021). Identification and treatment of T2-low asthma in the era of biologics. *ERJ Open Res*, *7*(2). https://doi.org/10.1183/23120541.00309-2020

Laffont, S., Blanquart, E., & Guéry, J. C. (2017). Sex Differences in Asthma: A Key Role of Androgen-Signaling in Group 2 Innate Lymphoid Cells. *Front Immunol*, *8*, 1069. https://doi.org/10.3389/fimmu.2017.01069

Lambrecht, B. N., & Hammad, H. (2015). The immunology of asthma. *Nature immunology*, *16*(1), 45-56. https://doi.org/10.1038/ni.3049

Langdon, C., & Mullol, J. (2016). Nasal polyps in patients with asthma: prevalence, impact, and management challenges. *J Asthma Allergy*, *9*, 45-53. https://doi.org/10.2147/jaa.S86251

Langfelder, P., & Horvath, S. (2008b). WGCNA: an R package for weighted correlation network analysis. *BMC bioinformatics*, *9*(1), 559. https://doi.org/10.1186/1471-2105-9-559

Lavoie, G., Howell, I., Melhorn, J., Borg, C., Bermejo-Sanchez, L., Seymour, J., Jabeen, M. F., Fries, A., Hynes, G., & Pavord, I. D. (2025). Effects of azithromycin in severe eosinophilic asthma with concomitant monoclonal antibody treatment. *Thorax*, *80*(2), 113-116.

Lavoie, G., Howell, I., Melhorn, J., Borg, C., Bermejo-Sanchez, L., Seymour, J., Jabeen, M. F., Fries, A., Hynes, G., Pavord, I. D., Petousi, N., & Hinks, T. S. (2025). Effects of azithromycin in severe eosinophilic asthma with concomitant monoclonal antibody treatment. *Thorax*, *80*(2), 113-116. https://doi.org/10.1136/thorax-2024-221977

Lee-Sarwar, K., Kelly, R. S., Lasky-Su, J., Kachroo, P., Zeiger, R. S., O'Connor, G. T., Sandel, M. T., Bacharier, L. B., Beigelman, A., Laranjo, N., Gold, D. R., Weiss, S. T., & Litonjua, A. A. (2019). Dietary and Plasma Polyunsaturated Fatty Acids Are Inversely Associated with Asthma and Atopy in Early Childhood. *J Allergy Clin Immunol Pract*, *7*(2), 529-538.e528. https://doi.org/10.1016/j.jaip.2018.07.039

Li, L., Zhang, H., Wang, X., Wang, Z., Cui, L., Xu, Y., & Guan, K. (2022). Upregulated antimicrobial immune response mediated by neutrophils in the development from allergic rhinitis to asthma. *Front Immunol*, *13*, 1026121. https://doi.org/10.3389/fimmu.2022.1026121

Lindahl, M., Stahlbom, B., & Tagesson, C. (2001). Identification of a new potential airway irritation marker, palate lung nasal epithelial clone protein, in human nasal lavage fluid with two-dimensional electrophoresis and matrix-assisted laser desorption/ionization-time of flight. *Electrophoresis*, *22*(9), 1795-1800. https://doi.org/10.1002/1522-2683(200105)22:9<1795::AID-ELPS1795>3.0.CO;2-J

Liu, F., Zhou, H., Gu, B., Zou, X., Huang, J., Wu, J., Li, Y., Chen, S. S., Hua, Y., & Zhou, P. (2025). Application of large language models in medicine. *Nature Reviews Bioengineering*, 1-20.

Loewenthal, L., Busby, J., McDowell, R., Brown, T., Burhan, H., Chaudhuri, R., Dennison, P., Dodd, J. W., Doe, S., Faruqi, S., Gore, R., Idris, E., Jackson, D. J., Patel, M., Pantin, T., Pavord, I., Pfeffer, P. E., Price, D. B., Rupani, H., . . . Menzies-Gow, A. (2024). Impact of sex on severe asthma: a cross-sectional retrospective analysis of UK primary and specialist care. *Thorax*, *79*(5), 403-411. https://doi.org/10.1136/thorax-2023-220512

LoMauro, A., & Aliverti, A. (2021). Sex and gender in respiratory physiology. *European Respiratory Review*, *30*(162).

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Loza, M. J., Foster, S., Bleecker, E. R., Peters, S. P., & Penn, R. B. (2010). Asthma and gender impact accumulation of T cell subtypes. *Respiratory research*, *11*(1), 103. https://doi.org/10.1186/1465-9921-11-103

Lundbäck, B., Backman, H., Lötvall, J., & Rönmark, E. (2016). Is asthma prevalence still

increasing? *Expert Rev Respir Med*, *10*(1), 39-51. https://doi.org/10.1586/17476348.2016.1114417

Macowan, M., Pattaroni, C., Bonner, K., Chatzis, R., Daunt, C., Gore, M., Custovic, A., Shields, M. D., Power, U. F., Grigg, J., Roberts, G., Ghazal, P., Schwarze, J., Turner, S., Bush, A., Saglani, S., Lloyd, C. M., & Marsland, B. J. (2025). Deep multiomic profiling reveals molecular signatures that underpin preschool wheeze and asthma. *J Allergy Clin Immunol*, *155*(1), 94-106. https://doi.org/10.1016/j.jaci.2024.08.017

Malvisi, L., Taddei, L., Yarraguntla, A., Wilkinson, T. M. A., Arora, A. K., & the, A. S. G. (2021). Sputum sample positivity for Haemophilus influenzae or Moraxella catarrhalis in acute exacerbations of chronic obstructive pulmonary disease: evaluation of association with positivity at earlier stable disease timepoints. *Respiratory research*, *22*(1), 67. https://doi.org/10.1186/s12931-021-01653-8

Mann-Nuttel, R., Mandal, S., Armbruster, M., Puttagunta, L., & Forsythe, P. (2025). Human Pulmonary Neuroendocrine Cells Respond to House Dust Mite Extract With PAR-1 Dependent Release of CGRP. *Allergy*, *80*(4), 976-985. https://doi.org/10.1111/all.16416

Manning, J., Dunne, E. M., Wescombe, P. A., Hale, J. D. F., Mulholland, E. K., Tagg, J. R., Robins-Browne, R. M., & Satzke, C. (2016). Investigation of Streptococcus salivarius-mediated inhibition of pneumococcal adherence to pharyngeal epithelial cells. *BMC Microbiology*, *16*(1), 225. https://doi.org/10.1186/s12866-016-0843-z

Mannion, J. M., McLoughlin, R. M., & Lalor, S. J. (2023). The Airway Microbiome-IL-17 Axis: a Critical Regulator of Chronic Inflammatory Disease. *Clin Rev Allergy Immunol*, *64*(2), 161-178. https://doi.org/10.1007/s12016-022-08928-y

Marathe, S. J., Snider, M. A., Flores-Torres, A. S., Dubin, P. J., & Samarasinghe, A. E. (2022). Human matters in asthma: Considering the microbiome in pulmonary health. *Frontiers in Pharmacology*, *13*, 1020133.

