An interferon-inducible signature of airway disease from blood gene expression profiling

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Airway wall thickness in chronic obstructive pulmonary disease (COPD) is associated with type 1 interferon signalling from peripheral blood gene expression https://bit.ly/2WEjvFH

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Abstract

Background The molecular basis of airway remodelling in chronic obstructive pulmonary disease (COPD) remains poorly understood. We identified gene expression signatures associated with chest computed tomography (CT) scan airway measures to understand molecular pathways associated with airway disease.

Methods In 2396 subjects in the COPDGene Study, we examined the relationship between quantitative CT airway phenotypes and blood transcriptomes to identify airway disease-specific genes and to define an airway wall thickness (AWT) gene set score. Multivariable regression analyses were performed to identify associations of the AWT score with clinical phenotypes, bronchial gene expression and genetic variants.

Results Type 1 interferon (IFN)-induced genes were consistently associated with AWT, square root wall area of a hypothetical airway with 10 mm internal perimeter (Pi10) and wall area percentage, with the strongest enrichment in AWT. A score derived from 18 genes whose expression was associated with AWT was associated with COPD-related phenotypes including reduced lung function (forced expiratory volume in 1 s percentage predicted $\beta = -3.4$; $p<0.05$) and increased exacerbations (incidence rate ratio 1.7; $p<0.05$). The AWT score was reproducibly associated with AWT in bronchial samples from 23 subjects ($\beta=3.22$; $p<0.05$). The blood AWT score was associated with genetic variant rs876039, an expression quantitative trait locus for *IKZF1*, a gene that regulates IFN signalling and is associated with inflammatory diseases.

Conclusions A gene expression signature with IFN-stimulated genes from peripheral blood and bronchial brushings is associated with CT AWT, lung function and exacerbations. Shared genes and genetic associations suggest viral responses and/or autoimmune dysregulation as potential underlying mechanisms of airway disease in COPD.

Introduction

Emphysema and airway disease are two distinct pathological processes that independently lead to airflow obstruction in chronic obstructive pulmonary disease (COPD). However, unlike emphysema which has been extensively studied using animal models, there are considerable differences between species in airway anatomy. Murine models generally do not develop airway remodelling or mucus hypersecretion. Thus, the biological pathways related to airway disease processes remain largely unknown.

Recent advances in chest computed tomography (CT) scan quantification allow distinction between emphysema and airway disease for phenotyping and understanding heterogeneity in the disease process. In addition, CT measures of airways correlate with physiological and clinical characteristics. Airway wall thickness (AWT), square root wall area of a hypothetical airway with 10 mm internal perimeter (Pi10) and airway wall area percentage (WA%) are inversely correlated with forced expiratory volume in 1 s (FEV_1) [1], and positively associated with exacerbation risk [2], bronchodilator response, and respiratory symptoms such as chronic bronchitis and St George's Respiratory Questionnaire score [3, 4].

The Genetic Epidemiology of COPD Study (COPDGene) is a multicentre study that extensively characterised participants, and includes quantitative chest CT scans, genetic analysis and gene expression profiling. Prior studies from COPDGene showed that blood gene expression profiles track with lung gene expression [5] and identified blood gene expression signatures of eosinophilic COPD [6], providing molecular insights.

Therefore, to understand the pathways related to airway disease in COPD, we utilised RNA sequencing and CT quantification in a large sample size. We hypothesised that blood gene expression profiling would identify molecular signatures associated with CT airway measures. We further hypothesised that a blood airway gene expression score would be associated with specific clinical phenotypes, with bronchial gene expression and with genetic risk.

Methods

Study population

COPDGene (ClinicalTrials.gov: NCT00608764) is a multicentre observational study which enrolled 10 192 smokers with and without COPD (defined as post-bronchodilator FEV_1/forced vital capacity (FVC) <0.7) in Phase 1 [7]. Approximately 5800 returned for a Phase 2 visit 5 years later (supplementary material).

