Clustering-based COPD subtypes have distinct longitudinal outcomes and multi-omics biomarkers

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ABSTRACT

Introduction Chronic obstructive pulmonary disease (COPD) can progress across several domains, complicating the identification of the determinants of disease progression. In our previous work, we applied k-means clustering to spirometric and chest radiological measures to identify four COPD-related subtypes: 'relatively resistant smokers (RRS)', 'mild upper lobe-predominant emphysema (ULE)', 'airway-predominant disease (AD)' and 'severe emphysema (SE)'. In the current study, we examined the associations of these subtypes to longitudinal COPD-related health measures as well as blood transcriptomic and plasma proteomic biomarkers.

Methods We included 8266 non-Hispanic white and African-American smokers from the COPDGene study. We used linear regression to investigate cluster associations to 5-year prospective changes in spirometric and radiological measures and to gene expression and protein levels. We used Cox-proportional hazard test to test for cluster associations to prospective exacerbations, comorbidities and mortality.

Results The RRS, ULE, AD and SE clusters represented 39%, 15%, 26% and 20% of the studied cohort at baseline, respectively. The SE cluster had the greatest 5-year FEV1 (forced expiratory volume in 1 s) and emphysema progression, and the highest risks of exacerbations, cardiovascular disease and mortality. The AD cluster had the highest diabetes risk. After adjustments, only the SE cluster had an elevated respiratory mortality risk, while the ULE, AD and SE clusters had elevated all-cause mortality risks. These clusters also demonstrated differential protein and gene expression biomarker associations, mostly related to inflammatory and immune processes.

Conclusion COPD k-means subtypes demonstrate varying rates of disease progression, prospective comorbidities, mortality and associations to transcriptomic and proteomic biomarkers. These findings emphasise the clinical and biological relevance of these subtypes, which call for more study for translation into clinical practice.

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WHAT IS ALREADY KNOW ON THIS TOPIC

⇒ Previous reports have grouped patients with chronic obstructive pulmonary disease (COPD) based on their cross-sectional spirometric, imaging and clinical features. However, few studies have explored the longitudinal outcomes and transcriptomic and protein biomarker characteristics of COPD subtypes.

WHAT THIS STUDY ADDS

⇒ COPD k-means subtypes show notable differences in regard to 5-year changes in FEV1, (forced expiratory volume in 1 s) and CT-quantified emphysema, as well as prospective risks of respiratory exacerbations, cardiovascular disease, diabetes and mortality. These subtypes also exhibit differences in their associations to whole blood transcriptomic and plasma proteomic biomarkers.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The heterogeneity of COPD progression presents major clinical challenges in risk-stratifying patients. The differences in cross-sectional and longitudinal associations of COPD subtypes may shed light on the biology of the disease and help with individualised care.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disorder, with a wide variety of clinical manifestations.1 Additionally, disease progression in COPD occurs across multiple domains, such as lung function decline, worsening of emphysema and development of comorbidities.2-4 These challenges complicate COPD subtyping and the identification of the determinants of COPD progression.

Subtyping beyond the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria is essential. Numerous investigations have revealed that many smokers without spirometry-defined obstruction experience respiratory symptoms, emphysema and airway thickening that are similar to those with traditionally defined COPD based on the GOLD criteria.5-8 COPD subtyping presents an opportunity to advance toward more
individualised treatment for this complex and heterogeneous disease.

Several reports have shown that factors, such as COPD exacerbation history, reduced pulmonary function, and a low body mass index (BMI), are associated with an elevated risk of respiratory exacerbations, accelerated spirometric decline and emphysema changes. Additionally, studies which have subtyped subjects with COPD based on spirometry, respiratory symptoms and other characteristics have revealed that these subgroups differ in their risks for exacerbations, hospital admissions and forced expiratory volume in 1 s (FEV₁) and emphysema changes. However, many of these studies have modest sample size and limited longitudinal follow-up. Furthermore, while comorbid conditions such as cardiovascular disease (CVD) and type 2 diabetes mellitus are common in subjects with COPD, the specific COPD-related characteristics associated with the risk of developing these comorbidities have not been fully described. According to earlier studies, more severe airflow obstruction and COPD exacerbation history are associated with higher risks of COPD exacerbations and CVD. In addition, Hersh et al. have previously demonstrated in cross-sectional data that COPD with limited emphysema and high airflow obstruction is associated with diabetes. However, the particular COPD-related traits linked to the likelihood of acquiring these comorbidities have not yet been thoroughly defined. Similarly, while transcriptomic signatures have been identified in COPD, the differences in such patterns between COPD subtypes have not been rigorously investigated. Moreover, different protein biomarkers have been identified in subjects with COPD, but few studies have assessed subtype-specific proteomic signatures.

In our previous work, we applied k-means clustering to spirometric and chest radiological measures and identified four COPD-related subtypes: ‘Relatively resistant smokers (ie, no/mild airflow obstruction and minimal emphysema despite heavy smoking) (RRS)’, ‘mild upper lobe-predominant emphysema (ULE)’, ‘airway-predominant disease (AD)’ and ‘severe emphysema (SE)’, which had differing cross-sectional profiles and genetic associations. In the current study, we included up to 12.7 years of prospective data to investigate subtype-specific rates of progression in spirometric measures and radiological emphysema and to quantify the risks of prospective COPD exacerbations, CVD events, diabetes and mortality. We also performed transcriptome and proteome-wide discovery analyses between the subtypes using high-throughput platforms. We hypothesised that these subtypes would have different disease progression profiles and associations to biomarkers. Some of these results have been previously reported as an abstract and in the form of a preprint.

**METHODS**

**Study description**

The COPDGene study is a prospective, multicentre, longitudinal study investigating the genetic and epidemiological characteristics of COPD across 21 centres in the USA (NCT00608764, www.copdgene.org). Institutional review board approval was obtained at each study centre. Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research. This study enrolled non-Hispanic whites and African-Americans, who were 45–80 years old and had at least 10 pack-years of lifetime smoking history. Subjects were recruited across the full spectrum of disease severity as defined by the GOLD spirometric grading system. Normal spirometry (GOLD grade 0) was defined as a postbronchodilator FEV₁/forced vital capacity (FVC) ≥0.7 and FEV₁ ≥80% predicted. GOLD 1–4 were defined as FEV₁/FVC <0.70 and postbronchodilator FEV₁ ≥80% predicted (GOLD 1), FEV₁/FVC <0.70 and postbronchodilator FEV₁ 50%–79% predicted (GOLD 2), FEV₁/FVC <0.70 and postbronchodilator FEV₁ 50%–49% predicted (GOLD 3), and FEV₁/FVC <0.70 and postbronchodilator FEV₁ <30% predicted (GOLD 4). COPDGene conducted two study visits (Visit 1 and Visit 2) approximately 5 years apart. Subjects were also contacted every 3–6 months through the COPDGene Longitudinal Follow-up programme via phone or online surveys to collect data on incident COPD-related events, comorbidities and mortality. COPD-related events and comorbidities included self-reported COPD exacerbations (defined as the acute worsening of respiratory symptoms that required systemic steroids and/or antibiotics), CVD events (defined as a composite endpoint of stroke, heart attack, coronary artery disease, coronary artery bypass graft surgery, peripheral artery disease and/or cardiac angina) and type 2 diabetes mellitus. All-cause mortality was determined through a combination of longitudinal follow-up and a search of the social security death index. Cause-specific mortality was categorised as respiratory related, CVD related, cancer related (any type) or due to other causes and determined through systematic adjudication process based on the methods used in the Towards a Revolution in COPD Health (TORCH) trial. More information about the COPDGene is available elsewhere.

Demographics, spirometry, imaging, smoking burden, respiratory symptoms and comorbidities were collected at Visits 1 and 2. In addition to GOLD grades 0–4, we included subjects with Preserved Ratio Impaired Spirometry (PRISm), defined as FEV₁/FVC ≥0.70 but with FEV₁ <80% predicted. Thirona software (www.thirona.eu) was used to quantify emphysema as the percentage of lung voxels with an attenuation of < −950 HU (Hounsfield unit) at maximal inspiration (%LAA-950). The Hounsfield units at the 15th percentile of the CT density histogram at end inspiration corrected for the depth of inspiratory variation (adjusted Perc15 density) were used for longitudinal changes in emphysema. Per convention, adjusted Perc15 density values are
reported as HU+1000. Total blood RNA was collected at Visit 2. The levels of 1305 protein biomarkers (SOMAscan Human Plasma 1.3K assay) were obtained from plasma samples collected at Visit 1.