Marchi, E., Hinks, T. S. C., Richardson, M., Khalfaoui, L., Symon, F. A., Rajasekar, P., Clifford, R., Hargadon, B., Austin, C. D., MacIsaac, J. L., Kobor, M. S., Siddiqui, S., Mar, J. S., Arron, J. R., Choy, D. F., & Bradding, P. (2024). The effects of inhaled corticosteroids on healthy airways. *Allergy*, *79*(7), 1831-1843. https://doi.org/10.1111/all.16146

Marri, P. R., Stern, D. A., Wright, A. L., Billheimer, D., & Martinez, F. D. (2013). Asthma-associated differences in microbial composition of induced sputum. *J Allergy Clin Immunol*, *131*(2), 346-352.e341-343. https://doi.org/10.1016/j.jaci.2012.11.013

Martin, M. J., Zain, N. M. M., Hearson, G., Rivett, D. W., Koller, G., Wooldridge, D. J., Rose, G., Gharbia, S. E., Forbes, B., Bruce, K. D., & Harrison, T. W. (2020). The airways microbiome of individuals with asthma treated with high and low doses of inhaled corticosteroids. *PLoS One*, *15*(12), e0244681. https://doi.org/10.1371/journal.pone.0244681

Martinez, F. D., & Vercelli, D. (2013). Asthma. *Lancet*, *382*(9901), 1360-1372. https://doi.org/10.1016/s0140-6736(13)61536-6

Martinez, F. J., Curtis, J. L., Sciurba, F., Mumford, J., Giardino, N. D., Weinmann, G., Kazerooni, E., Murray, S., Criner, G. J., Sin, D. D., Hogg, J., Ries, A. L., Han, M.,

Fishman, A. P., Make, B., Hoffman, E. A., Mohsenifar, Z., & Wise, R. (2007). Sex differences in severe pulmonary emphysema. *Am J Respir Crit Care Med*, *176*(3), 243-252. https://doi.org/10.1164/rccm.200606-828OC

Maspero, J., Adir, Y., Al-Ahmad, M., Celis-Preciado, C. A., Colodenco, F. D., Giavina-Bianchi, P., Lababidi, H., Ledanois, O., Mahoub, B., & Perng, D.-W. (2022). Type 2 inflammation in asthma and other airway diseases. *ERJ open research*, *8*(3).

Mattiuzzi, C., & Lippi, G. (2020). Worldwide asthma epidemiology: insights from the Global Health Data Exchange database. *Int Forum Allergy Rhinol*, *10*(1), 75-80. https://doi.org/10.1002/alr.22464

McCallister, J. W., Holbrook, J. T., Wei, C. Y., Parsons, J. P., Benninger, C. G., Dixon, A. E., Gerald, L. B., & Mastronarde, J. G. (2013). Sex differences in asthma symptom profiles and control in the American Lung Association Asthma Clinical Research Centers. *Respiratory medicine*, *107*(10), 1491-1500. https://doi.org/https://doi.org/10.1016/j.rmed.2013.07.024

McCleary, N., Nwaru, B. I., Nurmatov, U. B., Critchley, H., & Sheikh, A. (2018). Endogenous and exogenous sex steroid hormones in asthma and allergy in females: A systematic review and meta-analysis. *J Allergy Clin Immunol*, *141*(4), 1510-1513.e1518. https://doi.org/10.1016/j.jaci.2017.11.034

McDonald, V. M., Fingleton, J., Agusti, A., Hiles, S. A., Clark, V. L., Holland, A. E., Marks, G. B., Bardin, P. P., Beasley, R., Pavord, I. D., Wark, P. A. B., & Gibson, P. G. (2019). Treatable traits: a new paradigm for 21st century management of chronic airway diseases: Treatable Traits Down Under International Workshop report. *Eur Respir J*, *53*(5). https://doi.org/10.1183/13993003.02058-2018

McDonald, V. M., Hiles, S. A., Jones, K. A., Clark, V. L., & Yorke, J. (2018). Health-related quality of life burden in severe asthma. *Medical Journal of Australia*, *209*(S2), S28-S33.

McDonnell, M. J., Jary, H. R., Perry, A., MacFarlane, J. G., Hester, K. L., Small, T., Molyneux, C., Perry, J. D., Walton, K. E., & De Soyza, A. (2015). Non cystic fibrosis bronchiectasis: A longitudinal retrospective observational cohort study of Pseudomonas persistence and resistance. *Respir Med*, *109*(6), 716-726. https://doi.org/10.1016/j.rmed.2014.07.021

McGrath, K. W., Icitovic, N., Boushey, H. A., Lazarus, S. C., Sutherland, E. R., Chinchilli, V. M., & Fahy, J. V. (2012). A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. *Am J Respir Crit Care Med*, *185*(6), 612-619. https://doi.org/rccm.201109-1640OC [pii];10.1164/rccm.201109-1640OC [doi] (Not in File)

McGregor, M. C., Krings, J. G., Nair, P., & Castro, M. (2019). Role of biologics in asthma. *American journal of respiratory and critical care medicine*, *199*(4), 433-445.

McNicholl, D. M., Stevenson, M., McGarvey, L. P., & Heaney, L. G. (2012). The utility of fractional exhaled nitric oxide suppression in the identification of nonadherence in difficult asthma. *Am J Respir Crit Care Med*, *186*(11), 1102-1108. https://doi.org/rccm.201204-0587OC [pii];10.1164/rccm.201204-0587OC [doi] (Not in File)

Medoff, B. D., Sauty, A., Tager, A. M., Maclean, J. A., Smith, R. N., Mathew, A., Dufour, J. H., & Luster, A. D. (2002). IFN-gamma-inducible protein 10 (CXCL10) contributes to airway hyperreactivity and airway inflammation in a mouse model of asthma. *J Immunol*, *168*(10), 5278-5286. https://doi.org/10.4049/jimmunol.168.10.5278

Mena, K. D., & Gerba, C. P. (2009). Risk assessment of Pseudomonas aeruginosa in water. *Rev Environ Contam Toxicol*, *201*, 71-115. https://doi.org/10.1007/978-1-4419-0032-6_3

Mersha, T. B., Martin, L. J., Biagini Myers, J. M., Kovacic, M. B., He, H., Lindsey, M., Sivaprasad, U., Chen, W., & Khurana Hershey, G. K. (2015). Genomic architecture of asthma differs by sex. *Genomics*, *106*(1), 15-22. https://doi.org/https://doi.org/10.1016/j.ygeno.2015.03.003

Miethe, S., Karsonova, A., Karaulov, A., & Renz, H. (2020). Obesity and asthma. *Journal of Allergy and Clinical Immunology*, *146*(4), 685-693.

Modena, B. D., Bleecker, E. R., Busse, W. W., Erzurum, S. C., Gaston, B. M., Jarjour, N. N., Meyers, D. A., Milosevic, J., Tedrow, J. R., & Wu, W. (2017). Gene expression correlated with severe asthma characteristics reveals heterogeneous mechanisms of severe disease. *American journal of respiratory and critical care medicine*, *195*(11), 1449-1463.

