Dysregulation of lipid mediators in patients with frequent exacerbations of COPD

Marie Fisk [1,2], Esteban A. Gomez[3], Yuan Sun[2], Monika Mickute[1,2], Carmel McEniery[2], John R. Cockcroft[4], Charlotte Bolton [5,6], Jonathan Fuld[1], Joseph Cheriyan[1], Yasmin[2], William MacNee[7], Ruth Tal-Singer [8], Michael Polkey[9], Ian Wilkinson[2,10] and Jesmond Dalli[3,10]

[1]Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK. [2]University of Cambridge, Cambridge, UK. [3]William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK. [4]Cardiff Metropolitan University, Cardiff, UK. [5]Centre for Respiratory Research, Translational Medical Sciences, School of Medicine, University of Nottingham, Nottingham, UK. [6]NIHR Nottingham Biomedical Research Centre, Nottingham, UK. [7]University of Edinburgh, Edinburgh, UK. [8]COPD Foundation, Coral Gables, FL, USA. [9]Royal Brompton Hospital, London, UK. [10]Joint senior authors.

Corresponding author: Marie Fisk (mf503@medschl.cam.ac.uk)

Shareable abstract (@ERSpublications)
There is downregulation of specialised pro-resolving mediators across metabolomes in COPD patients with frequent exacerbations, compared with stable COPD patients. In those with frequent exacerbations, there is an inverse association between DHA resolvins and severe exacerbation risk. https://bit.ly/3Xtr9Q2

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Abstract

Introduction Specialised pro-resolving mediators (SPMs) are endogenously produced lipid mediators (LMs) that regulate the propagation of inflammation and promote tissue repair. We hypothesised that SPM production is dysregulated in COPD and is associated with disease severity, defined by patients with stable COPD (no exacerbations) *versus* patients with frequent exacerbations.

Methods LMs were measured in plasma samples from patients with COPD (stable patients and patients with frequent exacerbations) and from healthy controls, matched for age, sex and body mass index, using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The LM profiles of controls were compared with those of stable COPD patients, and the LM profiles of stable COPD patients were compared with those of COPD patients with frequent exacerbations. We explored whether or not there was an association between LM profile and ever having a severe COPD exacerbation over 4.1 years of follow-up. Data are presented as mean±SEM in pg·mL^{-1} for LMs, or mean±SD.

Results 49 stable COPD patients had increased levels of pro-inflammatory mediators and some SPMs, compared with 28 controls (prostaglandin (PG)D_2: 13.97±2.44 *versus* 0.53±0.13; p<0.001; lipoxins: 226.83±23.84 *versus* 59.84±20.25; p<0.01, respectively). 52 patients with frequent exacerbations had lower levels of PGD_2 (3.07±0.97 *versus* 13.97±2.44; p<0.01) and SPMs (D-resolvins: 8.73±1.25 *versus* 34.53±8.95; p<0.01; lipoxins: 53.93±9.23 *versus* 226.83±23.84; p<0.01) than stable COPD patients, despite having a higher neutrophil count (5.28±2.16×10^9 L^{-1} *versus* 4.28±1.60×10^9 L^{-1}; p=0.004). Among patients with frequent exacerbations, D-resolvin levels were independently inversely associated with occurrence of severe exacerbation (OR 0.88, 95% confidence interval (CI) 0.79–0.97; p=0.03) during follow-up.

Conclusion These findings demonstrate distinct LM profiles of stable COPD patients and patients with frequent exacerbations. In those with exacerbations, D-resolvins were downregulated, compared with stable COPD patients, and associated with future risk of severe exacerbations during follow-up. Further work is needed to understand these findings.

Introduction

Specialised pro-resolving mediators (SPMs) are endogenously produced anti-inflammatory lipid mediators (LMs) [1] that orchestrate the active process of inflammation resolution following injury or infection. They signal *via* specific cell receptors to switch off acute inflammatory responses and enable active tissue repair [2–4]. SPMs have anti-inflammatory and pro-resolution properties, inhibit transmigration of

polymorphonuclear cells, enhance macrophage phagocytosis and efferocytosis and are critical in modulating T-cell responses [5, 6]. SPMs include mediators biosynthesised *via* lipoxygenase (LOX) enzymatic pathways, from parent compounds of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), n3-docosapentaenoic acid (n3-DPA) and arachidonic acid (AA). Different families of SPMs (D- and E-series resolvins, maresins and lipoxins, for example) have been identified, which are involved in the specific responses of different cell types [4]. Several G protein-coupled receptors for SPMs have also been identified, for example ALX/FPR2, ERV1/Chem23 and DRV1/GPR32, that have ligand receptor specificity and display potent pro-resolution properties. Elucidation of SPMs and their receptors provides the basis for advancing understanding of inflammation resolution mechanisms and potential future novel therapeutic approaches to treat inflammatory-related conditions [1, 4, 7–9].

The inflammatory pathogenesis of COPD is well defined, but less is known about the role of anti-inflammatory mechanisms in this condition. Small clinical studies have shown reduced lipoxin (LX) A_4 levels in induced sputum and exhaled breath condensate of COPD patients, compared with healthy controls [10, 11], and reduced serum LXA_4 levels in severe asthma and bronchiectasis patients, compared with healthy controls [12, 13]. In preclinical studies relevant to COPD, murine models showed that treatment with resolvin (Rv)D1 attenuated the inflammatory effects of cigarette smoke, reduced emphysema and inflammatory cell infiltration and enhanced resolution of lung inflammation caused by *Pseudomonas aeruginosa* and *Haemophilus influenzae* infections [14–19]. RvD1 suppressed the production of pro-inflammatory mediators by human lung fibroblasts, small airway epithelial cells and blood monocytes exposed to cigarette smoke [14], and decreased inflammatory cytokine production from human alveolar macrophages exposed to cigarette smoke [11]. A very small clinical study of COPD patients (n=6) showed reduced levels of serum and bronchoalveolar lavage (BAL) samples of RvD1, compared with controls, and increased receptor expression in lung tissue [11, 15]. Together, these findings support a potential dysregulation in the production of these autacoids as a mechanism underlying the pathogenesis of COPD. To our knowledge, no previous study has evaluated LMs in relation to COPD severity, defined by patients who are stable in their COPD (*i.e.*, no exacerbations) *versus* patients with frequent exacerbations. Improving the understanding of differences between patients with stable COPD and those with frequent exacerbations is important, given that a history of recurrent exacerbations is the strongest predictor of a future exacerbation, and leads to a spiral of decline in health and increased mortality [20–22]. Persistent inflammation (and chronic infection or colonisation with certain bacteria) may be important factors in the "frequent exacerbator" phenotype [23, 24]. Therefore, understanding the mechanisms of resolution of inflammation and infection may also be important.