**Cluster generation**

We used the k-means clusters that were generated in our previously published work.\textsuperscript{26} FEV\textsubscript{1}, percent predicted, CT-quantified emphysema, percent airway wall thickness and apicobasal emphysema distribution (log of the lung upper third to lower third ratio of emphysema) were the input features that were used for clustering at Visit 1. Using the same approach, we also performed k-means clustering at Visit 2 to assess cluster assignment stability between the two visits and to conduct differential gene expression analyses using RNA-Seq data available at Visit 2. The percentage of subjects in a cluster at Visit 1 who are still in the same cluster at Visit 2 was used to assess cluster assignment stability.

**Statistical analyses**

Data distributions were reported as medians with IQRs or counts with percentages, where appropriate. We calculated FEV\textsubscript{1} and emphysema changes as either absolute or relative annualised changes. We computed absolute annualised changes by subtracting Visit 1 from Visit 2 values and dividing the difference by the time in years between visits for each subject. Relative annualised changes were obtained by dividing the absolute annualised changes by Visit 1 values and multiplying by 100. Negative values indicate worsening of the disease between visits. Lower adjusted Perc15 density (emphysema) values indicate worse emphysema. Emphysema change resembles FEV\textsubscript{1} change in that negative change values indicate a lower value of the outcome at Visit 2 (ie, lower adjusted Perc15 density/worsening emphysema at Visit 2). We used the Kruskal-Wallis and \chi\textsuperscript{2} tests for continuous and categorical variables, respectively. We subsequently performed post hoc pairwise comparisons between the clusters using the Nemenyi and \chi\textsuperscript{2} tests for continuous and categorical variables, respectively. Additionally, we constructed univariable and multivariable linear regression models to relate changes in FEV\textsubscript{1} and emphysema as well as plasma proteins to cluster assignment. We assessed risks of incident COPD exacerbations, CVD events, diabetes and mortality using Cox proportional hazards models and obtained survival curves using the Kaplan-Meier method. For the analyses of incident CVD events and diabetes, we excluded subjects who had a history of CVD or diabetes at Visit 1. We used the RRS cluster as the reference group. Linear regression and Cox models were adjusted for relevant baseline physiological, clinical and demographic characteristics. For analyses investigating the risk of diabetes, we adjusted for age, sex, race, smoking pack-years and systemic steroid use. We additionally adjusted for metabolic syndrome in the CVD multivariable Cox models and for BMI, airflow obstruction and exercise capacity (BODE index) and GOLD in the mortality models. To ensure that cluster associations to emphysema changes are not confounded by CT scanner type, a sensitivity analysis was performed by adding scanner type as a covariate in a subgroup analysis limited to subjects who underwent scans with the same scanner type between visits.

To test for differential gene expression between clusters, we used the linear modelling approach implemented in the limma R package (V.3.38.3)\textsuperscript{36} adjusting for age, sex, current smoking, white cell count proportions and library preparation batch. Gene ontology (GO) functional enrichment of the gene sets was calculated using the weighted Fisher test in the topGO Bioconductor package that accounts for the dependency between terms in the GO topology.\textsuperscript{37} We reported only the GO pathways with at least three significant genes. For the protein analyses, we adjusted for age, sex, race and current smoking status. We conducted an additional analysis where we limited the study population to subjects who were in the SE cluster at Visit 1 and remained in the SE cluster at Visit 2. We then built multivariable regression models relating SOMAscan plasma proteins to absolute and relative emphysema changes. We also tested for differential gene expression and protein associations by comparing subjects who transitioned from RRS to those who stayed within RRS at Visit 2 using multivariable linear regression.

All tests for the clinical outcomes were two-tailed with a significance threshold of p-value <0.05. For the protein and RNA-Seq analyses, we corrected for multiple comparisons using the Benjamini-Hochberg method and applied a threshold of significance of false discovery rate (FDR) of 10%\textsuperscript{38}. Significantly enriched GO pathways were identified using the weighted Fisher p-value <0.005.

Additional methods are available in the Online supplemental file.
RESULTS
The overview of the study is shown in figure 1, and the study flow diagram is outlined in online supplemental figure 1. Subjects who were not included in the analyses of longitudinal changes in FEV₁ and emphysema, 935 of whom died between the first and second study visit, had a higher proportion of GOLD spirometric grade 4 disease (online supplemental table S1).

Cluster characteristics at baseline and stability of cluster assignments between visits
The RRS cluster represented 39% of the studied population at the baseline visit and was characterised by a history of heavy smoking without significant airflow obstruction, emphysema or airway wall thickness compared with the other clusters (p<0.05) (online supplemental table S2). Additionally, the majority of the subjects in the RRS cluster were GOLD 0–1. At Visit 2, RRS cluster membership was stable as 76.3% of subjects were still assigned to this cluster (online supplemental figure 2). The ULE cluster consisted of 15% of all subjects and had moderate airflow obstruction and mild emphysema predominantly in the upper lung lobes. The ULE cluster was relatively unstable, with only 38% of subjects remaining within this cluster at their follow-up visits. The AD cluster, 26% of all participants, was characterised by high BMI and had the highest proportion of subjects with PRISm (p<0.05). The AD cluster was more stable than the ULE cluster with 53% of subjects staying within this cluster at their follow-up visits. The SE cluster, 20% of the studied cohort, exhibited high emphysema and gas trapping and had the highest proportion of GOLD 3–4 smokers (p<0.05). The SE cluster was very stable as 92.2% of subjects remained within the SE cluster at Visit 2. 7.8% of the subjects in the SE cluster at Visit 1 did transition to a different cluster at Visit 2. Of those, 2.4% transitioned to the RRS cluster, 4.3% transitioned to the ULE cluster and 1.1% transitioned to the AD cluster (online supplemental figure 2). For subsequent analyses of longitudinal outcomes and protein data by k-means clusters, subjects were analysed according to their cluster assignment at Visit 1.

Cluster-specific rates of spirometric and emphysema progression
The 5-year change values for FEV₁ (measured as absolute change in FEV₁ and percent change relative to baseline) and emphysema are shown in figure 2, and the results from the univariable and multivariable models are shown in table 1. The RRS cluster, which has the least impaired spirometry and emphysema at baseline, had the greatest loss in absolute FEV₁. The AD cluster had the lowest absolute loss in FEV₁, significantly less than the RRS cluster in both univariable and multivariable models. Both the ULE and SE clusters had similar absolute FEV₁ changes relative to the RRS cluster. However, when adjusted for relevant covariates, the SE cluster had significantly less absolute FEV₁ decline than the RRS cluster. While absolute FEV₁ changes were notably higher in the RRS cluster, percent changes in FEV₁ relative to baseline were most pronounced in the SE cluster. Pairwise comparisons between all clusters showed that both emphysema-related clusters (ULE and SE) had significantly larger relative changes in percent FEV₁ relative to baseline than both the RRS and AD clusters (p<0.05; online supplemental table S3). Compared with FEV₁ changes, the pattern of CT-quantified emphysema progression was less sensitive to the metric being used (absolute vs relative). The SE cluster had the most rapid while the RRS cluster had the least rapid relative progression in both univariable and multivariable models. The ULE and AD clusters had significantly greater absolute and relative emphysema changes than the RRS cluster. The ULE cluster had significantly greater relative emphysema progression than the AD cluster (pairwise p-value: 0.03; online supplemental table S4). We noted similar cluster associations to both absolute and relative emphysema changes when we added scanner type as a covariate in the subgroup analysis limited to subjects who underwent scans with the same scanner type between visits (online supplemental table S6).