Moffatt, M. F., Gut, I. G., Demenais, F., Strachan, D. P., Bouzigon, E., Heath, S., von Mutius, E., Farrall, M., Lathrop, M., & Cookson, W. (2010). A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med*, *363*(13), 1211-1221. https://doi.org/10.1056/NEJMoa0906312

Moffatt, M. F., Kabesch, M., Liang, L., Dixon, A. L., Strachan, D., Heath, S., Depner, M., von Berg, A., Bufe, A., Rietschel, E., Heinzmann, A., Simma, B., Frischer, T., Willis-Owen, S. A., Wong, K. C., Illig, T., Vogelberg, C., Weiland, S. K., von Mutius, E., . . . Cookson, W. O. (2007). Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature*, *448*(7152), 470-473. https://doi.org/10.1038/nature06014

Moradali, M. F., Ghods, S., & Rehm, B. H. (2017). Pseudomonas aeruginosa Lifestyle: A Paradigm for Adaptation, Survival, and Persistence. *Front Cell Infect Microbiol*, *7*, 39. https://doi.org/10.3389/fcimb.2017.00039

Morice, A. H., Jakes, A. D., Faruqi, S., Birring, S. S., McGarvey, L., Canning, B., Smith, J. A., Parker, S. M., Chung, K. F., Lai, K., Pavord, I. D., van den Berg, J., Song, W. J., Millqvist, E., Farrell, M. J., Mazzone, S. B., & Dicpinigaitis, P. (2014). A worldwide survey of chronic cough: a manifestation of enhanced somatosensory response. *Eur Respir J*, *44*(5), 1149-1155. https://doi.org/10.1183/09031936.00217813

Muc, M., Mota-Pinto, A., & Padez, C. (2016). Association between obesity and asthma–epidemiology, pathophysiology and clinical profile. *Nutrition research reviews*, *29*(2), 194-201.

Mukherjee, M., Stoddart, A., Gupta, R. P., Nwaru, B. I., Farr, A., Heaven, M., Fitzsimmons, D., Bandyopadhyay, A., Aftab, C., & Simpson, C. R. (2016). The epidemiology, healthcare and societal burden and costs of asthma in the UK and its member nations: analyses of standalone and linked national databases. *BMC medicine*, *14*, 1-

15.
Mumby, S., Kermani, N. Z., Garnett, J. P., Pavlidis, S., Wilson, S. J., Howarth, P. J., Thomas, M. J., Adcock, I. M., & Lopez-Garcia, C. (2022). CEACAM5 is an IL-13-regulated epithelial gene that mediates transcription in type-2 (T2) high severe asthma. *Allergy*, *77*(11), 3463-3466. https://doi.org/10.1111/all.15465
Nagasaki, T., Schuyler, A. J., Zhao, J., Samovich, S. N., Yamada, K., Deng, Y., Ginebaugh, S. P., Christenson, S. A., Woodruff, P. G., Fahy, J. V., Trudeau, J. B., Stoyanovsky, D., Ray, A., Tyurina, Y. Y., Kagan, V. E., & Wenzel, S. E. (2022). 15LO1 dictates glutathione redox changes in asthmatic airway epithelium to worsen type 2 inflammation. *J Clin Invest*, *132*(1). https://doi.org/10.1172/JCI151685
Nair, P., Martin, J. G., Cockcroft, D. C., Dolovich, M., Lemiere, C., Boulet, L.-P., & O'Byrne, P. M. (2017). Airway hyperresponsiveness in asthma: measurement and clinical relevance. *The Journal of Allergy and Clinical Immunology: In Practice*, *5*(3), 649-659. e642.
Nassaji, M., Ghorbani, R., & Afshar, R. K. (2015). The Effect of Statins Use on the Risk and Outcome of Acute Bacterial Infections in Adult Patients. *J Clin Diagn Res*, *9*(11), Oc09-12. https://doi.org/10.7860/jcdr/2015/14538.6773
Newcomb, D. C., Cephus, J. Y., Boswell, M. G., Fahrenholz, J. M., Langley, E. W., Feldman, A. S., Zhou, W., Dulek, D. E., Goleniewska, K., Woodward, K. B., Sevin, C. M., Hamilton, R. G., Kolls, J. K., & Peebles, R. S., Jr. (2015). Estrogen and progesterone decrease let-7f microRNA expression and increase IL-23/IL-23 receptor signaling and IL-17A production in patients with severe asthma. *J Allergy Clin Immunol*, *136*(4), 1025-1034.e1011. https://doi.org/10.1016/j.jaci.2015.05.046
Newcomb, D. C., & Peebles, R. S., Jr. (2013). Th17-mediated inflammation in asthma. *Curr Opin Immunol*, *25*(6), 755-760. https://doi.org/10.1016/j.coi.2013.08.002
Newman, A. M., Liu, C. L., Green, M. R., Gentles, A. J., Feng, W., Xu, Y., Hoang, C. D., Diehn, M., & Alizadeh, A. A. (2015). Robust enumeration of cell subsets from tissue expression profiles. *Nature methods*, *12*(5), 453-457.
Niessen, N. M., Fricker, M., McDonald, V. M., & Gibson, P. G. (2022). T2-low: what do we know?: Past, present, and future of biologic therapies in noneosinophilic asthma. *Annals of Allergy, Asthma & Immunology*, *129*(2), 150-159. https://doi.org/10.1016/j.anai.2022.04.020
Normansell, R., Sayer, B., Waterson, S., Dennett, E. J., Del Forno, M., & Dunleavy, A. (2018). Antibiotics for exacerbations of asthma. *Cochrane Database Syst Rev*, *6*(6), Cd002741. https://doi.org/10.1002/14651858.CD002741.pub2
Nunes, C., Pereira, A. M., & Morais-Almeida, M. (2017). Asthma costs and social impact. *Asthma research and practice*, *3*(1), 1.
O'Byrne, P., Fabbri, L. M., Pavord, I. D., Papi, A., Petruzzelli, S., & Lange, P. (2019). Asthma progression and mortality: the role of inhaled corticosteroids. *European Respiratory Journal*, *54*(1), 1900491. https://doi.org/10.1183/13993003.00491-2019
Ober, C., & Yao, T. C. (2011). The genetics of asthma and allergic disease: a 21st century perspective. *Immunol Rev*, *242*(1), 10-30. https://doi.org/10.1111/j.1600-065X.2011.01029.x

Odimba, U., Senthilselvan, A., Farrell, J., & Gao, Z. (2023). Identification of Sex-Specific Genetic Polymorphisms Associated with Asthma in Middle-Aged and Older Canadian Adults: An Analysis of CLSA Data. *J Asthma Allergy*, *16*, 553-566. https://doi.org/10.2147/jaa.S404670

Oh, J., Kim, S., Kim, M. S., Abate, Y. H., Abd ElHafeez, S., Abdelkader, A., Abdi, P., Abdulah, D. M., Aboagye, R. G., & Abolhassani, H. (2025). Global, regional, and national burden of asthma and atopic dermatitis, 1990–2021, and projections to 2050: a systematic analysis of the Global Burden of Disease Study 2021. *The Lancet Respiratory Medicine*, *13*(5), 425-446.

Ohno, S. (2013). *Sex chromosomes and sex-linked genes* (Vol. 1). Springer Science & Business Media.