The aim of this study was to evaluate LM levels (both pro-inflammatory and SPMs) in a clinical cohort of COPD patients. In particular, we also sought to explore whether the levels of these molecules and LM profiles were linked with COPD disease severity, defined by patients stable in their COPD (no exacerbations) *versus* patients with frequent exacerbations.

Methods

All data supporting the findings of this study are available from the corresponding author on reasonable request. The data for this study come from two prospectively studied cohorts based in the UK: the Evaluation of the Role of Inflammation in Chronic Airways disease (ERICA) study cohort of patients with COPD, which evaluated biomarkers and extrapulmonary assessments in relation to health outcomes in COPD, and the Anglo–Cardiff Collaborative Trial (ACCT), which is a large epidemiological study of vascular function in East Anglia and Wales, from which healthy volunteers were selected for this study. Research ethics committee approvals for both cohorts have previously been described [25–27]. Expanded methods are provided in the online supplementary material.

Patients with COPD

Patients with COPD were aged ⩾40 years, had a smoking history of ⩾10 pack-years, had a clinical diagnosis of COPD, had a postbronchodilator forced expiratory volume in 1 s (FEV_1) value of <80% and had a FEV_1 to forced vital capacity ratio of <0.7. Patients had to be free of exacerbations in the preceding 4 weeks before enrolment in the study. For this analysis, patients were defined as having stable COPD if they did not have a COPD exacerbation in the year preceding study enrolment. In contrast, patients were defined as having frequent exacerbations if they had two or more exacerbations requiring treatment with additional antibiotic and/or oral corticosteroid courses in the year before entering the study [28]. Patients had their study assessments, including blood samples, taken at baseline; the subsequent health outcomes of hospitalisation due to COPD exacerbation (severe exacerbation), time to first severe exacerbation and severe exacerbation rate were followed up prospectively from linked Hospital Episode Statistics data provided by NHS Digital. Codes used for defining an exacerbation are shown in supplementary table S1 [29].

The median duration of follow-up for selected participants in these analyses until study censor was 1784 (interquartile range (IQR) 1450–1993) days for stable COPD participants and 1155 (IQR 328–1614) for COPD participants with frequent exacerbations. See the supplementary material for further information.

Healthy volunteers (controls)

Participants were selected from the ACCT database to be matched to COPD participants in age, gender and body mass index (BMI). They were defined as healthy volunteers if they had no known significant background medical conditions such as cardiovascular disease (*i.e.*, hypertension, dyslipidaemia, ischaemic heart disease or stroke), respiratory conditions (*i.e.*, a record of a diagnosis such as asthma, COPD, pulmonary fibrosis or medications associated with respiratory conditions such as inhalers), cancer or inflammatory conditions. The data variables of spirometry and BMI were recorded for this group of participants. Due to enriching this LM profiling analysis for COPD participants, fewer healthy volunteers were selected than participants with COPD.

Lipid mediator profiling

Liquid chromatography–tandem mass spectrometry (LC-MS/MS)-based LM profiling was performed on plasma samples from the research cohorts. This analysis has been previously described [30] and is summarised in the supplementary material. Owing to technical issues with the LM analysis of four plasma samples (three from stable COPD participants and one from the group with frequent exacerbations), these samples were not included in the analysis. The total number of plasma samples, and therefore of participants, included in this study was 129 (each participant had a corresponding plasma LM analysis): 28 healthy volunteers, 49 stable COPD participants and 52 COPD participants with frequent exacerbations.

Data and statistical analysis

Data are expressed as mean±SD, mean±SEM, median (IQR) or percentages. Quantification analysis of mediator levels in plasma were measured as described previously [30], and individual mediator levels are reported in $pg \cdot mL^{-1}$. Group data are summarised as mean±SEM together with the total number of samples.

Data are represented visually using partial least squares discriminant analysis (PLS-DA) [30]. Variables with >75% missing values were excluded. Missing (zero) values were replaced by one fifth of the minimum positive value of each variable across the samples, features with a constant value were deleted and data were autoscaled. PLS-DA is a multivariate analysis that creates a linear regression model and accounts for multicollinearity to identify the relationship between samples, *i.e.*, to evaluate whether plasma LM concentrations were different between groups. The score plots illustrate the systematic clusters among the observations (*i.e.* mediators): plots that are closer together represent greater similarity in the data.

Variable importance in projection (VIP) scores were calculated for each group comparison to estimate the importance of each variable (LM) in a partial least squares model. An LM with a VIP score >1 can be considered important in each model, in contributing to a difference between groups.

To understand further group differences in LMs, biosynthetic pathway analyses were performed for LMs with VIP scores >1; this analysis evaluates LM data based on the LMs' substrate and enzymatic functional pathway. Pathway analyses were built using Cytoscape v.3.7.1, see the supplementary material for further information.

This was a hypothesis-driven study, and, as such, clinical associations with LMs are exploratory. Therefore, to explore associations with clinical variables of interest in COPD patients, cross-sectional associations between LMs with VIP >1 (and/or statistically significant differences in levels between groups) with clinically important baseline variables relating to COPD severity were evaluated using regression models adjusted for expected confounders. Please see the supplementary material for expanded information. Statistical analysis was performed using SPPS Statistics v.29.

Results

Demographic data for 101 patients with COPD (49 stable and 52 with frequent exacerbations) and 28 healthy volunteers are summarised in table 1. Participants were matched for age, gender and BMI.