Cluster-specific risk of incident comorbidities and COPD-related events
Starting from Visit 1, the median follow-up time was 9.2 years for prospective CVD and diabetes, 5.3 years for respiratory exacerbations, 9.5 years for all-cause mortality and 7.8 years for cause-specific mortality. When we analysed prospective risks of various health outcomes by subtype, we observed that the SE cluster had the highest
risk for prospective COPD exacerbations and incident CVD (figure 3). After multivariable adjustment, the SE cluster had a three times higher likelihood of having a COPD exacerbation when compared with the RRS cluster (HR 2.98 (SE: 0.05), p<0.001) (online supplemental table S5). The AD cluster had the highest risk of incident diabetes (HR 1.97 (SE: 0.09), p<0.001), and this association remained significant after correcting for age, sex, race, BMI, smoking pack-years and systemic steroid treatment. COPD exacerbation and CVD risks were higher in the ULE cluster compared with the RRS cluster (p<0.05).

Survival curves by subtype are shown for all-cause mortality (figure 3) and for cause-specific mortality (online supplemental figure 3). Results of the survival models are presented in online supplemental table S6. In the univariable models, subjects in the SE cluster had the highest risks of all-cause, respiratory-related, CVD-related, cancer-related and other cause-related mortality relative to the RRS cluster (6, 50, 3, 3 and 2 times higher, respectively; p<0.0001) (online supplemental table S6). The risks for all-cause and CVD mortality were also elevated for the ULE and AD clusters relative to RRS (p<0.05). In multivariable models adjusting for age, sex, race, smoking pack-years and BODE, statistical significance was maintained for the associations of the ULE, AD and SE clusters with all-cause mortality, the association of the SE cluster with respiratory mortality, the associations of the ULE and AD clusters with CVD mortality and the association of the ULE cluster with cancer mortality (p<0.05). When adjusting for GOLD, age, sex race, BMI, smoking pack-years and the BODE index, the all-cause mortality risk of the ULE, AD and SE clusters remained significantly elevated (p<0.05). The respiratory mortality risk of the SE cluster also remained elevated (p<0.05). On the other hand, the addition of GOLD as a covariate led to non-significantly elevated CVD mortality risk of the ULE and AD clusters, and the ULE cluster was no longer significantly associated with an elevated cancer mortality risk.

Clusters’ associations to gene expression and protein biomarkers
Using blood RNA sequencing data from 2072 subjects at Visit 2, we identified significant associations to 3, 2,105 and 148 genes for the ULE, AD and SE clusters, respectively, with the RRS group as the reference. The Bland-Altman plots are shown in figure 4, and the complete set of association results is reported in online supplemental table 7. Compared with the RRS cluster, the ULE cluster was associated with the upregulation of the GPR15, AHRR and GPR55 genes, and GO pathway enrichment analysis results did not identify any significantly enriched pathways (table 2). The AD cluster had particularly strong differences in gene expression with 22 and 34 enriched pathways relative to the RRS and SE clusters, respectively. Compared with the RRS cluster, the AD cluster showed significant associations to pathways involved in innate immunity, cellular defence response, and NF-kB (nuclear factor kappa B) signalling. Relative to the SE cluster, the AD cluster demonstrated associations to processes involved in both innate and adaptive immunity.
The SE cluster also had many differentially expressed genes, with significant pathway enrichment for positive regulation of synapse assembly and cell adhesion. The complete set of pathway enrichment results is reported in online supplemental table 8.

Using SOMAscan plasma protein measurements at baseline from 1047 subjects, we tested for differential protein associations between clusters. Using the RRS group as the reference, we identified significant associations in adjusted models to 16, 65 and 219 proteins for the ULE, AD and SE clusters, respectively (FDR 10%) (online supplemental table 9). The most strongly associated proteins for the ULE cluster were related to mitochondrial function (ATP synthase peripheral stalk subunit OSCP and glucokinase regulatory protein) and cytoskeleton rearrangement (serine/threonine protein kinases MRCK-beta and PAK 6) (table 3). Top proteins associations for the AD cluster were primarily involved in fatty acid metabolism, such as elevated fatty acid-binding protein, leptin and retinoic acid receptor responder protein 2 and decreased apolipoprotein M. For the SE cluster, top associated proteins were related to innate immunity, such as bactericidal permeability-increasing protein, complement component C9 and protein S100-A12. The overlap of the protein associations between subtypes is shown in online supplemental figure 4.

In subjects in the SE cluster at Visit 1 that remained in the SE cluster at Visit 2, there were 80 and 64 significant protein biomarker associations to absolute and relative emphysema changes (online supplemental table 10). Of the 80 associations, the top five included decreased metalloproteinase inhibitor 3 (TIMP-3), PKC-G, secreted protein acidic and cysteine rich (ON), amyloid precursor protein and nidogen (online supplemental table S11). Of the 64 associations, the top five included decreased TIMP-3, IGFBP-3 (IGF Binding Protein-3), plasminogen activator inhibitor 1, nidogen and increased serine/threonine protein kinase Chk2 (Chk2) (online supplemental table S11). There was a total of 41 shared significant associations that were mostly related to inflammatory
processes (toll-like receptor 4, mast/stem cell growth factor receptor kit, interleukin 10, interleukin 17-soluble receptor and toll-like receptor 4 (TLR4)/myeloid differentiation factor 2 (MD-2) complex).

The sample sizes of subjects who stayed within RRS at Visit 2 were 646 for the gene expression analysis and 248 for the protein association analysis. The sample sizes of subjects who transitioned from RRS at Visit 1 to any other cluster at Visit 2 were 275 for the gene expression analysis and 81 for the protein association analysis. When comparing subjects who transitioned from RRS to those who stayed within RRS at Visit 2, no significant associations to genes or GO pathways were observed. In terms of significant protein associations for this same comparison, the protein spectrin alpha chain, non-erythrocytic 1 (UniProt ID=Q13813) was the only one to produce a significant result (beta coefficient 0.14, SE 0.03, FDR=0.05) (online supplemental table S12).

DISCUSSION

In this study, we demonstrated that k-means subgroups of smokers enriched for COPD have varying disease progression patterns, development of prospective comorbidities and distinct associations to plasma protein and blood transcriptomic biomarkers. The two clusters at the extremes of the lung health spectrum (the RRS and SE clusters) showed high cluster assignment stability between the two visits. Spirometric progression was sensitive to the progression metric being used (absolute vs relative) with the SE subtype showing the most rapid rate of FEV₁ decline relative to baseline FEV₁ level. Emphysema progression however was less sensitive to the use of absolute versus relative metrics of progression. In general, the SE cluster had the highest risk for prospective adverse health events, though the AD cluster had the highest risk of incident diabetes and the most distinct gene expression patterns.

A wide array of risk factors have been associated with spirometric decline, such as low BMI, higher baseline FEV₁ and FVC, smoking exposure, bronchodilator reversibility, African-American race, female sex, previous history of exacerbations, CT-quantified emphysema, upper lobe-predominant emphysema and small airway abnormalities.1 2 39–43 Whereas previous studies have shown that mild-to-moderate COPD (GOLD 1–2) is associated with an increased loss in absolute FEV₁,2 39 our study also investigated changes in FEV₁ relative to baseline values and found that the SE cluster, which represents a subset of subjects with advanced disease, is associated with more rapid relative FEV₁ decline. To our knowledge, such differences in the metrics of progression used to assess COPD progression have not been previously reported. This finding emphasises the fact that disease activity and disease severity are distinct

**Figure 4**  Bland-Altman (MA) plots of the log ratio versus mean gene expression for the differential expression analysis results between k-means clusters. The cluster following the ‘versus’ is the reference group. (A) ULE versus RRS. (B) AD versus RRS. (C) SE versus RRS. (D) AD versus ULE. (E) SE versus ULE. (F) SE versus AD. AD, airway-predominant disease; RRS, relatively resistant smokers; SE, severe emphysema; ULE, upper lobe-predominant emphysema.
In our study, we evaluated emphysema changes and factors that are associated with emphysema progression. In contrast to FEV₁ changes, less is known about the factors that are associated with emphysema progression. In our study, we evaluated emphysema changes by COPD subtype, investigated both absolute and relative changes and adjusted the analysis for age, sex, race, BMI, smoking pack-years and CT scanner types. We showed that relative changes were the highest in the SE cluster, which has more advanced baseline emphysema, low BMI and more COPD exacerbations.