RNA sequencing

Whole-blood and bronchial epithelial RNA sequencing were performed from Phase 2 samples. Protocols have been described previously (supplementary material) [5, 8]. Bronchial epithelial cells were obtained by flexible bronchoscopy with brushing of the right mainstem bronchus.

Differential gene expression

Association analyses of CT airway phenotypes and transcript expression were performed with voom transformation in the limma R package [9]. Covariates in the linear models are specified in individual results tables. Differentially expressed genes were defined by false discovery rate (FDR) <0.05 in models with and without surrogate variable adjustment. Pathway analyses were performed using MSigDB [10]. Expression data have been deposited with the Database of Genotypes and Phenotypes (dbGaP; accession phs000765.v6.p2).

Interferon response genes

GEO datasets (GSE38351, GSE71634, GSE19491 and GSE60244) were analysed to identify differentially expressed genes associated with interferon (IFN) treatment or with disease conditions (supplementary table E1, supplementary material and supplementary material file E2).

Quantification of gene set scores

Gene set variation analysis (GSVA) was performed to calculate AWT and type 1 IFN enrichment scores [11]. Linear or negative binomial regression was performed to test the associations between scores and continuous variables. The strength of correlation was estimated by the regression coefficient (β), which is the change in the outcome variable associated with a 1-unit increase in the AWT score when all other variables are held constant. p-values were adjusted using the Holm–Bonferroni method.

Genome-wide association study

We performed linear regression of the AWT score (see Results) adjusting for age, sex, smoking status and genetic ancestry in non-Hispanic White subjects using Illumina OmniExpress genotypes, HRC 1.1 imputation [12] and PLINK version 2.00a2LM [13]. Functional annotation was conducted using FUMA version 1.3.5 [14] and the Open Targets Genetics platform [15].

Results

CT AWT is associated with IFN-stimulated genes

Peripheral blood RNA sequencing was performed on 2396 subjects with quantitative CT airway measurements (table 1). COPD subjects were older, and more likely to be male non-Hispanic White and former smokers. All three measures of CT airways (AWT, WA% and Pi10) were increased in COPD cases compared with controls and inversely associated with post-bronchodilator FEV_1 ($r=-0.16$, -0.38 and -0.55, respectively; $p<0.001$). We tested blood gene expression levels associated with CT airway measures using linear models adjusted for age, sex, race, pack-years of smoking, current smoking status, white blood cell counts and library construction batch. We found 18 genes whose expression was associated with AWT, 17 genes associated with WA% and 81 genes associated with Pi10 (supplementary material file E1). Notably, the AWT-associated genes were enriched for IFN-stimulated genes (ISGs) such as *OAS*, *RSAD2* and *IFI44*, with over-representation of type 1 IFN signalling and viral response pathways. These ISGs were

TABLE 1 Study subjects

	Non-COPD[#] (n=1368)	COPD (n=1028)	p-value
Age (years)	63.11±8.07	68.21±8.29	<0.01
Female	712 (52.9)	443 (43.1)	<0.01
Non-Hispanic White[¶]	958 (70.0)	832 (80.9)	<0.01
FEV_1 (% pred)	91.68±15.95	62.17±22.45	<0.01
Bronchodilator response	129 (9.4)	298 (29)	<0.01
ICS use[+]	124 (9.1)	402 (39.1)	<0.01
Airway wall thickness (mm)	0.98±0.20	1.10±0.24	<0.01
Pi10 (mm)	2.04±0.45	2.54±0.58	<0.01
Wall area percentage (%)	47.60±7.55	53.06±8.30	<0.01
Current smoker	533 (39.0)	331 (32.2)	<0.01
Smoking history (pack-years)	39.10±21.06	50.61±26.05	<0.01
White blood cells ($\times 10^3$ μL^{-1})	6.82±1.96	7.37±1.99	<0.01
Neutrophils (%)	57.27±9.80	61.23±10.24	<0.01
Lymphocytes (%)	31.43±9.38	27.11±9.33	<0.01
Monocytes (%)	8.01±2.36	8.29±2.45	0.01
Eosinophils (%)	2.66±1.92	2.75±2.11	0.28
Basophils (%)	0.64±0.60	0.61±0.57	0.38