Okuyama, K., Wada, K., Chihara, J., Takayanagi, M., & Ohno, I. (2008). Sex-related splenocyte function in a murine model of allergic asthma. *Clin Exp Allergy*, *38*(7), 1212-1219. https://doi.org/10.1111/j.1365-2222.2008.03015.x

Ostling, J., van Geest, M., Schofield, J. P. R., Jevnikar, Z., Wilson, S., Ward, J., Lutter, R., Shaw, D. E., Bakke, P. S., Caruso, M., Dahlen, S. E., Fowler, S. J., Horvath, I., Krug, N., Montuschi, P., Sanak, M., Sandstrom, T., Sun, K., Pandis, I., . . . Group, U. B. S. (2019). IL-17-high asthma with features of a psoriasis immunophenotype. *J Allergy Clin Immunol*, *144*(5), 1198-1213. https://doi.org/10.1016/j.jaci.2019.03.027

Papi, A., Blasi, F., Canonica, G. W., Morandi, L., Richeldi, L., & Rossi, A. (2020). Treatment strategies for asthma: reshaping the concept of asthma management. *Allergy, Asthma & Clinical Immunology*, *16*(1), 75.

Papi, A., Brightling, C., Pedersen, S. E., & Reddel, H. K. (2018). Asthma. *Lancet*, *391*(10122), 783-800. https://doi.org/10.1016/s0140-6736(17)33311-1

Park, H. W., & Weiss, S. T. (2020). Understanding the Molecular Mechanisms of Asthma through Transcriptomics. *Allergy Asthma Immunol Res*, *12*(3), 399-411. https://doi.org/10.4168/aair.2020.12.3.399

Pate, C. A., Zahran, H. S., Qin, X., Johnson, C., Hummelman, E., & Malilay, J. (2021). Asthma Surveillance - United States, 2006-2018. *MMWR Surveill Summ*, *70*(5), 1-32. https://doi.org/10.15585/mmwr.ss7005a1

Patel, M., Kurade, M., Rajalingam, S., Bhavsar, R., Mustafa, S. J., & Ponnoth, D. S. (2019). Role of angiotensin II type 1 (AT1) and type 2 (AT2) receptors in airway reactivity and inflammation in an allergic mouse model of asthma. *Immunopharmacol Immunotoxicol*, *41*(3), 428-437. https://doi.org/10.1080/08923973.2019.1609026

Patel, P. S., & Kearney, J. F. (2017). CD36 and Platelet-Activating Factor Receptor Promote House Dust Mite Allergy Development. *J Immunol*, *199*(3), 1184-1195. https://doi.org/10.4049/jimmunol.1700034

Patel, R., Solatikia, F., Zhang, H., Wolde, A., Kadalayil, L., Karmaus, W., Ewart, S., Arathimos, R., Relton, C., Ring, S., Henderson, A. J., Arshad, S. H., & Holloway, J. W. (2021). Sex-specific associations of asthma acquisition with changes in DNA methylation during adolescence. *Clin Exp Allergy*, *51*(2), 318-328. https://doi.org/10.1111/cea.13776

Pavord, I. D., Beasley, R., Agusti, A., Anderson, G. P., Bel, E., Brusselle, G., Cullinan, P.,

Custovic, A., Ducharme, F. M., & Fahy, J. V. (2018). After asthma: redefining airways diseases. *The Lancet*, *391*(10118), 350-400.

Pavord, I. D., Beasley, R., Agusti, A., Anderson, G. P., Bel, E., Brusselle, G., Cullinan, P., Custovic, A., Ducharme, F. M., Fahy, J. V., Frey, U., Gibson, P., Heaney, L. G., Holt, P. G., Humbert, M., Lloyd, C. M., Marks, G., Martinez, F. D., Sly, P. D., . . . Bush, A. (2018). After asthma: redefining airways diseases. *Lancet*, *391*(10118), 350-400. https://doi.org/10.1016/s0140-6736(17)30879-6

Pavord, I. D., & Corren, J. (2020). Biomarkers of type 2 airway inflammation in airway disease: and then there were two. *The Journal of Allergy and Clinical Immunology: In Practice*, *8*(8), 2640-2642.

Pelaia, G., Vatrella, A., Busceti, M. T., Gallelli, L., Calabrese, C., Terracciano, R., & Maselli, R. (2015). Cellular mechanisms underlying eosinophilic and neutrophilic airway inflammation in asthma. *Mediators Inflamm*, *2015*, 879783. https://doi.org/10.1155/2015/879783

Peng, B., Ye, W., Liu, S., Jiang, Y., Meng, Z., Guo, M., Zhi, L., Chang, X., & Shao, L. (2025). Sex differences in asthma: omics evidence and future directions. *Frontiers in Genetics*, *16*, 1560276.

Perencevich, E. N., McGregor, J. C., Shardell, M., Furuno, J. P., Harris, A. D., Morris, J. G., Fisman, D. N., & Johnson, J. A. (2008). Summer Peaks in the Incidences of Gram-Negative Bacterial Infection Among Hospitalized Patients. *Infection Control & Hospital Epidemiology*, *29*(12), 1124-1131. https://doi.org/10.1086/592698

Peters, M. C., Ringel, L., Dyjack, N., Herrin, R., Woodruff, P. G., Rios, C., O'Connor, B., Fahy, J. V., & Seibold, M. A. (2019). A transcriptomic method to determine airway immune dysfunction in T2-high and T2-low asthma. *American journal of respiratory and critical care medicine*, *199*(4), 465-477.

Peters, M. C., & Wenzel, S. E. (2020). Intersection of biology and therapeutics: type 2 targeted therapeutics for adult asthma. *The Lancet*, *395*(10221), 371-383. https://doi.org/10.1016/S0140-6736(19)33005-3

Qin, L., Yue, J., Guo, M., Zhang, C., Fang, X., Zhang, S., Bai, W., Liu, X., & Xie, M. (2023). Estrogen Receptor-α Exacerbates EGF-Inducing Airway Remodeling and Mucus Production in Bronchial Epithelium of Asthmatics. *Allergy Asthma Immunol Res*, *15*(5), 614-635. https://doi.org/10.4168/aair.2023.15.5.614

Rabe, K. F., & Schmidt, D. T. (2001). Pharmacological treatment of asthma today. *Eur Respir J Suppl*, *34*, 34s-40s. https://doi.org/10.1183/09031936.01.00252501

Radzikowska, U., & Golebski, K. (2023). Sex hormones and asthma: The role of estrogen in asthma development and severity. *Allergy*, *78*(3).

Ramakrishnan, S., & Couillard, S. (2021). Antibiotics for asthma attacks: masking uncertainty. *European Respiratory Journal*, *58*(1), 2100183. https://doi.org/10.1183/13993003.00183-2021

Ray, A., & Kolls, J. K. (2017). Neutrophilic Inflammation in Asthma and Association with Disease Severity. *Trends Immunol*, *38*(12), 942-954. https://doi.org/10.1016/j.it.2017.07.003

Reinke, S. N., Naz, S., Chaleckis, R., Gallart-Ayala, H., Kolmert, J., Kermani, N. Z., Tiotiu,

A., Broadhurst, D. I., Lundqvist, A., & Olsson, H. (2022). Urinary metabotype of severe asthma evidences decreased carnitine metabolism independent of oral corticosteroid treatment in the U-BIOPRED study. *European Respiratory Journal*, *59*(6).