In regard to prospective COPD events, prior reports have indicated that more severe airflow obstruction and COPD exacerbation history are associated with higher risks of COPD exacerbations and CVD.11,18-20 We demonstrated that when adjusted for covariates including airflow limitation and COPD exacerbation history, the SE cluster had three times higher risk of a COPD exacerbation, while the AD and ULE clusters had an HR of ~1.35 relative to the RRS group. The SE cluster was also associated with the highest risk of incident CVD, which may be explained by atherosclerosis or arterial stiffness mediated by inflammatory markers.48 Another novel finding from our study was that the AD cluster had the highest risk for the prospective development of diabetes, even after adjusting for BMI. This adds to the finding from the study by Hersh et al, which revealed that COPD with limited emphysema and high airflow obstruction is associated with diabetes in cross-sectional data.21 Our study provides further evidence linking airway-predominant COPD to diabetes and metabolic syndrome.

The age, dyspnoea and airflow obstruction (ADO) and BODE indices have been classically used to predict mortality in COPD, but less is known about the COPD characteristics that contribute to cause-specific mortality.49,50 CVD, airflow obstruction, low BMI, emphysema and poor exercise capacity were found to be associated with a high risk of all-cause mortality in COPD.51-53 We similarly observed that the SE cluster, which had these risk factors at baseline, had the highest risk of all-cause mortality. With regards to the CVD mortality, high Modified Medical Research Council Dyspnea Scale dyspnea score, exacerbation history, low FEV₁ and accelerated lung function decline have been shown to be contributors.54,55 In our paper, we showed that the SE, AD and ULE clusters had elevated risks of CVD-related mortality in unadjusted models, but when correcting for age, sex, race, BODE and smoking pack-years, only the AD and ULE clusters had significant risks, which indicates that airway disease and lobular emphysema distribution may be additional contributors to this risk. While most previously published COPD studies have examined the

Table 2  Top 10 significantly differentially expressed genes and enriched GO terms between k-means clusters

|       | Top 10 significant genes     | Top 10 significant GO terms                                      |
|-------|------------------------------|------------------------------------------------------------------|
| ULE versus RRS | ↑ GPR15                      | NS                                                              |
|       | ↑ AHRR                       |                                                                  |
|       | ↑ GPR55                      |                                                                  |
| AD versus RRS  | ↑ GPR15                      | Innate immune response                                          |
|       | ↓ SNRK                      | Mitotic chromosome movement towards spindle pole                |
|       | ↑ PTPN13                     | Negative regulation of NIK/nuclear factor-kappaB signalling     |
|       | ↓ ALG1L13P                   | Cellular defence response                                       |
|       | ↑ CD36                       | Cell proliferation                                              |
|       | ↓ MYLIP                      | Regulation of mitochondrial depolarisation                      |
|       | ↓ ETL3                       | Negative regulation of defence response to virus                |
|       | ↓ IL18R1                     | Mitotic cytokinesis                                             |
|       | ↓ ADPRHHL2                   | Fc-epsilon receptor signalling pathway                          |
|       | ↑ PTPRA                      | Vascular endothelial growth factor receptor signalling pathway  |
| SE versus RRS  | ↑ GPR15                      | Positive regulation of synapese assembly                        |
|       | ↑ MS4A2                      | Cell adhesion                                                   |
|       | ↑ TTL7                       |                                                                  |
|       | ↑ GCSAML                     |                                                                  |
|       | ↑ AKAP12                     |                                                                  |
|       | ↑ LINC02458                  |                                                                  |
|       | ↓ COG2                       |                                                                  |
|       | ↑ HDC                        |                                                                  |
|       | ↓ DLEU1                      |                                                                  |
|       | ↑ ENSG00000261055 (LncRNA)   |                                                                  |

For the gene expression analysis, covariates used were age, sex, race, current smoking status, white cell count proportions and library batch effects. A false discovery rate of 10% was used for multiple testing corrections. For the GO analysis, we only reported the pathways with at least three significant genes. Enriched GO terms between clusters were identified using the weighted Fisher’s test p-values <0.005. The cluster following the ‘versus’ is the reference group. ↑ = positive log fold change; ↓ = negative log fold change.

AD, airway-predominant disease; GO, gene ontology; NIK, NF-KappaB-inducing kinase; NS, non-significant; RRS, relatively resistant smokers; SE, severe emphysema; ULE, upper lobe-predominant emphysema.
studies. The inflammatory response in COPD involves the pathogenesis of COPD, according to a number of distinct gene expression signature that is enriched for systems. Additionally, COPD inflammation has a clear histology description, and COPD inflammation has a histology description, which may be potential mechanisms underlying these associations.

COPD subtype-specific associations to transcriptomic and protein biomarkers have only been studied in a few studies of relatively small size. Furthermore, although COPD inflammation has a clear histology description, the primary biological factors that are distinct to each COPD subtype are still not fully understood. At the transcriptomic level, COPD has been associated with inflammation, and pathways related to inflammation, and the severe emphysema cluster has the most significant enrichment for innate immunity-related pathways. Dysregulation of adipokine metabolism has also been widely implicated in COPD and emphysema pathophysiology.

While misclassifying subjects into their respective cluster at baseline is a potential limitation of k-means subtyping, it is also important to consider the sensitivity, specificity and reproducibility of spirometry and CT scans. From the existing literature, the coefficient of variation associated with repetitive measurements of FEV₁ in patients with obstructive lung disease was shown to be -0.04%–0.3% over a wide range of FEV₁. Additionally, using data from Shaker et al, the coefficient of variation of emphysema measurements on CT scans can be estimated at 3.7%.

This study has a number of strengths. Compared with previous publications, our study included a larger sample size and longer follow-up and investigated incident comorbidities in addition to lung health outcomes. Additionally, we included a well-phenotyped cohort of smokers across the full spectrum of disease severity, and we were able to test the association of subtypes to clinical, radiological and multi-omic molecular markers.

Table 3 Top five unique significantly differentially associated proteins between k-means clusters

| Protein ID | Beta coefficient (SE) | FDR |
|------------|----------------------|-----|
| P48047 | 0.33 (0.07) | 6.3x10⁻³ |
| Q14937 | 0.14 (0.04) | 4.2x10⁻² |
| Q9Y5S2 | 0.09 (0.02) | 4.2x10⁻² |
| Q9NQ5 | 0.37 (0.10) | 4.6x10⁻² |
| Q9BY79 | 0.14 (0.04) | 5.5x10⁻² |

SOMA scan plasma proteins significantly associated to Visit 1 k-means cluster membership from multivariable linear regression modelling (FDR 10%). We used linear regression and adjusted for age, sex, race and current smoking status. The top five significantly differentially associated proteins unique to each of the three comparisons (ULE vs RRS, AD vs RRS and SE vs RRS) are reported in this table. The cluster following the ‘versus’ is the reference group. The units of all proteins are relative fluorescence units.

AD, airway-predominant disease; RRS, relatively resistant smokers; SE, severe emphysema; ULE, upper lobe-predominant emphysema.
When studying progression, we considered both absolute and relative changes in lung function and CT-quantified emphysema. Furthermore, this is to our knowledge the first study demonstrating that airway-predominant COPD is independently associated with incident diabetes risk and has particularly strong associations to inflammatory biomarkers.

One of the limitations of our study is that because of its observational design, the statistical associations observed may not reflect causal relationships. Our study sample was limited to subjects who survived the 5-year observation period and as a result, our findings are not representative of subjects with very advanced COPD and limited life expectancy. Many of the participants in our study were already receiving treatment for their disease. The effect of pharmacotherapy on the proteome was described in the literature. Although it is not well-documented that specific pharmacotherapy impacts the course of the disease, these medications may have influenced our findings. However, medication use data in COPDGene are limited to self-report and do not include important information on adherence. As a result, we have not performed comprehensive adjustment for the various medications patients were using. The findings from our study are not yet ready for clinical applications. Nevertheless, they represent an important step towards identifying subtype-specific molecular signatures, which will open the door for later studies on treatments that target distinct patient subtypes and subtype-specific biological pathways. Analyses that jointly model death and other aspects of disease progression would provide additional useful information. As further follow-up data from COPDGene are obtained, our hypotheses from this study can be validated over longer follow-up.