Lipid mediator profiles

Stable COPD patients versus COPD patients with frequent exacerbations *versus* controls

In plasma samples from COPD patients (stable COPD patients and patients with frequent exacerbations) and healthy volunteers (controls), LMs from the four fatty metabolomes were identified. Differences in LM

TABLE 1 Characteristics of groups

Variable	Healthy controls (n=28)	Stable COPD patients (n=49)	COPD patients with frequent exacerbations (n=52)	Statistical difference assessed, p-value
Age, years, median (IQR)	66 (63–70)	64 (62–74)	67 (63–72)	0.95
Male	15 (54)	35 (66)	27 (51)	0.16
BMI, kg·m^{-2}	27.08±4.04	26.87±5.48	26.76±6.72	0.97
Ethnicity				0.51
White British or other white	28 (100)	51 (96)	53 (100)	
Asian		1 (2)		
Black		1 (2)		
Smoker status				
Current smoker	1 (3)	24 (45)	20 (38)	<0.001 0.33[#]
Former smoker	15 (54)	29 (55)	33 (62)	
Never smoked	12 (43)	0	0	
Hypertension	–	21 (40)	25 (47)	0.43
Dyslipidaemia	–	21 (40)	18 (34)	0.55
Diabetes	–	4 (8)	4 (8)	1.0
Peripheral vascular disease	–	1 (2)	3 (6)	0.31
Angina	–	8 (15)	2 (4)	0.05
Myocardial infarction	–	4 (8)	3 (6)	0.68
Stroke	–	5 (9)	3 (6)	0.45
FEV_1, L	2.61±0.60	1.42±0.49	1.14±0.48	<0.001 0.003[#]
FEV_1, %	104±16	55±15	45±16	<0.001 0.005[#]
FVC, L	3.10±0.87	2.79±0.96	2.76±0.91	<0.001 0.97[#]
FVC, %	97±18	84±18	86±23	<0.001 0.54[#]
Exacerbations (number in last 12 months)	–	0	3.93±2.00	<0.001
CRP, mg·mL^{-1}	–	4.57±5.58	8.07±9.17	0.01
White cell count		7.12±2.09	8.19±2.62	0.01
Neutrophils, ×10^9 L^{-1}	–	4.28±1.60	5.29±2.16	0.004
Fibrinogen, g·L^{-1}	–	3.27±0.86	3.56±0.83	0.04
6MWD, m	–	374±124	343±136	0.12
Inhaled triple therapy[¶]	–	11 (22)	39 (75)	<0.001
ICS	–	19 (39)	44 (85)	<0.001
Outcome data				
Duration of follow-up, days, median (IQR)	–	1867 (1608–2002)	1625 (1490–1730)	
Participants hospitalised due to COPD exacerbation over follow-up	–	7 (14)	28 (54)	
Time to first hospitalised exacerbation, days, median (IQR)	–	1784 (1450–1993)	1155 (329–1614)	

Data are presented as mean±SD and n (%), unless otherwise stated. Chi-squared test, t-tests and Kruskal–Wallis test or one-way ANOVA were used to assess for differences between groups depending on whether categorical, two-group or three-group analysis. IQR: interquartile range; BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; CRP: C-reactive protein; 6MWD: 6-min walk distance; ICS, inhaled corticosteroid. [#]: Comparison between stable COPD patients and COPD patients with frequent exacerbations. [¶]: ICS, long-acting beta agonist and long-acting muscarinic antagonist.

concentrations across the three groups were evaluated and are shown in the supplementary table S2. In summary, RvD2, aggregate levels of D-resolvins, D-protectins, D-maresins, the DHA metabolome and the n-3 DPA metabolome were lower in those with exacerbations than in both the stable COPD group and controls. Aggregate levels of lipoxins, RvD1, RvD3, RvD5 and cyclooxygenase (COX) products were higher in stable COPD participants than in participants with frequent exacerbations and in controls.

PLS-DA cluster analysis and biosynthetic pathway analyses were conducted for group comparisons of stable COPD patients *versus* controls, stable COPD patients *versus* COPD patients with frequent exacerbations and COPD patients with frequent exacerbations *versus* controls; see figures 1–4 and supplementary figures S1 and S2.

Stable COPD versus controls

Levels of LMs in patients with stable COPD were distinct from those found in healthy controls, as demonstrated by a separation between the clusters representing each of these groups in PLS-DA. Evaluation of the VIP scores, which identify those mediators that are differentially expressed between the two groups, demonstrated an upregulation of PGE_2, 15-epi-lipoxin A4 (15-epi-LXA_4), lipoxin B4 (LXB_4), RvD1, RvD3, RvE1 and leukotriene (LT) D_4, and a downregulation of RvD2 and 4S,14S-dihydroxydocosahexaenoic acid (diHDHA), in stable COPD patients, compared with controls (figure 1). Pathway analysis demonstrated an overall upregulation in arachidonate 5-lipoxygenase (ALOX5) activity in patients with stable COPD, which contributes to an upregulation in the formation of both SPMs, namely RvD and LX, as well as LTD_4 (figure 2).

Stable COPD patients versus COPD patients with frequent exacerbations

We next sought to evaluate whether plasma LM concentrations were linked with COPD severity according to exacerbation status. Plasma LM concentrations were distinct between the two COPD patient groups. (figure 3). Here we observed an overall downregulation of SPMs, including 15-epi-LXA_4 and RvD1 in the exacerbation group *versus* the stable COPD group (figure 3 and supplementary table S2). Pathway analysis identified broad downregulation of ALOX15–ALOX5 interaction products in patients with frequent exacerbations, compared with those with stable COPD, across the different fatty acid metabolomes (figure 4).

There was a pattern of reduced activity across the DHA metabolome (D-resolvins and maresins) and AA metabolome in both anti-inflammatory (lipoxins) and pro-inflammatory (PGE_2) mediators in participants with frequent exacerbations, compared with stable COPD participants. $PGF_{2\alpha}$ was the only LM upregulated in those with exacerbations, compared with stable COPD participants (supplementary table S2).

For COPD patients with frequent exacerbations *versus* controls, see the supplementary material.