Data are presented as mean±SD or n (%), unless otherwise stated. COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in 1 s; ICS: inhaled corticosteroid; Pi10: square root wall area of a hypothetical airway with 10 mm internal perimeter. [#]: including 306 subjects with preserved ratio and impaired spirometry (reduced FEV_1 in the setting of preserved FEV_1/forced vital capacity [40]); [¶]: remainder African American; [+]: including ICS and long-acting β-agonist combination inhalers. Quantitative phenotyping of segmental airways using Thirona software was performed by measuring airway dimensions in six bronchial paths: right upper lobe apical bronchus (RB1), right middle lobe lateral bronchus (RB4), right lower lobe posterior basal bronchus (RB10), left upper lobe apicoposterior bronchus (LB1), superior lingular bronchus (LB4) and left lower lobe posterior basal bronchus (LB10). p-values are based on the t-test or Chi-squared test for proportions.

positively associated with AWT, indicating higher expression was associated with increased AWT. Similar associations of IFN response genes were observed with WA% and Pi10. WA% was associated with ISGs *RSAD2* and *IFI44L*, and Pi10-associated genes included *LMO2*, *GBP2* and *RABGAP1L* induced by IFN-α (supplementary material file E1) that were all positively associated with CT airway measures. The association of ISGs was strongest with AWT (table 2). WA%-associated genes did not show any significant pathways, while Pi10-associated genes were enriched for haemoglobin biosynthetic process and regulation of phosphorus metabolic process (Gene Ontology Biological Process terms). Enrichment of ISGs was unique for airway-related phenotypes and was not observed with lung function or emphysema [16]. Unlike ISGs, the expression of genes encoding IFNs themselves was not associated with CT airway measures.

TABLE 2 Genes and pathways associated with computed tomography (CT) airway phenotypes

	AWT	WA%	Pi10
Differentially expressed genes (n)	18	17	81
Enrichment analysis			
Gene Ontology Biological Process	Regulation of nuclease activity; response to type I IFN; response to virus; viral life cycle; defence response to other organism	NS	Haemoglobin biosynthetic process; regulation of phosphorus metabolic process
MSigDB	Genes upregulated in ovarian cancer progenitor cells in response to IFN-α; IFN-induced antiviral module in sputum during asthma exacerbations; genes upregulated in SARS-CoV-2 infection	NS	Genes upregulated in primary fibroblast culture after treatment with IFN-α

Genes associated with CT airway phenotypes at false discovery rate <0.05. AWT: (segmental) airway wall thickness; WA%: wall area percentage; Pi10: square root wall area of a hypothetical airway with 10 mm internal perimeter; IFN: interferon; MSigDB: Molecular Signatures Database [10]; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; NS: nonsignificant.

TABLE 3 Associations between the blood airway wall thickness (AWT) score and computed tomography (CT) AWT

	β (95% CI)	Adjusted p-value
All subjects (n=2396)	0.25 (0.16–0.34)	<0.001
COPD (n=1028)	0.26 (0.11–0.42)	0.036
Control (n=1368)	0.15 (0.05–0.26)	0.13

COPD: chronic obstructive pulmonary disease. Linear regression adjusted for age, sex, race, body mass index, smoking status, CT scanner model and emphysema (%). CT AWT standardised using z-scores. p-value adjusted for multiple testing using Holm–Bonferroni correction.