CONCLUSIONS

COPD-related subtypes defined by spirometric and radiographic measures at baseline have different rates of disease progression and are differentially associated with prospective health outcomes. They also exhibit distinct biomarker profiles indicative of underlying biological differences. In the future, these subtypes could be used as the basis for targeted drug development, studies of differential treatment response or the enrolment of specific subgroups in clinical trials.

Contributors AB and PJC had full access to all the data in the study, take responsibility for the integrity of the data and the accuracy of the data analysis, had authority over manuscript preparation and the decision to submit the manuscript for publication. AB and PJC accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish. Study concept and design: AG, AB, PJC. Acquisition, analysis or interpretation of data: All authors. Drafting of the manuscript: AG, AB, PJC. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: AG, ZX, PJC, AB. Obtained funding: AB, PJC, ES. Study design: AG, AB, PJC. Critical revision of the manuscript: AG, AB, PJC. Study concept and design: AG, AB, PJC. Acquisition, analysis or interpretation of data: All authors. Drafting of the manuscript: AG, AB, PJC. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: AG, ZX, PJC, AB. Obtained funding: AB, PJC, ES. Study...
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Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Brigham and Women's Hospital (2007P000554). Participants gave informed consent to participate in the study before taking part.

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Data availability statement Data are available in a public, open access repository.

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Figure S1

NHW and AA smokers (45-80 years old; ≥10 pack-years of lifetime smoking history) in the COPDGene study at Visit 1 (n = 10,263)

Subjects with available k-means cluster assignment (n = 8,288)

Subjects who did not have lung volume reduction surgery, lung transplant, or more than a 1L increase in FEV₁ between Visits 1 and 2 (n = 8,266)

Subjects with available data for the following outcomes:
- 5-year changes in FEV₁: n = 4,679
- 5-year changes in adjusted Perc15 density: n = 4,387
- Prospective COPD exacerbations: n = 7,310
- Prospective CVD: n = 6,019
- Prospective diabetes: n = 6,355
- Prospective survival: n = 8,266 (all-cause), n = 7,364 (ascertained cause-specific)
- Plasma proteins: n = 1,047
- Whole blood RNA: n = 2,072
Figure S2

Cluster assignment – Visit 1

Cluster assignment – Visit 2
Figure S3

A

Proportion survival (respiratory)

P-value < 0.001

Follow-up time (years)

Number at risk

RRS 2909 2869 2816 2190 166
ULE 1060 1045 1006 809 59
AD 1900 1961 1796 1418 69
SE 1495 1400 1228 862 93

B

Proportion survival (CVD)

P-value < 0.001

Follow-up time (years)

Number at risk

RRS 2909 2869 2816 2190 166
ULE 1060 1045 1006 809 59
AD 1900 1961 1796 1418 69
SE 1495 1400 1228 862 93

C

Proportion survival (cancer)

P-value < 0.001

Follow-up time (years)

Number at risk

RRS 2909 2869 2816 2190 166
ULE 1060 1045 1006 809 59
AD 1900 1961 1796 1418 69
SE 1495 1400 1228 862 93

D

Proportion survival (other causes)

P-value < 0.001

Follow-up time (years)

Number at risk

RRS 2909 2869 2816 2190 166
ULE 1060 1045 1006 809 59
AD 1900 1961 1796 1418 69
SE 1495 1400 1228 862 93
Figure S4

Number of proteins

193
47
17
8
7
1

SE vs. RRS
AD vs. RRS
ULE vs. RRS
Clustering-based COPD Subtypes Have Distinct Longitudinal Outcomes and Multi-omics Biomarkers

**Supplementary Materials**

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Methods

COPDGene

Exclusion criteria of subjects in the COPDGene study included a history of other lung diseases except asthma, prior lobectomy or lung volume reduction surgery, active cancer undergoing treatment, known or suspected lung cancer, or pregnancy. A severe exacerbation was defined as a COPD exacerbation that required a hospital admission or an emergency room visit. Quality of life and respiratory disease-related health impairments were determined using the St George’s Respiratory Questionnaire (SGRQ)\(^1\). Dyspnea was assessed by the Modified Medical Research Council (MMRC) dyspnea score system\(^2\). Metabolic syndrome was defined as having at least 3 of the following conditions: BMI > 30, diabetes, hypertension, and high cholesterol. During both visits in the COPDGene study, spirometry was performed before and after 180 mcg of albuterol was administered (ndd Easy-One spirometer, Andover, MA). The Hankinson NHANES reference equations were used to calculate the percent predicted values\(^3\).

Radiologic assessment

The log of the lung upper third to lower third ratio of emphysema (%LAA-950) was used to evaluate the distribution of apico-basal emphysema. VIDA software (www.vidadiagnostics.com) measured airway disease as gas trapping (percentage of low attenuation units < -856 HU at end-expiration), airway wall thickness (obtained along the center line of the lumen, in the middle third of the airway segment, for one segmental airway of each lung lobe; the mean value across all lobes was used for analysis), and Pi10 (the square root of the wall area of a hypothetical airway of 10-mm internal perimeter).
**Plasma protein biomarkers**

At baseline, participants who agreed and consented to participate in an omics ancillary study provided an additional sample of blood, collected using 8.5 mL p100 tubes (Becton Dickinson and Company). National Jewish Health stored and determined protein levels of 1,305 proteins using the SOMAscan Human Plasma 1.3K assay (SomaLogic, Boulder, Colorado). SOMAscan is a multiplex aptamer-based assay. Aptamers are single-stranded deoxyoligonucleotides that bind with high affinity and specificity to specific protein structures. SOMAscan data was standardized by SomaLogic per their protocol. It consists of within plate hybridization to control for variability across array signals, median signal normalization to control for technical variability of replicates within a run, and plate scaling and calibration of SOMAmers to control for inter-assay variation between analytes and batch differences between plates.

**Total RNA extraction**

Total blood RNA was extracted from subjects at Visit 2 and collected in PAXgene TM Blood RNA tubes from the Qiagen PreAnalytiX PAXgene Blood miRNA Kit (Qiagen, Valencia, CA). The extraction protocol was performed either with the Qiagen QIAcube extraction robot according to the company’s standard operating procedure or manually. RNA samples with a concentration of $\geq 25 \mu g/ul$ and RNA integrity number (RIN) $> 6$ were sequenced.

**cDNA library construction and sequencing**

Total RNA Globin reduction and cDNA library preparation were performed with the Illumina TruSeq Stranded Total RNA with Ribo-Zero Globin kit (Illumina, Inc., San Diego, CA). Quantification with picogreen, size analysis on an Agilent Bioanalyzer or Tapestation.
2200 (Agilent, Santa Clara, CA), and qPCR quantitation against a standard curve assisted with library quantity control. Samples were sequenced to an average depth of 20 million 75bp paired end reads on Illumina HiSeq 2500 sequencers.

Sequencing read alignment, quality control and expression quantification

Skewer with default parameters trimmed reads of TruSeq adapters. The STAR (version 2.5.2b) aligner aligned the trimmed reads to the GRCh38 genome. Transcript GTF and gene annotations were downloaded from the Biomart Ensembl database (Ensembl Genes release 94, GRCh38.p12 assembly). Quality control was performed with the FastQC and RNA-SeQC programs and samples were included for further analysis if they had > 10 million total reads, > 80% of reads mapped to the reference genome, XIST and Y chromosome expression was consistent with reported sex, < 10% of R1 reads in the sense orientation, Pearson correlation ≥ 0.9 with samples in the same library construction batch, and concordant genotype calls between variants called from RNA sequencing reads and DNA genotyping. Sequencing read counts were obtained from the featureCounts function in the Rsubread R package (v1.32.2) and the gene count data used for this analysis are available in GEO (accession number GSE158699).

Differential gene expression

Genes were filtered to remove very low expressed genes (average counts per million (CPM) < 0.2 or number of subjects with CPM < 0.5 was < 50) or outlying extremely highly expressed genes (the number of subjects with CPM > 50,000 was less than 50). The trimmed mean of M values (TMM) procedure from the edgeR R package (v3.24.3) was applied to account for differences in sequencing depth. Subsequently, counts were transformed to log2 CPM values and quantile-normalized to further remove systematic noise from the data.
Figure Legends:

Figure S1: Study flow chart. Abbreviations: NHW = non-Hispanic whites. AA = African-Americans.