Clinical perspective

In this exploratory analysis, we did not observe any cross-sectional association between FEV_1 (L) or % predicted, 6-min walk distance, C-reactive protein (CRP), fibrinogen, neutrophils or total white cell count and individual LMs identified by VIP or found to have a statistically significant difference in concentrations

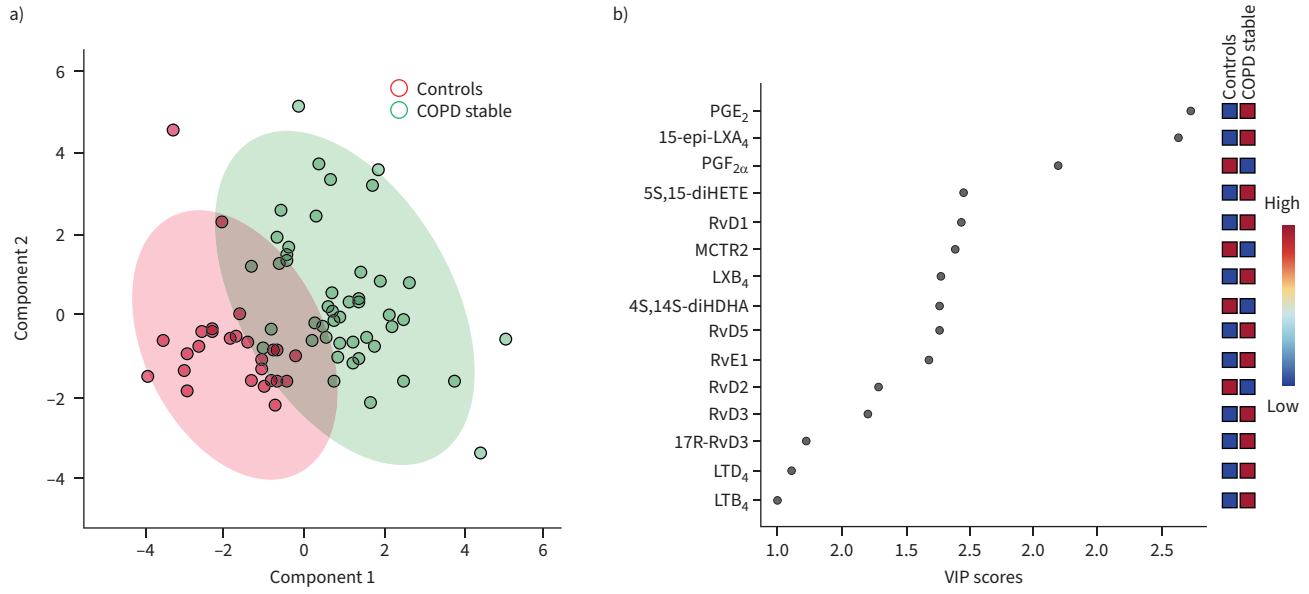

FIGURE 1 Distinct plasma lipid mediator (LM) profiles in stable COPD patients when compared with controls. LMs were identified and quantified in plasma from patients with COPD who were stable and matched controls using liquid chromatography–tandem mass spectrometry-based methodologies. Differences in LM concentrations between the two groups were evaluated using partial least squares discriminant analysis. a) Score plot denoting the clustering obtained between LM levels in stable COPD patients and LM levels in healthy controls. The shaded regions denote the 95% confidence regions. b) Variable importance in projection (VIP) scores, where VIP scores >1 denote mediators that contribute to the separation between the two groups. epi: epimer; LX: lipoxin; PG: prostaglandin; Rv: resolvin.