Generation of a score associated with CT AWT

To further understand pathways and phenotypes associated with airway gene expression, we generated a gene signature score with the 18 genes associated with CT AWT using GSVA which we termed the "blood AWT (gene set) score" (supplementary material file E1). As expected, the blood AWT score was associated with AWT after adjusting for age, sex, race, body mass index, smoking status, emphysema percentage and CT scanner model, and it was also associated with WA%, Pi10 and emphysema percentage to a lesser degree (table 3, supplementary table E2 and supplementary figure E1). These correlations between CT AWT and the AWT score were maintained in former and current smokers. When stratified by case–control status, the association between CT AWT and the blood AWT score was stronger in subjects with COPD (table 3).

AWT score relationship with the type 1 IFN signature

We compared the AWT score with type 1 IFN-induced genes in blood from previously published studies. The type 1 IFN signature was defined as 110 upregulated genes after treatment of peripheral blood monocytes with IFN-α2 and peripheral blood mononuclear cells with IFN-β, using blood samples from healthy donors (supplementary material file E2) [17, 18]. Nine genes (*IFI44*, *IFI44L*, *RSAD2*, *CMPK2*, *DDX60*, *RIN2*, *OAS2*, *OAS3* and *HERC5*) overlapped between AWT genes and type 1 IFN-induced genes; the AWT score and type 1 IFN score showed high correlation (Spearman $\rho=0.89$; $p<2.2\times10^{-16}$), supporting enrichment of ISGs (figure 1). Stratified differential expression analysis of low *versus* high AWT score subjects also confirmed enrichment of ISG pathways (supplementary material and supplementary table E3).

The blood-derived AWT score is recapitulated in bronchial gene expression

Using RNA sequencing data from bronchial epithelial brushings from 23 COPDGene subjects (supplementary table E4), we recreated the AWT gene set score with the same genes from the blood signature, except immunoglobin genes *IGHG3* and *IGLV3-21*, which were not expressed in bronchial epithelial cells. The bronchial AWT score was positively associated with AWT and WA% in multivariable linear regression analysis, reproducing the relationship observed in blood. We did not find the AWT score to be associated with Pi10, which showed the weakest association with the AWT score in blood (table 4).

The AWT score is associated with reduced lung function and lung function decline

A previous COPDGene publication has shown CT airway measures to be inversely associated with lung function [1]. We found that the AWT score was inversely associated with FEV_1 % pred and FEV_1/FVC measured at the Phase 2 visit (concurrent with RNA collection) as well as spirometry measurements from the Phase 1 visit, ~5 years prior. Higher AWT score was also associated with greater lung function decline over 5 years (table 5).

The AWT score overlaps with autoimmune and respiratory viral infection signatures and predicts exacerbations

First discovered as an essential component of the antiviral host response, type 1 IFN can also be induced by bacterial pathogens and drive chronic inflammation in autoimmune diseases. To understand whether type 1 IFN signalling in the AWT score may represent active infection or an autoimmune-like process, we compared the AWT gene set with two published blood gene expression studies: 1) in patients with systemic lupus erythematosus (SLE) or systemic bacterial infection [19] and 2) in patients with lower respiratory viral or bacterial infections (supplementary material file E2) [20]. SLE is an extensively studied autoimmune disease with dysregulation of type 1 IFN response genes. We found the AWT score overlapped with SLE gene expression but not with systemic bacterial infection ($p<1.0\times10^{-6}$). Within lower respiratory infection, the AWT score overlapped with viral respiratory infection ($p<2.8\times10^{-14}$) but not with