Figure S2: Sankey diagram showing changes in cluster assignments between Visits 1 and 2. Abbreviations: RRS = Relatively resistant smokers; ULE = Upper lobe predominant emphysema; AD = Airway-predominant disease; SE = Severe emphysema.

Figure S3: Kaplan-Meier plots of mortality by k-means cluster. (A) Risk of a respiratory-related mortality. (B) Risk of CVD-related mortality. (C) Risk of cancer-related mortality. (D) Risk of mortality due to other causes. Limited data is available for subjects at the 8-year time point, since the mortality adjudication process is still ongoing. Abbreviations: RRS = Relatively resistant smokers; ULE = Upper lobe predominant emphysema; AD = Airway-predominant disease; SE = Severe emphysema.

Figure S4: UpSet plots of SOMAscan plasma proteins significantly associated with k-means cluster membership. Reference group was the RRS cluster. Covariates used were age, sex, race, and current smoking status. We corrected for multiple comparisons with the Benjamini-Hochberg method. Proteins were selected if they reached a false discovery rate (FDR) of 10%. Abbreviations: RRS = Relatively resistant smokers; ULE = Upper lobe predominant emphysema; AD = Airway-predominant disease; SE = Severe emphysema.
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Table S1. Visit 1 characteristics of subjects who were included vs. excluded in the analyses of longitudinal changes in FEV$_1$ and emphysema.

|                  | Included subjects (n = 4,679) | Excluded subjects (n = 3,587) | P-value |
|------------------|-------------------------------|-------------------------------|---------|
| Age              | 59.60 (13.70)                 | 57.90 (15.30)                 | < 0.0001|
| Sex, % male      | 51.10                         | 57.04                         | < 0.0001|
| BMI              | 28.25 (7.61)                  | 27.71 (8.09)                  | < 0.0001|
| Smoking pack-years | 38.70 (27.30)            | 40.40 (27.70)                 | < 0.0001|
| FEV$_1$ (mL)     | 2,326 (1,178)                 | 2,173.50 (1,470)              | < 0.0001|
| FEV$_1$, % predicted | 83.50 (29.95)              | 76.90 (40.70)                 | < 0.0001|
| FVC (mL)         | 3,299 (1,368)                 | 3,159 (1,436)                 | < 0.0001|
| GOLD             | PRISm                         | 558 (54.81 %)                 | 460 (45.19 %) | 0.2 |
|                  | GOLD 0                        | 2,220 (61.26 %)               | 1,404 (38.74 %) | < 0.0001 |
|                  | GOLD 1                        | 419 (64.07 %)                 | 235 (35.93 %) | < 0.0001 |
|                  | GOLD 2                        | 918 (57.3 %)                  | 684 (42.70 %) | 0.5 |
|                  | GOLD 3                        | 466 (50.27 %)                 | 461 (49.73 %) | < 0.0001 |
|                  | GOLD 4                        | 98 (22.37 %)                  | 340 (77.63 %) | < 0.0001 |
| Variable                                      | Value 1       | Value 2       | p-value |
|----------------------------------------------|---------------|---------------|---------|
| Bronchodilator responsiveness (% FEV₁)      | 4.29 (8.47)   | 4.55 (9.65)   | 0.2     |
| Bronchodilator responsiveness (% FVC)       | 1.86 (10.02)  | 2.46 (11.36)  | 0.03    |
| Adjusted Perc15 density                      | 86.08 (29.91) | 85.59 (33.89) | 0.01    |
| %LAA-950                                     | 1.91 (5.39)   | 2.04 (7.86)   | 0.02    |
| Upper/lower emphysema ratio                  | 1.00 (1.55)   | 1.20 (1.74)   | < 0.0001|
| % Segmental airway wall thickness            | 49.12 (11.57) | 52.27 (12.22) | < 0.0001|
| Gas trapping (%)                             | 13.54 (20.77) | 14.91 (29.97) | < 0.0001|
| Pi10                                         | 2.15 (0.75)   | 2.38 (0.89)   | < 0.0001|
| Exacerbation history (%)                     | 17.97         | 23.98         | < 0.0001|
| Severe exacerbations history (%)             | 8.21          | 15.25         | < 0.0001|
| SGRQ symptom score                           | 23.39 (38.24) | 34.11 (43.29) | < 0.0001|
| MMRC dyspnea score                           |               |               |         |
| 0                                            | 2,346 (63.27 %)| 1,362 (36.73 %)| < 0.0001|
| 1                                            | 717 (60.4 %)  | 470 (39.6 %)  | 0.004   |
| 2                                            | 604 (55.72 %) | 480 (44.28 %) | 0.5     |
| 3                                            | 710 (47.24 %) | 793 (52.76 %) | < 0.0001|
| 4                                            | 302 (38.52 %) | 482 (61.48 %) | < 0.0001|
| CVD (%) | 16.48 % | 17.74 % | 0.1 |
|----------|---------|---------|-----|
| Diabetes (%) | 12.44 % | 13.49 % | 0.2 |
| Hypertension (%) | 42.45 % | 44.24 % | 0.1 |
| Mortality, n | 0 | 935 | < 0.0001 |

Continuous variables are reported as medians (interquartile ranges). Categorical variables are reported as percentages. Chi-square tests were used for categorical variables. 

Kruskal-Wallis rank sum tests were used for continuous variables.

BMI: Body mass index; Bronchodilator responsiveness (%) FEV₁: Percentage of subjects with post-bronchodilator increase in FEV₁ of at least 12% from baseline; Bronchodilator responsiveness (%) FVC: Percentage of subjects with post-bronchodilator increase in FVC of at least 12% from baseline; CVD: Cardiovascular disease (composite endpoint of stroke, heart attack, coronary artery disease, coronary artery bypass graft surgery, peripheral artery disease, and/or cardiac angina); Exacerbation history: At least one COPD exacerbation (acute worsening of respiratory symptoms that required systemic steroids and/or antibiotics) in the previous year; Severe exacerbation history: COPD exacerbation requiring an emergency department visit or hospital admission; FEV₁ (mL): Forced expiratory volume in 1 second; FEV₁, % predicted: Percent of the normal FEV₁ based on height, weight, and race; FVC: Forced vital capacity; GOLD: Global Initiative for Chronic Obstructive Lung Disease; GOLD 0: Normal spirometry (defined as post-bronchodilator FEV₁/FVC ≥ 0.7 and FEV₁ ≥ 80% predicted); GOLD 1: FEV₁/FVC < 0.70 and post-bronchodilator FEV₁ ≥ 80% predicted; GOLD 2: FEV₁/FVC < 0.70 and post-bronchodilator FEV₁ 50-79% predicted; GOLD 3: FEV₁/FVC < 0.70 and post-bronchodilator FEV₁ 30-49% predicted; GOLD 4: FEV₁/FVC < 0.70 and post-bronchodilator FEV₁ < 30% predicted; MMRC: Modified medical research council dyspnea scoring system; PRISm: Preserved Ratio Impaired Spirometry (defined as FEV₁/FVC ≥ 0.70 but with FEV₁ < 80% predicted); SGRQ: St. George’s respiratory questionnaire score.

Adjusted Perc15 density: Cut off value in Hounsfield units (HU) below which 15% of all voxels are distributed on a lung CT scan (per convention, adjusted Perc15 density values are reported as the HU + 1000); Gas trapping (%): Percentage of lung voxels with a density less than -856 HU at end exhalation; % LAA-950: Percentage of CT low attenuation less than -950 HU at end-inspiration using Thirona software; Pi10: Square root of the wall area of a hypothetical airway of a 10-mm internal perimeter; % Segmental airway wall thickness: Percentage of the wall relative to the total bronchial area for the segmental airways; Upper/lower emphysema ratio: Log of the lung upper third to lower third ratio of emphysema.