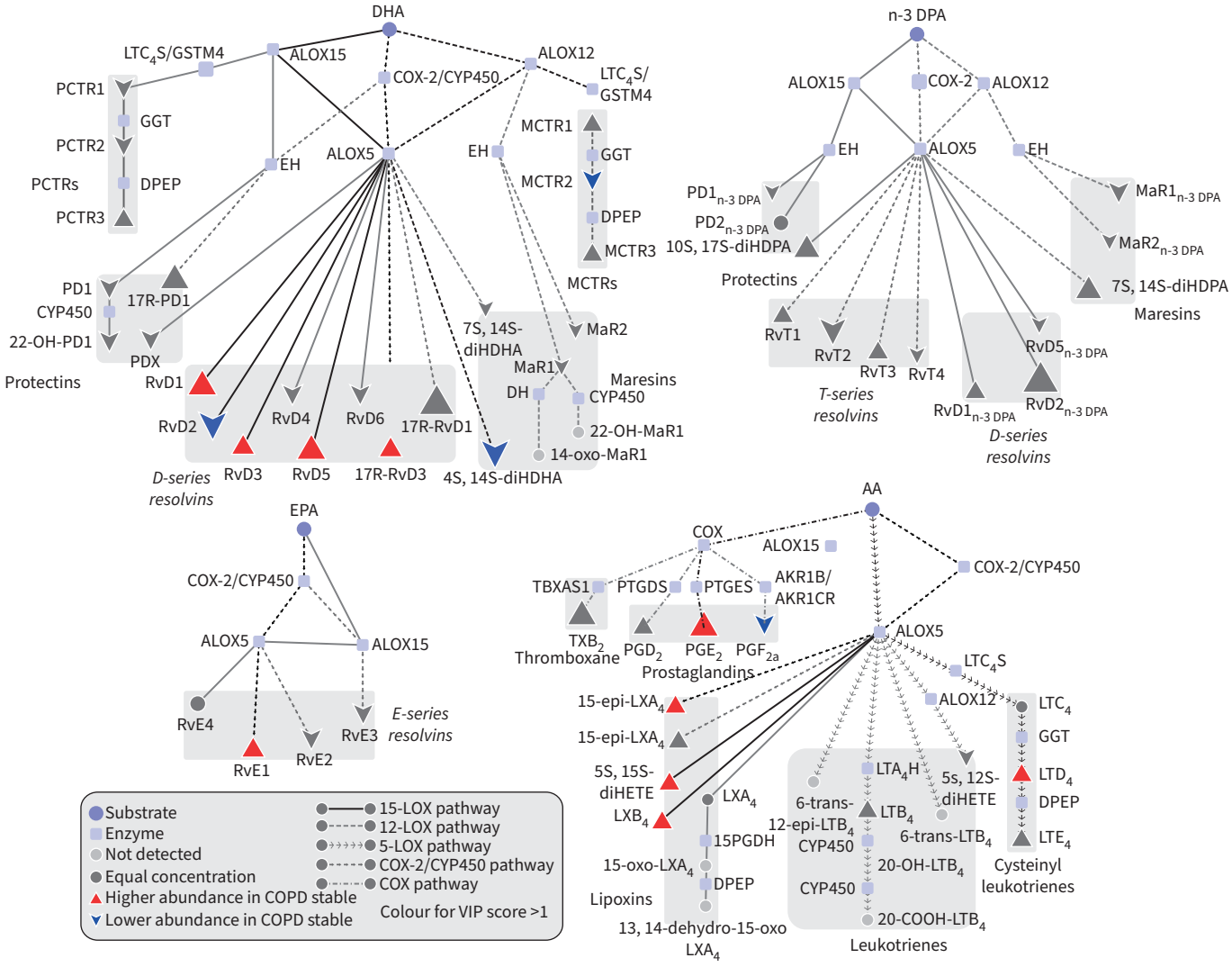

FIGURE 2 Differential regulation of lipid mediator (LM) biosynthetic pathways in stable COPD patients, compared with controls. Analysis highlighting the relative regulation of mediators identified in figure 1 with VIP scores >1 and their biosynthetic pathways in stable COPD patients when compared with controls. Stable COPD patients have general upregulation (higher abundance) of the following LMs, compared with controls: PGE_2, 15-epi-LXA_4, LXB_4, LTD4, RvE1, RvD1, RvD3, RvD5 and 17R-RvD3. There are lower levels of the following LMs in stable patients than in controls: $PGF_{2\alpha}$, RvD2, 4S,14S-diHDHA and MCTR2. DHA: docosahexaenoic acid; n3-DPA: n3-docosapentaenoic acid; EPA: eicosapentaenoic acid; AA: arachidonic acid; LOX: lipoxygenase; COX: cyclooxygenase; CYP450: cytochromes P450; PG: prostaglandin; epi: epimer; LX: lipoxin; LT: leukotriene; Rv: resolvin; diHDHA: dihydroxydocosahexaenoic acid; MCTR: maresin conjugates in tissue regeneration.

(including aggregated data) across COPD patients or within stable and exacerbation groups (data not shown). Associations with inhaled corticosteroid (ICS) use are provided in the supplementary material.

Outcome data

Analysis of the entire COPD cohort data combined did not show any significance of LMs to predict future exacerbations over follow-up. Given the differences in LM profiles observed in stable and COPD patients, outcome analyses within each group of patients were also undertaken.

Stable COPD

There were no significant associations between individual mediators or mediator families, in particular, lipoxins, resolvins or PGD_2, and outcomes of severe exacerbation, time to first severe exacerbation or exacerbation rate in stable COPD patients, despite these mediators being upregulated in this group.

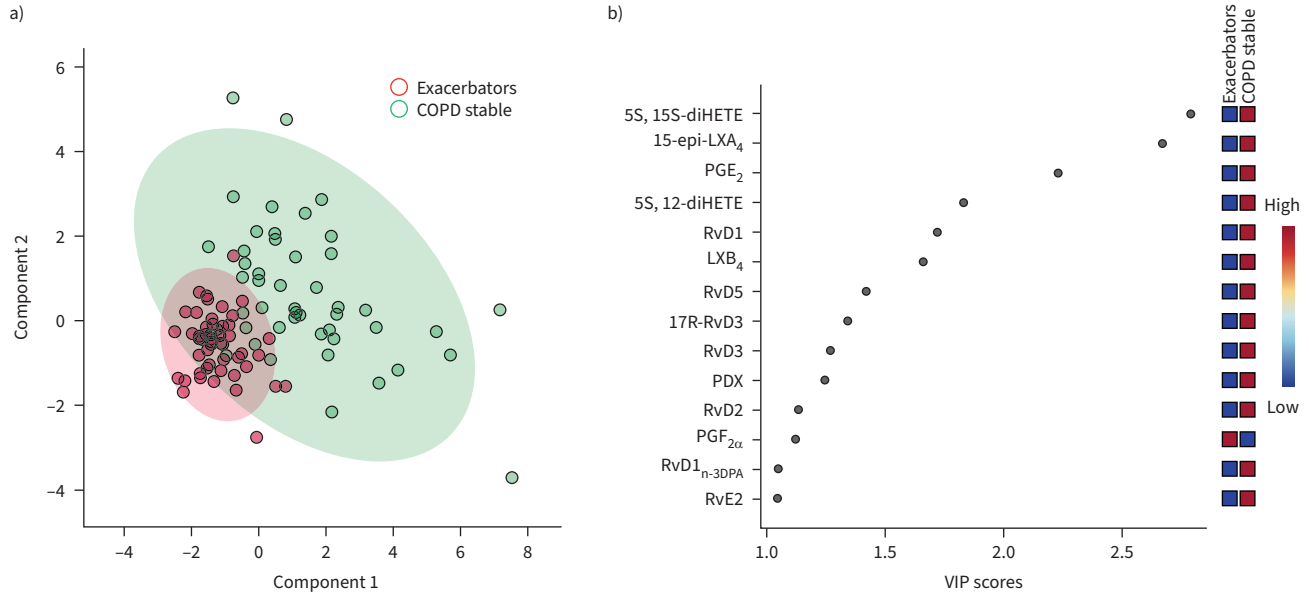

FIGURE 3 Distinct plasma lipid mediator (LM) profiles in COPD patients with frequent exacerbations, compared with stable COPD patients. LMs were identified and quantified in plasma from COPD patients with frequent exacerbations and stable COPD patients using liquid chromatography–tandem mass spectrometry-based methodologies. Differences in LM concentrations between the two groups were evaluated using partial least squares discriminant analysis. a) Score plot denoting the clustering obtained between LM levels in patients with frequent exacerbations and LM levels in stable COPD patients. The shaded regions denote the 95% confidence regions. b) Variable importance in projection (VIP) scores, where VIP scores >1 denote mediators that contribute to the separation between the two groups. epi: epimer; LX: lipoxin; PG: prostaglandin; Rv: resolvin.

Patients with frequent exacerbations

D-resolvins were inversely associated with occurrence of future severe COPD exacerbation, time to first severe exacerbation and rate of severe COPD exacerbations over follow-up, after adjusting for baseline exacerbation history, age, FEV_1 % predicted, CRP, smoking and ICS use (table 2).