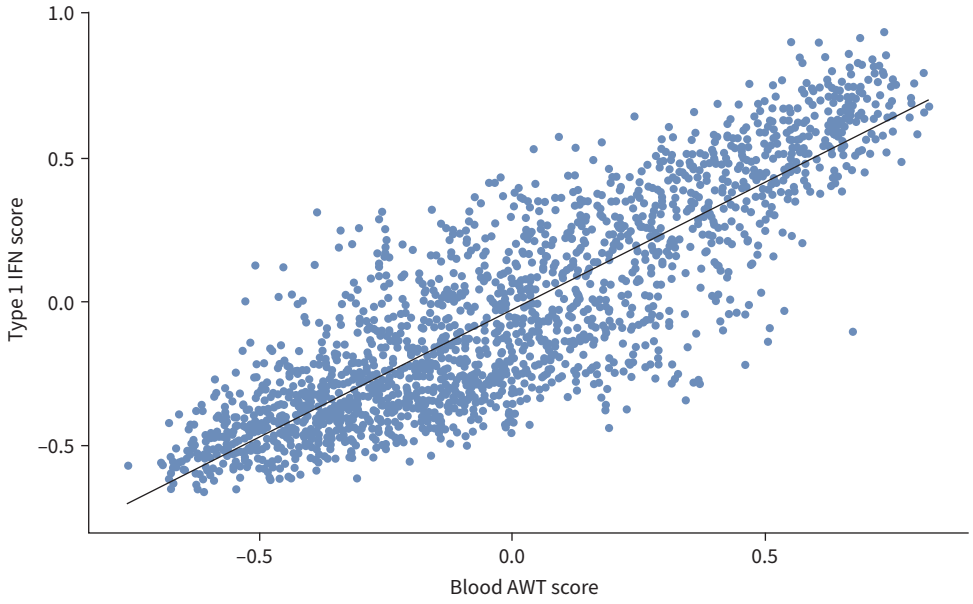

FIGURE 1 Blood airway wall thickness (AWT) and type 1 interferon (IFN) scores are highly correlated. Gene set enrichment scores are derived from gene set variation analysis. The blood AWT score was derived from 18 genes associated with computed tomography AWT. The type 1 IFN score was derived from upregulated genes after treatment of peripheral blood with IFN-α and -β.

bacterial infection (supplementary figure E2). The airway genes overlapping with SLE and viral lower respiratory infection genes were nine identical IFN response genes (*OAS1*, *OAS2*, *OAS3*, *RSAD2*, *IFI44*, *IFI44L*, *DDX60*, *CMPK2* and *HERC5*), supporting the shared molecular pathways between viral infection and autoimmune processes (supplementary material file E2).

Next we applied the published 396-gene meta-virus signature (MVS) that could distinguish respiratory viral infections from bacterial infections or healthy controls to our blood gene expression data [21]. A strong association between the AWT score and the MVS was found (Spearman $\rho=0.43$; $p<2.2\times10^{-16}$). As COPD exacerbations are commonly triggered by viral infections [22], we examined whether the AWT score or the MVS was associated with exacerbations. The AWT score was correlated with the number of exacerbations in the year prior to enrolment (Spearman $\rho=0.059$; $p<0.005$). In a subset of subjects who had prospective follow-up (n=2181), the blood AWT score and the MVS score predicted future exacerbations (table 5 and supplementary table E5).

Subgroup and sensitivity analysis

We performed stratified analysis for potential confounders influencing gene expression (sex, smoking, inhaled corticosteroids (ICSs) and asthma history) (supplementary material and supplementary tables E6–E10). Male sex and lack of ICS use were associated with stronger association of AWT gene sets with AWT. The blood AWT score was not significantly associated with CT AWT in ICS users, suggesting that ICSs suppress ISG expression in blood (supplementary table E6).

TABLE 4 Association between the bronchial epithelial airway wall thickness (AWT) score and computed tomography (CT) airway measurements

	AWT		WA%		Pi10	
	β (95% CI)	Adjusted p-value	β (95% CI)	Adjusted p-value	β (95% CI)	Adjusted p-value
Bronchial AWT score (n=23)	3.22 (1.21–5.24)	0.01	2.82 (0.76–4.88)	0.02	1.39 (−0.91–3.69)	0.2

CT airway measurements are standardised using z-scores. AWT: (segmental) airway wall thickness; WA%: wall area percentage; Pi10: square root wall area of a hypothetical airway with 10 mm internal perimeter. Linear regression adjusted for age, sex, race, body mass index, smoking status and emphysema (%). CT scanner model was identical for all subjects. p-value adjusted for multiple testing using Holm–Bonferroni correction.