P-values correspond to global P-values for comparisons across all 4 k-means clusters. P-values < 0.05 are italicized.
**Table S2.** Visit 1 characteristics of k-means clusters

|                      | Relatively Resistant Smokers (n = 3,192) | Mild Upper Lobe Predominant Emphysema (n = 1,242) | Airway Predominant Disease (n = 2,176) | Severe Emphysema (n = 1,656) | P-value |
|----------------------|----------------------------------------|-------------------------------------------------|--------------------------------------|----------------------------|---------|
| Age                  | 57.90 (13.70)                          | 57.40 (12.48)                                   | 55.20 (12.65)                       | 66.00 (11.53)              | < 0.0001|
| Sex, % male          | 56.45                                  | 48.15                                           | 47.70                               | 60.33                      | < 0.0001|
| BMI                  | 27.74 (6.88)                           | 26.83 (7.39)                                    | 30.91 (8.95)                        | 26.26 (7.41)               | < 0.0001|
| Smoking pack-years   | 35.00 (24.20)                          | 41.70 (25.85)                                   | 38.00 (25.80)                       | 50.00 (34.45)              | < 0.0001|
| FEV₁ (mL)            | 2,838.50 (1,017.50)                    | 2,296.00 (963.75)                               | 2,127.00 (920.50)                   | 1,094.00 (682.75)          | < 0.0001|
| FEV₁, % predicted    | 95.30 (18.90)                          | 81.45 (21.17)                                   | 74.90 (22.50)                       | 40.50 (22.65)              | < 0.0001|
| FVC (mL)             | 3,720 (1,347)                          | 3,250 (1,224)                                   | 2,972 (1,206)                       | 2,681 (1,210)              | < 0.0001|
| GOLD                 |                                        |                                                 |                                     |                            |         |
| PRISm                | 224 (22 %)                             | 186 (18.27 %)                                   | 596 (58.55 %)                       | 12 (1.18 %)                |         |
| GOLD 0               | 2,412 (66.56 %)                        | 487 (13.44 %)                                   | 723 (19.95 %)                       | 2 (0.06 %)                 | < 0.0001|
| GOLD 1               | 346 (52.91 %)                          | 186 (28.44 %)                                   | 108 (16.51 %)                       | 14 (2.14 %)                |         |
| GOLD 2 | 204 (12.73 %) | 361 (22.53 %) | 572 (35.71 %) | 465 (29.03 %) |
|--------|---------------|---------------|---------------|---------------|
| GOLD 3 | 6 (0.65 %)    | 21 (2.27 %)   | 158 (17.04 %) | 742 (80.04 %) |
| GOLD 4 | 0 (0 %)       | 1 (0.23 %)    | 18 (4.11 %)   | 419 (95.66 %) |

|                  | Bronchodilator responsiveness (% FEV<sub>1</sub>) |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 3.35 (6.43)                                   | 3.89 (8.36)      | 4.74 (10.59)     | 7.93 (13.53)     |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | Bronchodilator responsiveness (% FVC)         |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 0.51 (7.53)                                   | 1.64 (9.83)      | 2.92 (11.86)     | 6.58 (14.54)     |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | Adjusted Perc15 density                       |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 88.95 (24.48)                                 | 89.77 (27.56)    | 96.59 (27.23)    | 52.04 (28.38)    |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | Emphysema (%LAA-950)                          |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 1.40 (3.08)                                   | 2.10 (3.97)      | 0.69 (1.62)      | 18.85 (16.83)    |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | Upper/lower emphysema ratio                   |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 0.69 (0.47)                                   | 3.87 (6.51)      | 0.52 (0.41)      | 1.43 (1.73)      |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | % Segmental airway wall thickness             |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 44.73 (7.65)                                  | 50.33 (10.54)    | 56.89 (9.78)     | 54.54 (10.34)    |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | Gas trapping (%)                              |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 9.87 (12.86)                                  | 13.87 (15.63)    | 9.91 (13.76)     | 52.32 (22.25)    |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | Pi10                                          |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 1.86 (0.41)                                   | 2.23 (0.63)      | 2.65 (0.76)      | 2.7 (0.69)       |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | Exacerbation history (%)                     |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 8.77                                          | 17.39            | 21.65            | 44.32            |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | Severe exacerbations history (%)             |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 3.26                                          | 10.55            | 13.28            | 24.58            |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | SGRQ symptom score                           |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 15.01 (28.78)                                 | 28.61 (40.06)    | 33.88 (42.96)    | 47.68 (35.80)    |
|                  | < 0.0001                                      |                  |                  |                  |

|                  | MMRC dyspnea score                           |                  |                  |                  |
|------------------|-----------------------------------------------|------------------|------------------|------------------|
|                  | 0                                             | 2,089 (56.34 %)  | 866 (23.35 %)    | 209 (5.64 %)     |
|                  | < 0.0001                                      |                  |                  |                  |
|                  | 1                                             | 466 (39.26 %)    | 319 (26.87 %)    | 191 (16.09 %)    |
Continuous variables are reported as medians (interquartile ranges). Categorical variables are reported as percentages. Kruskal-Wallis rank sum tests were used for continuous variables. Chi-square tests were used for categorical variables.

BMI: Body mass index; Bronchodilator responsiveness (%) FEV$_1$: Percentage of subjects with post-bronchodilator increase in FEV$_1$ of at least 12% from baseline; Bronchodilator responsiveness (%) FVC: Percentage of subjects with post-bronchodilator increase in FVC of at least 12% from baseline; CVD: Cardiovascular disease (composite endpoint of stroke, heart attack, coronary artery disease, coronary artery bypass graft surgery, peripheral artery disease, and/or cardiac angina); Exacerbation history: At least one COPD exacerbation (acute worsening of respiratory symptoms that required systemic steroids and/or antibiotics) in the previous year; Severe exacerbation history: COPD exacerbation requiring an emergency department visit or hospital admission; FEV$_1$ (mL): Forced expiratory volume in 1 second; FEV$_1$, % predicted: Percent of the normal FEV$_1$ based on height, weight, and race; FVC: Forced vital capacity; GOLD: Global Initiative for Chronic Obstructive Lung Disease; GOLD 0: Normal spirometry (defined as post-bronchodilator FEV$_1$/FVC ≥ 0.7 and FEV$_1$ ≥ 80% predicted); GOLD 1: FEV$_1$/FVC < 0.70 and post-bronchodilator FEV$_1$ ≥ 80% predicted; GOLD 2: FEV$_1$/FVC < 0.70 and post-bronchodilator FEV$_1$ 50-79% predicted; GOLD 3: FEV$_1$/FVC < 0.70 and post-bronchodilator FEV$_1$ 30-49% predicted; GOLD 4: FEV$_1$/FVC < 0.70 and post-bronchodilator FEV$_1$ < 30% predicted; MMRC: Modified medical research council dyspnea scoring system; PRISm: Preserved Ratio Impaired Spirometry (defined as FEV$_1$/FVC ≥ 0.70 but with FEV$_1$ < 80% predicted); SGRQ: St. George’s respiratory questionnaire score. Systemic corticosteroid treatment was defined as subjects having any history of systemic corticosteroid treatment.

Adjusted Perc15 density: Cut off value in Hounsfield units (HU) below which 15% of all voxels are distributed on a lung CT scan (per convention, adjusted Perc15 density values are reported as the HU + 1000); Gas trapping (%): Percentage of lung voxels with a density less than -856 HU at end exhalation; % LAA-950: Percentage of CT low attenuation less than -950 HU at end-inspiration using Thirona software; Pi10: Square root of the wall area of a hypothetical airway of a 10-mm internal perimeter; % Segmental airway wall thickness: Percentage of the wall relative to the total bronchial area for the segmental airways; Upper/lower emphysema ratio: Log of the lung upper third to lower third ratio of emphysema.

P-values correspond to global P-values for comparisons across all 4 k-means clusters. P-values < 0.05 are italicized.
### Table S3. Pairwise P-values between k-means clusters for annualized changes in FEV<sub>1</sub> and adjusted Perc15 density

|                           | RRS  | ULE  | AD   |
|---------------------------|------|------|------|
| Absolute annualized FEV<sub>1</sub> changes | ULE  | 0.6  | -    |
|                           | AD   | < 0.0001 | 0.004 | -    |
|                           | SE   | 0.4  | 1.0  | 0.01 |
| Relative annualized FEV<sub>1</sub> changes (percent changes from baseline) | ULE  | 0.02 | -    | -    |
|                           | AD   | 1.0  | 0.04 | -    |
|                           | SE   | < 0.0001 | < 0.0001 | < 0.0001 |
| Absolute annualized adjusted Perc15 density changes | ULE  | < 0.0001 | -    | -    |
|                           | AD   | < 0.0001 | 0.06 | -    |
|                           | SE   | < 0.0001 | 1.0  | 0.1  |
| Relative annualized adjusted Perc15 density changes (percent changes from baseline) | ULE  | < 0.0001 | -    | -    |
|                           | AD   | < 0.0001 | 0.03 | -    |
|                           | SE   | < 0.0001 | 0.02 | < 0.0001 |

Absolute annualized changes were computed by subtracting Visit 1 values from Visit 2 values and dividing by the time in years between both visits for each subject. Relative annualized changes were calculated by dividing absolute annualized changes by Visit 1 values and multiplying by 100.