Discussion

To our knowledge, this is the first comprehensive evaluation of SPMs in the plasma of patients with COPD, inclusive of both stable COPD patients and patients with frequent exacerbations, compared with age-, gender- and BMI-matched healthy controls. These data show that there is a differentiation in the clustering of LM profiles between healthy controls and both stable COPD patients and patients with frequent exacerbations, and, interestingly, within COPD, between patients with stable COPD and those with frequent exacerbations. This suggests a distinct molecular difference in LM profiles between these two groups of COPD patients. In stable patients, there was a pattern of general upregulation in mediators (archetypal pro-inflammatory eicosanoids, leukotrienes and prostaglandins), combined with parallel upregulation in SPMs from different substrates (DHA, EPA and AA), in comparison to both healthy controls and those with exacerbations. In patients with frequent exacerbations, there was a pattern of downregulation in LMs across metabolomes, compared with stable COPD patients (despite higher levels of systemic inflammation). In addition, comparison of LM levels across all three groups, showed a general downregulation in DHA substrates in those with exacerbations, compared with both stable COPD patients and controls. Interestingly, low D-resolvin levels in those with exacerbations were independently associated with occurrence of future severe COPD exacerbation, time to first severe exacerbation and rate of exacerbations over follow-up, although these are exploratory analyses.

Resolution is an active, tightly controlled process that, in health, ensures that the acute inflammatory response is self-limiting and results in return of tissue homeostasis. SPMs are bioactive metabolites enzymatically derived from essential fatty acids in a LOX-dependent formation that are fundamental to this regulated process. They are produced *via* the enzymatic conversion of essential fatty acids by different cell types during the acute phase of inflammation, whereby orchestrated LM class-switching from pro-inflammatory mediators (*i.e.*, prostaglandins and leukotrienes) to anti-inflammatory/pro-resolving mediators (*i.e.*, lipoxins), together with the production of other families of SPMs (D-resolvins, maresins,

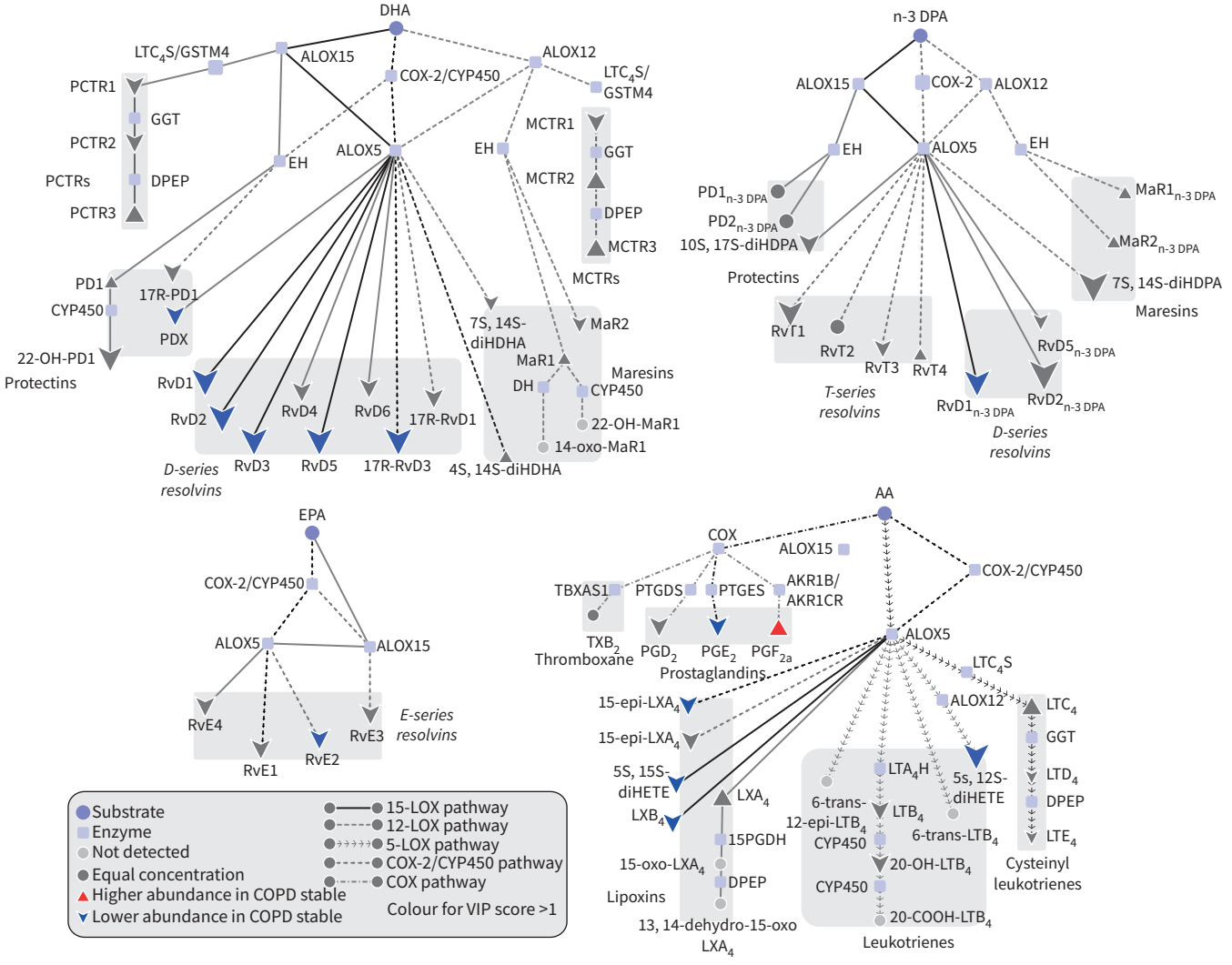

FIGURE 4 Differential regulation of lipid mediator (LM) biosynthetic pathways in COPD patients with frequent exacerbations, compared with stable COPD patients. Analysis highlighting the relative regulation of mediators identified in figure 3 with VIP scores >1 and their biosynthetic pathways in stable COPD patients, compared with patients with exacerbations. Downregulation of ALOX5–ALOX15 pathways was observed in patients with frequent exacerbations, compared with stable COPD patients. There was general downregulation of the following LMs (lower abundance) in COPD patients with exacerbations (*versus* stable COPD patients): PGE_2, 15-epi-LXA_4, LXB_4, 5S, 15S-diHETE, 5S-12S-diHETE, RvE2, PDX, RvD1, RvD2, RvD3, RvD5 and 17R-RvD3, n3-RvD3. DHA: docosahexaenoic acid; n3-DPA: n3-docosapentaenoic acid; EPA: eicosapentaenoic acid; AA: arachidonic acid; LOX: lipoxygenase; COX: cyclooxygenase; CYP450: cytochromes P450; ALOX: arachidonate lipoxygenase; PG: prostaglandin; epi: epimer; LX: lipoxin; diHETE, dihydroxy-eicosatetraenoic acid; PDX: protecin DX (also referred to as 10S, 17S-dihydroxydocosahexaenoic acid (diHDHA)); Rv: resolvin.