Ricciardolo, F. L. M., Sprio, A. E., Baroso, A., Gallo, F., Riccardi, E., Bertolini, F., Carriero, V., Arrigo, E., & Ciprandi, G. (2021). Characterization of T2-Low and T2-High Asthma Phenotypes in Real-Life. *Biomedicines*, *9*(11). https://doi.org/10.3390/biomedicines9111684

Riemann, S., Meulmeester, F. L., Mailhot-Larouche, S., Ramakrishnan, S., Wechsler, M. E., Corren, J., Diver, S., Brightling, C., Castro, M., Hanania, N. A., Jackson, D. J., Marin, N., Clarke, D., Moore, A., Hardin, M. E., Holweg, C., Allu, S., T.S.C, H., Beasley, R., . . . Couillard, S. (2025). Clinical risk factors and Type-2 biomarkers predict asthma attacks regardless of sex: the ORACLE2 patient-level meta-analysis. *Preprint*.

Riffo-Vasquez, Y., Ligeiro de Oliveira, A. P., Page, C. P., Spina, D., & Tavares-de-Lima, W. (2007). Role of sex hormones in allergic inflammation in mice. *Clin Exp Allergy*, *37*(3), 459-470. https://doi.org/10.1111/j.1365-2222.2007.02670.x

Rijken, F., & Bruijnzeel, P. L. B. (2009). The Pathogenesis of Photoaging: The Role of Neutrophils and Neutrophil-Derived Enzymes. *Journal of Investigative Dermatology Symposium Proceedings*, *14*(1), 67-72. https://doi.org/https://doi.org/10.1038/jidsymp.2009.15

Rogers, G. B., Bruce, K. D., Martin, M. L., Burr, L. D., & Serisier, D. J. (2014). The effect of long-term macrolide treatment on respiratory microbiota composition in non-cystic fibrosis bronchiectasis: an analysis from the randomised, double-blind, placebo-controlled BLESS trial. *The Lancet Respiratory Medicine*, *2*(12), 988-996. https://doi.org/https://doi.org/10.1016/S2213-2600(14)70213-9

Rogers, G. B., van der Gast, C. J., & Serisier, D. J. (2015). Predominant pathogen competition and core microbiota divergence in chronic airway infection. *Isme j*, *9*(1), 217-225. https://doi.org/10.1038/ismej.2014.124

Roved, J., Westerdahl, H., & Hasselquist, D. (2017). Sex differences in immune responses: Hormonal effects, antagonistic selection, and evolutionary consequences. *Horm Behav*, *88*, 95-105. https://doi.org/10.1016/j.yhbeh.2016.11.017

Saglani, S., & Lloyd, C. M. (2015). Novel concepts in airway inflammation and remodelling in asthma. *European Respiratory Journal*, *46*(6), 1796-1804.

Saha, S., Majumdar, S., & Bhattacharyya, P. (2023). Asthma. In S. Saha, S. Majumdar, & P. Bhattacharyya (Eds.), *Pulmonomics: Omics Approaches for Understanding Pulmonary Diseases* (pp. 59-89). Springer Nature Singapore. https://doi.org/10.1007/978-981-99-3505-5_4

Sajjan, U. S., Jia, Y., Newcomb, D. C., Bentley, J. K., Lukacs, N. W., LiPuma, J. J., & Hershenson, M. B. (2006). H. influenzae potentiates airway epithelial cell responses to rhinovirus by increasing ICAM-1 and TLR3 expression. *FASEB J*, *20*(12), 2121-2123. https://doi.org/10.1096/fj.06-5806fje

Sánchez-Solís, M. (2019). Early lung function and future asthma. *Frontiers in Pediatrics*, *7*,

253.
Sathish, V., Martin, Y. N., & Prakash, Y. (2015). Sex steroid signaling: implications for lung diseases. *Pharmacology & therapeutics*, *150*, 94-108.
Schiavone, G., Epistolio, S., Martin, V., Molinari, F., Barizzi, J., Mazzucchelli, L., Frattini, M., & Wannesson, L. (2020). Functional and clinical significance of ROR1 in lung adenocarcinoma. *BMC Cancer*, *20*(1), 1085. https://doi.org/10.1186/s12885-020-07587-6
Schupp, J. C., Adams, T. S., Cosme, C., Jr., Raredon, M. S. B., Yuan, Y., Omote, N., Poli, S., Chioccioli, M., Rose, K. A., Manning, E. P., Sauler, M., DeIuliis, G., Ahangari, F., Neumark, N., Habermann, A. C., Gutierrez, A. J., Bui, L. T., Lafyatis, R., Pierce, R. W., . . . Kaminski, N. (2021). Integrated Single-Cell Atlas of Endothelial Cells of the Human Lung. *Circulation*, *144*(4), 286-302. https://doi.org/10.1161/circulationaha.120.052318
Schurz, H., Salie, M., Tromp, G., Hoal, E. G., Kinnear, C. J., & Möller, M. (2019). The X chromosome and sex-specific effects in infectious disease susceptibility. *Hum Genomics*, *13*(1), 2. https://doi.org/10.1186/s40246-018-0185-z
Senna, G., Latorre, M., Bugiani, M., Caminati, M., Heffler, E., Morrone, D., Paoletti, G., Parronchi, P., Puggioni, F., Blasi, F., Canonica, G. W., & Paggiaro, P. (2021). Sex Differences in Severe Asthma: Results From Severe Asthma Network in Italy-SANI. *Allergy Asthma Immunol Res*, *13*(2), 219-228. https://doi.org/10.4168/aair.2021.13.2.219
Shaw, D. E., Sousa, A. R., Fowler, S. J., Fleming, L. J., Roberts, G., Corfield, J., Pandis, I., Bansal, A. T., Bel, E. H., Auffray, C., Compton, C. H., Bisgaard, H., Bucchioni, E., Caruso, M., Chanez, P., Dahlén, B., Dahlen, S. E., Dyson, K., Frey, U., . . . Chung, K. F. (2015). Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur Respir J*, *46*(5), 1308-1321. https://doi.org/10.1183/13993003.00779-2015
Sheikhpour, M., Maleki, M., Ebrahimi Vargoorani, M., & Amiri, V. (2021). A review of epigenetic changes in asthma: methylation and acetylation. *Clinical Epigenetics*, *13*(1), 65. https://doi.org/10.1186/s13148-021-01049-x
Shikotra, A., Choy, D. F., Siddiqui, S., Arthur, G., Nagarkar, D. R., Jia, G., Wright, A. K., Ohri, C. M., Doran, E., Butler, C. A., Hargadon, B., Abbas, A. R., Jackman, J., Wu, L. C., Heaney, L. G., Arron, J. R., & Bradding, P. (2017). A CEACAM6-High Airway Neutrophil Phenotype and CEACAM6-High Epithelial Cells Are Features of Severe Asthma. *J Immunol*, *198*(8), 3307-3317. https://doi.org/10.4049/jimmunol.1600606
Shukla, S. D., Taylor, S. L., Gibson, P. G., Barker, D., Upham, J. W., Yang, I. A., Reynolds, P. N., Hodge, S., James, A. L., Rogers, G. B., & Simpson, J. L. (2021). Add-on azithromycin reduces sputum cytokines in non-eosinophilic asthma: an AMAZES substudy. *Thorax*, *76*(7), 733-736. https://doi.org/10.1136/thoraxjnl-2020-216331
Siddiqui, S., Shikotra, A., Richardson, M., Doran, E., Choy, D., Bell, A., Austin, C. D., Eastham-Anderson, J., Hargadon, B., Arron, J. R., Wardlaw, A., Brightling, C. E., Heaney, L. G., & Bradding, P. (2018). Airway pathological heterogeneity in asthma: Visualization of disease microclusters using topological data analysis. *J Allergy Clin*