TABLE 5 Association of the blood airway wall thickness score with lung function and exacerbations

	β or IRR (95% CI)	Adjusted p-value
FEV_1 (% pred)[#]	−3.4 (−5.44–−1.42)	0.04
FEV_1/FVC[#]	−0.03 (−0.04–−0.02)	<0.0001
ΔFEV_1 over 5 years (mL)[¶]	53.5 (−81.6–−25.4)	<0.01
Exacerbation frequency[+]	1.7 (1.24–2.38)	0.04

IRR: incidence rate ratio; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity. [#]: linear regression adjusted for age, sex, race, body mass index (BMI), smoking status, smoking history (pack-years) and emphysema (%). Chronic obstructive pulmonary disease (COPD) n=1026, control n=1367. [¶]: linear regression adjusted for age, sex, race, BMI, smoking status, smoking history (pack-years), baseline FEV_1 (% pred) and emphysema (%). COPD n=1024, control n=1360. [+]: acute respiratory disease defined by flare-up of chest troubles requiring antibiotic and/or steroid treatment [41]. Negative binomial regression adjusted for age, sex, race, smoking status, baseline FEV_1 (% pred) and history of previous exacerbation. COPD n=937, control n=1244. p-value adjusted for multiple testing using Holm–Bonferroni correction.

Genome-wide association of the blood AWT score

To understand the genetic factors that contribute to airway signature genes, we performed a genome-wide association study (GWAS) of the airway signature in 1788 non-Hispanic White subjects from COPDGene, adjusted for age, sex, current smoking status and genetic ancestry. We identified two significant loci (on chromosomes 9 and 7) associated with the AWT score at genome-wide significance ($p<5\times10^{-8}$) (figure 2, table 6 and supplementary material file E3). In the chromosome 7p12 locus, one lead single nucleotide polymorphism (SNP) rs876039 was identified, which is near the *IKZF1* gene and is also an expression quantitative trait locus (eQTL) for *IKZF1* in blood (eQTLGen; FDR <0.05) (supplementary figure E3) [23] and more recently described as the lead variant affecting the level of plasmacytoid dendritic cells (pDCs) [24]. This signal co-localises with susceptibility to SLE and coincides with several eQTLs acting *in trans*, suggesting other signals that could be mediated by the action of this gene. *IKZF1* encodes the transcription factor Ikaros that has previously been suggested as a candidate gene for SLE [25]. Other associations at this locus include monocyte percentage, non-albumin protein and albumin/globulin ratio [15]. We found the AWT score was positively associated with peripheral blood monocyte percentage in COPDGene, consistent with the GWAS direction of effect (β=1.39; $p_{adjusted}<0.001$). GWAS mapped genes were enriched for the response to type 1 IFN pathway, confirming the association found for the blood AWT score (supplementary material file E3).

Cellular origin of the AWT score

To identify the cell types expressing AWT score genes, we analysed matched blood and lung single-cell RNA sequencing data [26]. The majority of IFN-induced genes were highly expressed in nonclassical