EPA resolvins and n3-DPA resolvins), promote a number of anti-inflammatory, pro-resolving actions [1–5]. SPMs facilitate these mechanisms by molecular signalling at cognate receptors [7, 31–36]. In stable COPD, our data suggest that it is potentially appropriate to upregulate mediators (albeit the balance may still be pro-inflammatory) in response to the inflammatory processes of COPD. Patients with exacerbations generally had lower levels of some LMs, including pro-inflammatory mediators, than stable COPD patients, despite higher systemic markers of inflammation, such as white cell count and CRP, suggesting the potential for dysfunctional resolution pathways. It is postulated that this may be important in persistent inflammation and propensity for exacerbations, which are hallmarks of the "frequent exacerbator" phenotype. The clinical significance of these findings is uncertain, but a similar profile of reduced pro-inflammatory LMs was observed in critically ill COVID-19 patients [37].

Data from preclinical studies and very small clinical studies have suggested dysregulation of SPMs in COPD, in particular the LM RvD1, with reduced levels in serum and BAL samples and increased receptor

TABLE 2 Association of specialised pro-resolving mediators (SPMs) with severe COPD exacerbations over follow-up

Dependent variable	**Occurrence of severe COPD exacerbation**
Independent variables	**OR (95% confidence interval); p-value**
Baseline exacerbations history[#]	2.12 (1.14–3.96); 0.02
Age, years	0.95 (0.86–1.05); 0.31
FEV_1 % predicted	0.95 (0.90–0.99); 0.14
CRP, $mg{\cdot}mL^{-1}$	1.11 (0.99–1.25); 0.07
Current smoker	1.80 (0.38–8.62); 0.44
ICS use	2.33 (0.28–19.44); 0.44
D-resolvins, $pg{\cdot}mL^{-1}$	0.88 (0.80–0.97); 0.01
RvD3[¶]	0.03 (0.001–0.99); 0.05
Dependent variable	**Time to first severe COPD exacerbation**
Independent variables	**Risk ratio (95% confidence interval); p-value**
Baseline exacerbations history[#]	1.38 (1.14–1.67); <0.001
Age, years	0.96 (0.91–1.01); 0.10
FEV_1 % predicted	0.97 (0.94–1.00); 0.02
CRP, $mg{\cdot}mL^{-1}$	1.03 (0.99–1.07); 0.13
Current smoker	1.67 (0.66–4.24); 0.28
ICS use	1.56 (0.48–5.07); 0.46
D-resolvins, $pg{\cdot}mL^{-1}$	0.91 (0.85–0.97); 0.005
Dependent variable	**Severe exacerbation rate**
Independent variables	**Risk ratio (95% confidence interval); p-value**
Baseline exacerbations history[#]	1.58 (1.30–1.93); <0.001
Age, years	0.92 (0.87–0.98); 0.01
FEV_1 % predicted	0.96 (0.93–0.98); 0.001
CRP, $mg{\cdot}mL^{-1}$	1.07 (1.02–1.12); 0.004
Current smoker	0.82 (0.31–2.13); 0.68
ICS use	0.83 (0.22–3.11); 0.78
D-resolvins, $pg{\cdot}mL^{-1}$	0.92 (0.86–0.98); 0.01
17R-RvD1[¶]	0.86 (0.75–1.00); 0.04
RvD3[¶]	0.03 (0.003–0.30); 0.002

FEV_1: forced expiratory volume in 1 s; CRP: C-reactive protein; ICS: inhaled corticosteroid; Rv: resolvin. [#]: Reported at baseline entry to the Evaluation of the Role of Inflammation in Chronic Airways disease (ERICA) study of exacerbation frequency in the preceding 12 months. [¶]: Separate models to assess individual lipid mediator (LM) contributions to aggregate D-resolvins' inverse association with severe exacerbation (ever occurrence, time to first exacerbation and exacerbation rate). For time to first severe exacerbation, no individual D-resolvin LM met the threshold for statistical significance ($p<0.05$), although aggregate D-resolvins did. For severe exacerbation rate, 17R-RvD1 and RvD3 contributed most to the association of D-resolvins with severe exacerbation rate. Logistic regression, Cox proportional hazards regression analysis and negative binomial regression analysis were used to assess associations for ever occurrence of severe exacerbation, time to first severe exacerbation and exacerbation rate over follow-up. All models were adjusted for expected confounders shown in the models (age, FEV_1 % predicted, ICS use, CRP, current smoker status). Duration of follow-up for outcomes or censor: 1155 (329–1614) days (median ~3 years' follow-up). See online supplementary material for expanded methods.

expression in the lung tissue of COPD patients, compared with controls [11]. Upregulation of the inflammatory eicosanoids LTB_4, LTE_4 and PGE_2 in the exhaled condensate of mild–moderate COPD, compared with controls, has also been reported [38]. Reduced LXA_4 levels in the exhaled breath condensate of COPD patients *versus* controls, and in the serum of severe bronchiectasis patients and of severe *versus* moderate asthma, have been reported in small published studies [11–13, 39].