Immunol, *142*(5), 1457-1468. https://doi.org/10.1016/j.jaci.2017.12.982

Simpson, J. L., Daly, J., Baines, K. J., Yang, I. A., Upham, J. W., Reynolds, P. N., Hodge, S., James, A. L., Hugenholtz, P., Willner, D., & Gibson, P. G. (2016). Airway dysbiosis: Haemophilus influenzae and Tropheryma in poorly controlled asthma. *Eur Respir J*, *47*(3), 792-800. https://doi.org/10.1183/13993003.00405-2015

Simpson, J. L., Scott, R., Boyle, M. J., & Gibson, P. G. (2006). Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology*, *11*(1), 54-61. https://doi.org/10.1111/j.1440-1843.2006.00784.x

Singhania, A., Rupani, H., Jayasekera, N., Lumb, S., Hales, P., Gozzard, N., Davies, D. E., Woelk, C. H., & Howarth, P. H. (2017). Altered Epithelial Gene Expression in Peripheral Airways of Severe Asthma. *PLoS One*, *12*(1), e0168680. https://doi.org/10.1371/journal.pone.0168680

Singhania, A., Wallington, J. C., Smith, C. G., Horowitz, D., Staples, K. J., Howarth, P. H., Gadola, S. D., Djukanović, R., Woelk, C. H., & Hinks, T. S. C. (2018). Multitissue Transcriptomics Delineates the Diversity of Airway T Cell Functions in Asthma. *Am J Respir Cell Mol Biol*, *58*(2), 261-270. https://doi.org/10.1165/rcmb.2017-0162OC

Smith, D., Du Rand, I. A., Addy, C., Collyns, T., Hart, S., Mitchelmore, P., Rahman, N., & Saggu, R. (2020). British Thoracic Society guideline for the use of long-term macrolides in adults with respiratory disease. *BMJ Open Respiratory Research*, *7*(1).

Smith, D., Gill, A., Hall, L., & Turner, A. M. (2021). Prevalence, Pattern, Risks Factors and Consequences of Antibiotic Resistance in COPD: A Systematic Review. *Copd*, *18*(6), 672-682. https://doi.org/10.1080/15412555.2021.2000957

Smits, H. H., Hiemstra, P. S., Da Costa, C. P., Ege, M., Edwards, M., Garn, H., Howarth, P. H., Jartti, T., De Jong, E. C., & Maizels, R. M. (2016). Microbes and asthma: opportunities for intervention. *Journal of Allergy and Clinical Immunology*, *137*(3), 690-697.

Somayaji, R., & Chalmers, J. D. (2022). Just breathe: a review of sex and gender in chronic lung disease. *Eur Respir Rev*, *31*(163). https://doi.org/10.1183/16000617.0111-2021

Stefan, M. S., Shieh, M. S., Spitzer, K. A., Pekow, P. S., Krishnan, J. A., Au, D. H., & Lindenauer, P. K. (2019). Association of Antibiotic Treatment With Outcomes in Patients Hospitalized for an Asthma Exacerbation Treated With Systemic Corticosteroids. *JAMA Intern Med*, *179*(3), 333-339. https://doi.org/10.1001/jamainternmed.2018.5394

Stengel, P. W., Zeckner, D. J., Guo, W. K., Wolos, J. A., & Snyder, D. W. (2008). Methacholine-induced pulmonary gas trapping in a mouse model of allergic asthma: effect of inhaled budesonide and ciglitazone. *Eur J Pharmacol*, *578*(1), 65-75. https://doi.org/10.1016/j.ejphar.2007.08.040

Stikker, B., Trap, L., Sedaghati-Khayat, B., de Bruijn, M. J., van Ijcken, W. F., de Roos, E., Ikram, A., Hendriks, R. W., Brusselle, G., & van Rooij, J. (2024). Epigenomic partitioning of a polygenic risk score for asthma reveals distinct genetically driven disease pathways. *European Respiratory Journal*, *64*(2).

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., & Lander, E. S. (2005). Gene set

enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences*, *102*(43), 15545-15550.

Suissa, S., Ernst, P., Benayoun, S., Baltzan, M., & Cai, B. (2000). Low-dose inhaled corticosteroids and the prevention of death from asthma. *N Engl J Med*, *343*(5), 332-336. https://doi.org/10.1056/nejm200008033430504

Sze, E., Bhalla, A., & Nair, P. (2020). Mechanisms and therapeutic strategies for non-T2 asthma. *Allergy*, *75*(2), 311-325.

Takahashi, K., Pavlidis, S., Ng Kee Kwong, F., Hoda, U., Rossios, C., Sun, K., Loza, M., Baribaud, F., Chanez, P., Fowler, S. J., Horvath, I., Montuschi, P., Singer, F., Musial, J., Dahlen, B., Dahlen, S. E., Krug, N., Sandstrom, T., Shaw, D. E., . . . Chung, K. F. (2018). Sputum proteomics and airway cell transcripts of current and ex-smokers with severe asthma in U-BIOPRED: an exploratory analysis. *Eur Respir J*, *51*(5). https://doi.org/10.1183/13993003.02173-2017

Tam, A., Churg, A., Wright, J. L., Zhou, S., Kirby, M., Coxson, H. O., Lam, S., Man, S. F., & Sin, D. D. (2016). Sex Differences in Airway Remodeling in a Mouse Model of Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*, *193*(8), 825-834. https://doi.org/10.1164/rccm.201503-0487OC

Tarasidis, G. S., & Wilson, K. F. (2015). Diagnosis of asthma: clinical assessment. International forum of allergy & rhinology,

Taylor, S. L., Leong, L. E. X., Choo, J. M., Wesselingh, S., Yang, I. A., Upham, J. W., Reynolds, P. N., Hodge, S., James, A. L., Jenkins, C., Peters, M. J., Baraket, M., Marks, G. B., Gibson, P. G., Simpson, J. L., & Rogers, G. B. (2018). Inflammatory phenotypes in patients with severe asthma are associated with distinct airway microbiology. *J Allergy Clin Immunol*, *141*(1), 94-103.e115. https://doi.org/10.1016/j.jaci.2017.03.044

Terracciano, R., Pelaia, G., Preianò, M., & Savino, R. (2015). Asthma and COPD proteomics: current approaches and future directions. *PROTEOMICS–Clinical Applications*, *9*(1-2), 203-220.

Tessa, M. K., Susan, M., Monica, K., Salman, S., Leonardo, M. F., Klaus, F. R., Alberto, P., Chris, B., Dave, S., Thys van der, M., Martijn, C. N., Huib, A. M. K., & Maarten van den, B. (2024). Sex differences in asthma control, lung function and exacerbations: the ATLANTIS study. *BMJ Open Respiratory Research*, *11*(1), e002316. https://doi.org/10.1136/bmjresp-2024-002316

Travaglini, K. J., Nabhan, A. N., Penland, L., Sinha, R., Gillich, A., Sit, R. V., Chang, S., Conley, S. D., Mori, Y., Seita, J., Berry, G. J., Shrager, J. B., Metzger, R. J., Kuo, C. S., Neff, N., Weissman, I. L., Quake, S. R., & Krasnow, M. A. (2020). A molecular cell atlas of the human lung from single-cell RNA sequencing. *Nature*, *587*(7835), 619-625. https://doi.org/10.1038/s41586-020-2922-4

Trinh, H. K. T., Lee, S.-H., Cao, T. B. T., & Park, H.-S. (2019). Asthma pharmacotherapy: an update on leukotriene treatments. *Expert Review of Respiratory Medicine*, *13*(12), 1169-1178.