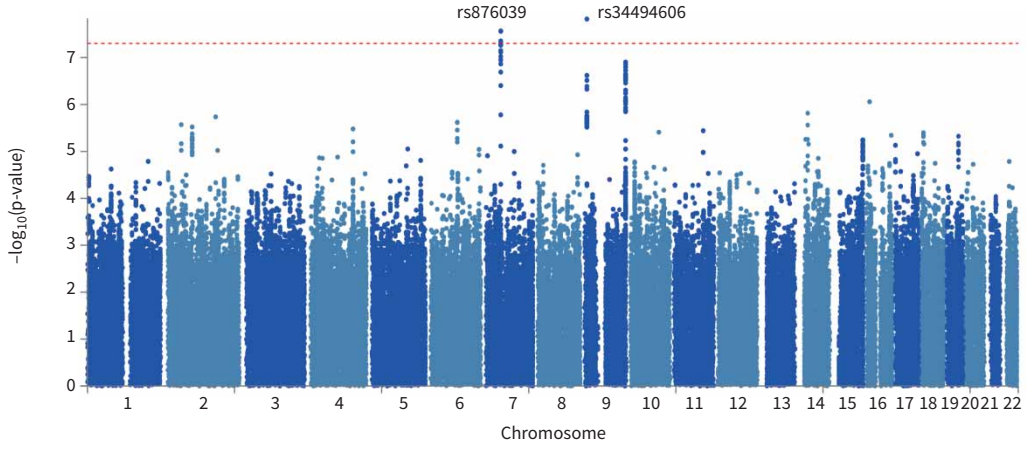

FIGURE 2 Genome-wide association study of the blood airway wall thickness (AWT) score. Manhattan plot showing the association of variants with the blood AWT score in 1788 non-Hispanic White individuals. The dashed red line marks the threshold for genome-wide significance ($p=5\times10^{-8}$).

TABLE 6 Genome-wide significant single nucleotide polymorphisms (SNPs) associated with the blood airway wall thickness score from non-Hispanic White subjects at two loci

SNP	Chromosome	Position	Effect allele	Minor allele frequency	β	p-value
rs34494606	9	7 872 062	C	0.10	0.32	1.5×10^{-8}
rs876039	7	50 308 811	C	0.31	−0.20	2.7×10^{-8}

monocytes in both blood and lung. In blood, some genes were expressed in neutrophils and pDCs, while in lung, AWT genes were also abundantly expressed in endothelial cells and bronchial epithelial cells (club cells and goblet cells) (supplementary figure E4).

Discussion

In a gene expression study of 2396 individuals with and without COPD, we identified an 18-gene molecular signature associated with CT AWT that is conserved between blood and bronchial epithelial cells. This AWT score is associated with type 1 IFN signalling. We also identified genetic loci associated with the blood AWT score, providing molecular and genetic insights into further understanding airway disease in COPD.

The CT airway measures were associated with IFN response genes. Among the three complementary CT airway measures, the enrichment was strongest with AWT. The blood AWT gene set was recapitulated in bronchial epithelial gene expression, where the bronchial AWT score was associated with AWT and WA%. Recent studies show dynamic changes in AWT, Pi10 and WA% in COPD, with an increase in AWT in smokers associated with COPD incidence [27]. AWT is a direct measure of wall thickness, compared with Pi10 and WA% that are composite measures of airway lumen and wall thickness. In COPD, the airway lumen is narrowed by inflammatory mucus, whereas wall thickness increases from infiltration of inflammatory cells and remodelling. This suggests that the IFN signalling may represent a specific molecular process involved in airway thickening, early airway inflammation or remodelling in COPD, or a subtype of COPD associated with such processes. Accordingly, the blood AWT score was associated with lung function decline adjusting for emphysema, suggesting that it may potentially serve as a biomarker for lung function decline related to airway disease. Conversely, we also found an association between the AWT score and emphysema after adjusting for AWT, supporting the interconnected relationship between airway disease and emphysema.

As a first line of defence against viral pathogens, IFN response genes encode antiviral effectors and also elicit a wide range of context-specific inflammatory mediators that could be protective or pathogenic. For example, in severe acute respiratory syndrome, early induction of type 1 IFN signalling was associated with reduced inflammation and milder disease, but delayed type 1 IFN signalling and persistent expression of ISGs were associated with sustained inflammation and fatal disease [28]. In coronavirus disease 2019 (COVID-19), a low type 1 IFN transcriptomic score from blood was associated with severe disease [29]. In asthma, impaired IFN-β response in airway epithelial cells upon virus infection was associated with greater viral load and worse symptoms during exacerbations [30]. In COPD, studies on the effect of type 1 IFN response yielded inconsistent results that appear to be context dependent. Some studies demonstrated increased expression of IFN-β in COPD epithelial cells infected with rhinovirus [31] and human metapneumovirus [32], whereas others reported deficiency of type 1 IFN upon rhinovirus infection in COPD bronchoalveolar lavage cells [33] and reduced expression of IFN-β in sputum samples from frequent exacerbators, without differential expression of ISGs [34]. Furthermore, in COPD, cigarette smoke and ICS can also impact the IFN response. Cigarette smoke suppresses induction of type 1 IFN, but cell death caused by smoking and emphysema can release DAMPs (damage-associated molecular patterns) which lead to type 1 IFN production [35]. We found ICS use is associated with low AWT score in COPD patients, consistent with ICS suppressing ISG expression in mouse viral infection models and primary airway epithelial cells from COPD patients [36].