Our findings are compatible with these studies and provide novel insights that deepen our understanding of LMs in COPD. We showed an expected increase in pro-inflammatory eicosanoids (prostaglandins (PGE_2) and leukotrienes (LTB_4 and LTD_4)) in COPD participants *versus* controls, with a pattern of higher levels in the stable COPD group specifically. With regard to SPMs, we reported increased D-resolvin levels in stable COPD participants *versus* controls, and increased levels in stable COPD patients *versus* patients with frequent exacerbations. We observed this same pattern with lipoxins, maresins, protectins, EPA resolvins and n3-DPA resolvins. These novel data signify the value of our well-characterised COPD cohort, with sufficient stable and "frequent exacerbator" phenotype patients to discern these differences. It is also important to highlight the group differences observed in individual LM levels in our analysis. Specifically,

why RvD2 is lower in both COPD groups than in controls, and relatively reduced in COPD patients with exacerbations compared with stable COPD patients, is uncertain. RvD2 is a potent regulator of leukocytes and controls microbial sepsis, as demonstrated in preclinical studies [40]. Recently published work showed that RvD1 and RvD2 resolved lung inflammation and enhanced macrophage phagocytosis in murine models [41]. LTD_4 was relatively increased in both stable participants and participants with frequent exacerbations, compared with healthy controls, in pathway analyses. Experimental studies have shown that LTD_4 may be important in airway eosinophilia and pulmonary inflammation [42, 43]. The relative upregulation of RvE1 in both stable participants and participants with frequent exacerbations, compared with controls, in pathway analyses is also of interest. RvE1 has protective effects in preclinical models of allergic lung inflammation and preserves macrophage function under cigarette smoke-induced oxidative stress [44, 45]. These differences in individual mediator profiles require further research to understand their relevance to COPD and different phenotypes of COPD.

We observed no cross-sectional baseline associations between mediators with spirometric parameters and other clinical variables measured within our cohort. This contrasts with asthma and bronchiectasis studies [12, 13]. However, in our study, the range and values of FEV_1 % predicted were considerably narrower and lower in our patients than reported in these previous studies.

Among patients with frequent exacerbations, we observed reduced D-resolvin levels, associated with occurrence of severe exacerbation, time to first severe exacerbation and rate of severe exacerbations over a median follow-up of about 4.1 years, adjusted for expected confounders. We emphasise that these are exploratory analyses; therefore, these interesting findings should be interpreted with caution, but they highlight the need for further study of SPMs in relation to clinically meaningful data, as well as mechanistic experimental studies in COPD. The individual LMs that contributed most to the association of D-resolvins with severe exacerbations over follow-up were 17R-RvD1 and RvD3, both of which have reported beneficial effects in preclinical studies [46, 47].

The exploratory association between lower DHA metabolome levels in stable COPD patients on ICS *versus* those not on ICS and higher DHA metabolome levels in COPD patients with exacerbations on ICS *versus* those not on ICS provides potentially interesting data. It highlights potential differences existing within COPD phenotypes and the possible impact of therapeutics on SPMs in COPD. However, it is emphasised that any clinical interpretation of such data is not possible; a randomised controlled trial would be needed to evaluate the impact of ICS on LMs. Further research work is needed to explain these findings.

For the first time, to our knowledge, we have demonstrated differences in plasma levels of SPMs and their profiles among COPD patients, stratified by exacerbations *versus* stable COPD and compared with healthy controls. Our study has strengths and limitations. The large cohort size for such an in-depth lipidomics study, which is well-matched for age, sex and BMI, is helpful to minimise biological factors that may influence LMs, and we have analysed data in the context of clinically important COPD severity variables and outcome data from the cohort. This has progressed our understanding of LMs and highlighted the need for further research. The potential for measured and unmeasured confounding factors that may align with the stable *versus* frequent exacerbation subtypes of COPD is recognised as a potential limitation, but this does reflect real-world patients with COPD. Further limitations include the fact that, although we have a large cohort, we have only one measurement per LM per patient, and no pairing with LMs in sputum, condensate or BAL, although paired sampling has been performed in prior smaller studies. Our findings do require validation in a different cohort. A further limitation is that we do not have information on diet or nutritional supplements for participants, nor measurement of blood fatty acid substrate (*i.e.*, DHA and EPA) levels. The impact of diet and essential fatty acid supplementation on substrate levels or LM metabolites in COPD are further important research questions that need to be understood. There is evidence that supplementation may impact metabolite levels [48]. Moreover, given the constraints of our clinical cohorts' collected data, we were unable to assess potential mechanisms underlying our results in further detail, for example, any relationship between LMs and the lung microbiome. A further limitation is the lack of ethnic diversity, with most participants being white British. However, this does reflect the current population with diagnosed COPD in the UK [49].

In summary, our results indicate differential clustering of LMs in COPD patients *versus* healthy controls and in patients with stable COPD *versus* COPD patients with frequent exacerbations, with relative downregulation of SPMs across metabolomes in those with frequent exacerbations compared with stable COPD patients. The potential clinical relevance of these findings of differences in LM profiles with COPD is suggested by the association of D-resolvins with severe exacerbation outcomes, suggesting dysfunctional resolution pathways in patients with frequent exacerbations.

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Author contributions: M. Fisk designed the study. The source datasets came from two studies. The ERICA study was conceived and directed by I. Wilkinson, M. Polkey and R. Tal-Singer. W. MacNee, C. Bolton, J.R. Cockroft, C. McEniery, J. Cheriyan and J. Fuld were part of the steering group and/or site principal investigators. M. Fisk contributed to data collection. The ACCT study was conceived and directed by I. Wilkinson, J.R. Cockroft and C. McEniery. Yasmin contributed to data collection. Lipid mediator identification and quantitation was performed by E.A. Gomez and J. Dalli, and they produced the figures. Y. Sun, M. Mickute and M. Fisk conducted the statistical analysis, and produced the results and tables. M. Fisk wrote the initial draft of the complete manuscript. All co-authors critiqued and commented on the manuscript. All authors have approved the final version of the manuscript prior to this submission and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest: I. Wilkinson held research grants with GSK and Innovate UK. R. Tal-Singer is a former GSK employee and shareholder, is a board member of ENA Respiratory on behalf of the COPD Foundation, and received personal fees from GSK, ImmunoMet, VOCALIS Health, ENA Respiratory and Teva. J. Cheriyan has received support from GSK, Evelo Biosciences, AstraZeneca, Alexion and Eli Lilly. J. Cheriyan is a full-time employee of Cambridge University Hospitals NHS Foundation Trust, but was seconded by the trust for 50% of his NHS salaried time to work on GSK clinical trials until October 2020. He received no employee benefits or shares/dividends or income from GSK. E.A. Gomez is an inventor on patents related to the utility of lipid mediators as biomarkers assigned to Queen Mary University of London. J. Dallis is an inventor on patents related to the composition of matter and/or use of pro-resolving mediators assigned to Brigham and Women's Hospital or Queen Mary University of London.

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