Tyagi, S., & Katara, P. (2024). Metatranscriptomics: A Tool for Clinical Metagenomics. *Omics:*

a journal of integrative biology, *28*(8), 394-407.

Upton, M. N., McConnachie, A., McSharry, C., Hart, C. L., Smith, G. D., Gillis, C. R., & Watt, G. C. (2000). Intergenerational 20 year trends in the prevalence of asthma and hay fever in adults: the Midspan family study surveys of parents and offspring. *Bmj*, *321*(7253), 88-92. https://doi.org/10.1136/bmj.321.7253.88

Vanderweil, S. G., Tsai, C. L., Pelletier, A. J., Espinola, J. A., Sullivan, A. F., Blumenthal, D., & Camargo, C. A., Jr. (2008). Inappropriate use of antibiotics for acute asthma in United States emergency departments. *Acad Emerg Med*, *15*(8), 736-743. https://doi.org/10.1111/j.1553-2712.2008.00167.x

Vernon, M. K., Wiklund, I., Bell, J. A., Dale, P., & Chapman, K. R. (2012). What do we know about asthma triggers? A review of the literature. *Journal of Asthma*, *49*(10), 991-998.

Vieira Braga, F. A., Kar, G., Berg, M., Carpaij, O. A., Polanski, K., Simon, L. M., Brouwer, S., Gomes, T., Hesse, L., Jiang, J., Fasouli, E. S., Efremova, M., Vento-Tormo, R., Talavera-López, C., Jonker, M. R., Affleck, K., Palit, S., Strzelecka, P. M., Firth, H. V., . . . Teichmann, S. A. (2019). A cellular census of human lungs identifies novel cell states in health and in asthma. *Nat Med*, *25*(7), 1153-1163. https://doi.org/10.1038/s41591-019-0468-5

Vijeyakumaran, M., Jawhri, M. A., Fortunato, J., Solomon, L., Shrestha Palikhe, N., Vliagoftis, H., & Cameron, L. (2023). Dual activation of estrogen receptor alpha and glucocorticoid receptor upregulate CRTh2-mediated type 2 inflammation; mechanism driving asthma severity in women? *Allergy*, *78*(3), 767-779.

Villedieu, A., Papesh, E., Weinberg, S. E., Teare, L., Radhakrishnan, J., & Elamin, W. F. (2018). Seasonal variation of Pseudomonas aeruginosa in culture positive otitis externa in South East England. *Epidemiol Infect*, *146*(14), 1811-1812. https://doi.org/10.1017/s0950268818001899

Vitiello, A., Blasi, F., Sabbatucci, M., Zovi, A., Miele, F., Ponzo, A., Langella, R., & Boccellino, M. (2024). The Impact of Antimicrobial Resistance in Cystic Fibrosis. *J Clin Med*, *13*(6). https://doi.org/10.3390/jcm13061711

Wadhwa, R., Dua, K., Adcock, I. M., Horvat, J. C., Kim, R. Y., & Hansbro, P. M. (2019). Cellular mechanisms underlying steroid-resistant asthma. *European Respiratory Review*, *28*(153), 190096. https://doi.org/10.1183/16000617.0096-2019

Wadsworth, S., Sin, D., & Dorscheid, D. (2011). Clinical update on the use of biomarkers of airway inflammation in the management of asthma. *J Asthma Allergy*, *4*, 77-86. https://doi.org/10.2147/jaa.S15081

Wan, X., Miao, R., Zhang, N., Huang, W., Wu, Z., Wang, H., Yang, Y., Xie, Y., & Du, Y. (2025). Global burden of antimicrobial resistance in lower respiratory infections in 2021: A systematic analysis. *International Journal of Antimicrobial Agents*, *65*(2), 107431. https://doi.org/https://doi.org/10.1016/j.ijantimicag.2024.107431

Wang, C., Jiang, S., Zhang, S., Ouyang, Z., Wang, G., & Wang, F. (2021). Research progress of metabolomics in asthma. *Metabolites*, *11*(9), 567.

Wang, E., Liu, X., Tu, W., Do, D. C., Yu, H., Yang, L., Zhou, Y., Xu, D., Huang, S. K., Yang, P., Ran, P., Gao, P. S., & Liu, Z. (2019). Benzo(a)pyrene facilitates dermatophagoides group 1 (Der f 1)-induced epithelial cytokine release through aryl hydrocarbon

receptor in asthma. *Allergy*, *74*(9), 1675-1690. https://doi.org/10.1111/all.13784
Wang, W., Li, J. J., Foster, P. S., Hansbro, P. M., & Yang, M. (2010). Potential therapeutic targets for steroid-resistant asthma. *Curr Drug Targets*, *11*(8), 957-970. https://doi.org/10.2174/138945010791591412
Welp, A. L., & Bomberger, J. M. (2020). Bacterial community interactions during chronic respiratory disease. *Frontiers in cellular and infection microbiology*, *10*, 213.
Wenzel, S. (2005). Severe asthma in adults. *Am. J. Respir. Crit. Care Med*, *172*(2), 149-160. http://ajrccm.atsjournals.org/cgi/content/abstract/172/2/149 (Not in File)
Wenzel, S., Ford, L., Pearlman, D., Spector, S., Sher, L., Skobieranda, F., Wang, L., Kirkesseli, S., Rocklin, R., Bock, B., Hamilton, J., Ming, J. E., Radin, A., Stahl, N., Yancopoulos, G. D., Graham, N., & Pirozzi, G. (2013). Dupilumab in persistent asthma with elevated eosinophil levels. *N. Engl. J Med*, *368*(26), 2455-2466. https://doi.org/10.1056/NEJMoa1304048 [doi] (Not in File)
Whetstone, C. E., Amer, R., Maqbool, S., Javed, T., & Gauvreau, G. M. (2025). Pathobiology and Regulation of Eosinophils, Mast Cells, and Basophils in Allergic Asthma. *Immunol Rev*, *331*(1), e70018. https://doi.org/10.1111/imr.70018
Wilkinson, T. M. A., Aris, E., Bourne, S., Clarke, S. C., Peeters, M., Pascal, T. G., Schoonbroodt, S., Tuck, A. C., Kim, V., Ostridge, K., Staples, K. J., Williams, N., Williams, A., Wootton, S., & Devaster, J. M. (2017). A prospective, observational cohort study of the seasonal dynamics of airway pathogens in the aetiology of exacerbations in COPD. *Thorax*, *72*(10), 919-927. https://doi.org/10.1136/thoraxjnl-2016-209023
Williams, C. G., Lee, H. J., Asatsuma, T., Vento-Tormo, R., & Haque, A. (2022). An introduction to spatial transcriptomics for biomedical research. *Genome medicine*, *14*(1), 68.
Wilson, S. J., Ward, J. A., Pickett, H. M., Baldi, S., Sousa, A. R., Sterk, P. J., Chung, K. F., Djukanovic, R., Dahlen, B., & Billing, B. (2021). Airway Elastin is increased in severe asthma and relates to proximal wall area: histological and computed tomography findings from the U-BIOPRED severe asthma study. *Clinical & Experimental Allergy*, *51*(2), 296-304.
Wilson, S. J., Ward, J. A., Pickett, H. M., Baldi, S., Sousa, A. R., Sterk, P. J., Chung, K. F., Djukanovic, R., Dahlen, B., Billing, B., Shaw, D., Krug, N., Sandström, T., Brightling, C., & Howarth, P. H. (2021). Airway Elastin is increased in severe asthma and relates to proximal wall area: histological and computed tomography findings from the U-BIOPRED severe asthma study. *Clin Exp Allergy*, *51*(2), 296-304. https://doi.org/10.1111/cea.13813
Woodruff, P. G., Boushey, H. A., Dolganov, G. M., Barker, C. S., Yang, Y. H., Donnelly, S., Ellwanger, A., Sidhu, S. S., Dao-Pick, T. P., Pantoja, C., Erle, D. J., Yamamoto, K. R., & Fahy, J. V. (2007). Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci U S A*, *104*(40), 15858-15863. https://doi.org/10.1073/pnas.0707413104
Woodruff, P. G., Modrek, B., Choy, D. F., Jia, G., Abbas, A. R., Ellwanger, A., Koth, L. L., Arron, J. R., & Fahy, J. V. (2009). T-helper type 2-driven inflammation defines major