Given the cross-sectional design of our study, it is not possible to determine whether the enriched IFN response is protective or pathogenic in the disease process, or if pathogenic, whether we observed secondary consequences of viral infections *versus* enhanced IFN signalling that is part of the disease pathogenesis. We did not detect the differential expression of IFN itself in our data. Due to the reduced sensitivity of detecting type 1 IFN gene expression, ISG expression is thought to be a more sensitive readout for activation of this pathway than the cytokine itself [37]. Furthermore, we cannot exclude the possibility that the observed ISGs may also be upregulated to some extent by IFN-γ. Alternatively, ISGs

may be induced independent of IFN as reported in certain viral infections or cell lines [38]. Nonetheless, the finding of genetic variants associated with this signature suggests that future studies may be able to identify potential mechanisms. The blood AWT score overlapped with transcriptomic signatures of respiratory viral infections and was predictive of future exacerbations. In COPDGene, blood was obtained during a stable state, at least 30 days since the last exacerbation, so we do not believe the signature reflects acute viral infection, but may represent sustained, chronic IFN response. Investigations into chronic viral infections and autoimmune diseases have shown that prolonged IFN signalling leads to chronic immune activation and inflammation, leading to immune exhaustion, autoimmunity and tissue damage. Therefore, we speculate that an elevated blood AWT score in the absence of active infection is likely pathological.

The overlap of the AWT score with the blood transcriptomic signature of SLE patients and the genetic association of the blood AWT score shared with monocyte counts and SLE align with the notion of pathological chronic immune dysregulation mediated by type 1 IFN response in COPD airway disease. The C allele in rs876039 was associated with reduced blood AWT score, reduced monocyte counts and lower risk of SLE, consistent with our findings. The putative causal gene, *IKZF1*, a SLE susceptibility gene, not only regulates immune cell development but also IFN pathways. Monocytes are critical producers, effectors and regulators of type 1 IFN that showed enrichment of AWT score gene expression from single-cell RNA sequencing data; type 1 IFN is produced largely by pDCs and inflammatory monocytes. In response to type 1 IFN, monocytes can differentiate into antigen-presenting dendritic cells that cause tissue damage, produce inflammatory cytokines and regulate pDC production of type 1 IFN. It would be informative to examine how IFN responses are modulated in different monocyte subsets and other cell types expressing AWT genes, such as bronchial epithelial and endothelial cells. Future studies will be required to validate the blood AWT score in independent cohorts. However, through cross-tissue analysis, we showed that the AWT score is conserved between blood and airways.

This study leverages recent advances in quantitative CT imaging analysis and RNA sequencing to dissect molecular processes associated with airway disease in COPD, and found IFN signalling genes as possible players in the disease process. Given the importance of ICSs, which suppress the ISG response, in COPD therapeutics and a recent study on IFN-β prophylaxis to modulate viral infection in COPD [39], an understanding of IFN signalling and airway phenotypes is needed to delineate the protective or pathological effect of IFN signalling on COPD airway disease. ICS or other modulation of IFN signalling may have potential to treat airway disease. Lastly, how this relates to the airway microbiome, outcomes of viral infections, and prospective changes in CT airway phenotypes and risk of COPD development remains to be demonstrated in future studies.

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