subphenotypes of asthma. *Am J Respir Crit Care Med*, *180*(5), 388-395. https://doi.org/10.1164/rccm.200903-0392OC

Yaghoubi, M., Adibi, A., Safari, A., FitzGerald, J. M., & Sadatsafavi, M. (2019). The Projected Economic and Health Burden of Uncontrolled Asthma in the United States. *Am J Respir Crit Care Med*, *200*(9), 1102-1112. https://doi.org/10.1164/rccm.201901-0016OC

Yan, Q., Zhang, X., Xie, Y., Yang, J., Liu, C., Zhang, M., Zheng, W., Lin, X., Huang, H.-t., & Liu, X. (2024). Bronchial epithelial transcriptomics and experimental validation reveal asthma severity-related neutrophilc signatures and potential treatments. *Communications Biology*, *7*(1), 181.

Yu, G., Wang, L.-G., Han, Y., & He, Q.-Y. (2012). clusterProfiler: an R package for comparing biological themes among gene clusters. *Omics: a journal of integrative biology*, *16*(5), 284-287.

Yuan, L., Tao, J., Wang, J., She, W., Zou, Y., Li, R., Ma, Y., Sun, C., Bi, S., & Wei, S. (2025). Global, regional, national burden of asthma from 1990 to 2021, with projections of incidence to 2050: a systematic analysis of the global burden of disease study 2021. *EClinicalMedicine*, *80*.

Yue, M., Gaietto, K., Han, Y. Y., Rosser, F. J., Xu, Z., Qoyawayma, C., Acosta-Perez, E., Canino, G., Forno, E., & Chen, W. (2025). Transcriptomic profiles in nasal epithelium and asthma endotypes in youth. *Jama*, *333*(4), 307-318.

Yung, J. A., Fuseini, H., & Newcomb, D. C. (2018). Hormones, sex, and asthma. *Ann Allergy Asthma Immunol*, *120*(5), 488-494. https://doi.org/10.1016/j.anai.2018.01.016

Zein, J., Gaston, B., Bazeley, P., DeBoer, M. D., Igo, R. P., Jr., Bleecker, E. R., Meyers, D., Comhair, S., Marozkina, N. V., Cotton, C., Patel, M., Alyamani, M., Xu, W., Busse, W. W., Calhoun, W. J., Ortega, V., Hawkins, G. A., Castro, M., Chung, K. F., . . . Sharifi, N. (2020). HSD3B1 genotype identifies glucocorticoid responsiveness in severe asthma. *Proc Natl Acad Sci U S A*, *117*(4), 2187-2193. https://doi.org/10.1073/pnas.1918819117

Zein, J. G., McManus, J. M., Sharifi, N., Erzurum, S. C., Marozkina, N., Lahm, T., Giddings, O., Davis, M. D., DeBoer, M. D., Comhair, S. A., Bazeley, P., Kim, H. J., Busse, W., Calhoun, W., Castro, M., Chung, K. F., Fahy, J. V., Israel, E., Jarjour, N. N., . . . Gaston, B. (2021). Benefits of Airway Androgen Receptor Expression in Human Asthma. *Am J Respir Crit Care Med*, *204*(3), 285-293. https://doi.org/10.1164/rccm.202009-3720OC

Zeng, X., Qing, J., Li, C.-M., Lu, J., Yamawaki, T., Hsu, Y.-H., Vander Lugt, B., Hsu, H., Busby, J., & McDowell, P. (2023). Blood transcriptomic signature in type-2 biomarker-low severe asthma and asthma control. *Journal of Allergy and Clinical Immunology*, *152*(4), 876-886.

Zhang, J. (2022). *ClusterGVis: One-step to Cluster and Visualize Gene Expression Matrix. https://github.com/junjunlab/ClusterGVis*.

Zhang, J., Li, X., Jia, X., Li, Y., Zheng, B., Yu, Y., Hu, F., Sun, Z., Chen, Z., Zhang, G., Kang, W., Wang, T., Li, J., Gao, H., Yi, Q., Yu, W., Chu, X., Xu, Y., & Yang, Q. (2025). Establishing Sitafloxacin Epidemiological Cut-off Values (ECOFFs) for Clinical

Bacterial Isolates. *Infect Drug Resist*, *18*, 1993-2004. https://doi.org/10.2147/idr.S501783

Zhang, Q., Cox, M., Liang, Z., Brinkmann, F., Cardenas, P. A., Duff, R., Bhavsar, P., Cookson, W., Moffatt, M., & Chung, K. F. (2016). Airway Microbiota in Severe Asthma and Relationship to Asthma Severity and Phenotypes. *PLoS One*, *11*(4), e0152724. https://doi.org/10.1371/journal.pone.0152724

Zhang, Q., Illing, R., Hui, C. K., Downey, K., Carr, D., Stearn, M., Alshafi, K., Menzies-Gow, A., Zhong, N., & Fan Chung, K. (2012). Bacteria in sputum of stable severe asthma and increased airway wall thickness. *Respir Res*, *13*(1), 35. https://doi.org/10.1186/1465-9921-13-35

Zhang, W., Zhang, Y., Li, L., Chen, R., & Shi, F. (2024). Unraveling heterogeneity and treatment of asthma through integrating multi-omics data. *Frontiers in Allergy*, *5*, 1496392.

Zhou, Y., Duan, Q., & Yang, D. (2023). In vitro human cell-based models to study airway remodeling in asthma. *Biomedicine & Pharmacotherapy*, *159*, 114218.

Zhu, G., Cai, H., Ye, L., Mo, Y., Zhu, M., Zeng, Y., Song, X., Yang, C., Gao, X., Wang, J., & Jin, M. (2021). Small Proline-Rich Protein 3 Regulates IL-33/ILC2 Axis to Promote Allergic Airway Inflammation. *Front Immunol*, *12*, 758829. https://doi.org/10.3389/fimmu.2021.758829