Per convention, adjusted Perc15 density values are reported as the HU + 1000.

Pairwise P-values were obtained using the Nemenyi test. P-values < 0.05 are italicized.

**Abbreviations:** RRS = Relatively resistant smokers; ULE = Upper lobe predominant emphysema; AD = Airway-predominant disease; SE = Severe emphysema.
Table S4. Associations between clusters for absolute and relative annualized emphysema changes after adding scanner types as a covariate in the subgroup analysis with only subjects who underwent scans with the same scanner type between visits

| K-means cluster | Absolute annualized emphysema (adjusted Perc15 density) changes | Relative annualized emphysema (% adjusted Perc15 density) changes |
|-----------------|-----------------------------------------------------------------|-----------------------------------------------------------------|
|                 | Univariable models                                               | Multivariable models                                            |
|                 | Beta (Std Err)         P-value                   Beta (Std Err)         P-value |
| ULE             | -0.66 (0.11)          < 0.0001                -0.58 (0.11)          < 0.0001 |
| AD              | -0.31 (0.09)          0.0008                  -0.29 (0.09)          0.003   |
| SE              | -1.06 (0.11)          < 0.0001                -1.02 (0.12)          < 0.0001 |
| ULE             | -0.75 (0.12)          < 0.0001                -0.64 (0.13)          < 0.0001 |
| AD              | -0.31 (0.11)          0.004                   -0.33 (0.11)          0.003   |
| SE              | -2.39 (0.13)          < 0.0001                -2.29 (0.13)          < 0.0001 |

Absolute annualized changes were computed by subtracting Visit 1 values from Visit 2 values and dividing by the time in years between both visits for each subject. Relative annualized changes were calculated by dividing absolute annualized changes by Visit 1 values and multiplying by 100. Negative values indicate worsening of the disease between visits.

Per convention, adjusted Perc15 density values are reported as the HU + 1000.

A total of 2,557 (from the 4,387 subjects with available Visit 1 and 2 adjusted Perc15 density values) had CT chest with identical scanner types in both Visits 1 and 2. Univariable linear regression models included only visit 1 k-means cluster assignment. Multivariable models also included adjustments for age, CT scanner type, sex, race, BMI, and smoking pack-years. The reference group was the relatively resistant smokers cluster (RRS) cluster.

Pairwise comparisons showed that the SE cluster had significantly greater absolute and relative emphysema changes than the RRS, ULE and AD clusters (P-values < 0.05), the ULE cluster had greater absolute and relative emphysema changes than the RRS and AD clusters (P-values < 0.05), and the AD cluster had significantly greater absolute and relative emphysema changes than the RRS cluster (P-values < 0.05).

P-values < 0.05 are italicized.

Abbreviations: Std Err = standard error; RRS = Relatively resistant smokers; ULE = Upper lobe predominant emphysema; AD = Airway-predominant disease; SE = Severe emphysema.
Table S5. Risks of COPD-related events and incident comorbidities between clusters

| K-means cluster | Univariable models | Multivariable models |
|-----------------|--------------------|----------------------|
| Hazard ratio (Std Err) | P-value | Hazard ratio (Std Err) | P-value |
| COPD exacerbations | | | |
| ULE | 1.48 (0.06) | < 0.0001 | 1.35 (0.06) | < 0.0001 |
| AD | 1.62 (0.05) | < 0.0001 | 1.34 (0.05) | < 0.0001 |
| SE | 3.96 (0.04) | < 0.0001 | 2.98 (0.05) | < 0.0001 |
| CVD | | | |
| ULE | 1.38 (0.11) | 0.003 | 1.30 (0.11) | 0.02 |
| AD | 1.18 (0.09) | 0.08 | 1.12 (0.10) | 0.2 |
| SE | 1.55 (0.10) | < 0.0001 | 1.25 (0.10) | 0.03 |
| Diabetes | | | |
| ULE | 1.05 (0.12) | 0.7 | 0.96 (0.12) | 0.7 |
| AD | 1.97 (0.09) | < 0.0001 | 1.38 (0.09) | < 0.0001 |
| SE | 1.25 (0.11) | 0.04 | 1.15 (0.12) | 0.02 |

Univariable Cox-proportional hazard models included only visit 1 k-means cluster assignment. The multivariable models for COPD exacerbations were also adjusted for COPD exacerbation history, age, sex, race, BMI, and smoking pack-years. The multivariable models for cardiovascular disease were also adjusted for age, sex, race, BMI, metabolic syndrome (defined as having at least 3 of the following conditions: BMI > 30, diabetes, hypertension, and high cholesterol) and smoking pack-years. The multivariable models for diabetes, all-cause mortality, cause-specific mortality, and mortality due to other causes were also adjusted for age, sex, race, BMI, smoking pack-years, and steroid treatment. The reference group was the relatively resistant smokers (RRS) cluster.

Log-rank tests were used to compare clusters and multiple comparisons were corrected with the Benjamini-Hochberg method. The log-rank tests revealed that the SE cluster had higher risks for COPD exacerbations than the RRS, ULE, and AD clusters (P-values < 0.05), and higher risks for CVD events than the RRS and AD clusters (P-values < 0.05). The ULE cluster had higher risks for COPD exacerbations and CVD events than the RRS cluster (P-values < 0.05). The AD cluster had higher risks for diabetes than the RRS, ULE, and SE clusters (P-values < 0.0001), and higher risks of COPD exacerbations and CVD than the RRS cluster (P-values < 0.05).

P-values < 0.05 are italicized.

Abbreviations: Std Err = standard error; ULE = Upper lobe predominant emphysema; AD = Airway-predominant disease; SE = Severe emphysema; CVD = Cardiovascular disease (defined as a composite endpoint of stroke, heart attack, coronary artery disease diagnosis, coronary artery bypass graft surgery, peripheral artery disease diagnosis, and/or cardiac angina).
Table S6. Risks of mortality between clusters

| K-means cluster | Univariable model | Multivariable model 1 | Multivariable model 2 | Multivariable model 3 |
|-----------------|-------------------|-----------------------|-----------------------|-----------------------|
|                 | HR (Std Err) | P-value | HR (Std Err) | P-value | HR (Std Err) | P-value | HR (SE) | P-value |
| All-cause mortality |          |          |          |          |          |          |          |          |
| ULE             | 1.91 (0.09) | <0.0001 | 1.73 (0.09) | <0.0001 | 1.48 (0.09) | <0.0001 | 1.42 (0.10) | <0.001 |
| AD              | 1.84 (0.08) | <0.0001 | 2.02 (0.08) | <0.0001 | 1.30 (0.08) | <0.0001 | 1.23 (0.09) | 0.02   |
| SE              | 5.87 (0.07) | <0.0001 | 4.34 (0.07) | <0.0001 | 1.64 (0.09) | <0.0001 | 1.58 (0.11) | <0.001 |
| COPD respiratory mortality |          |          |          |          |          |          |          |          |
| ULE             | 2.40 (0.41) | 0.03    | 2.12 (0.41) | 0.07    | 1.59 (0.41) | 0.3     | 1.01 (0.44) | 0.9    |
| AD              | 3.08 (0.34) | 0.001   | 3.77 (0.34) | 0.0001  | 1.51 (0.35) | 0.2     | 0.97 (0.40) | 0.9    |
| SE              | 50.1 (0.28) | <0.0001 | 34.7 (0.29) | <0.0001 | 5.75 (0.32) | <0.0001 | 2.36 (0.40) | 0.04   |
| CVD mortality   |          |          |          |          |          |          |          |          |
| ULE             | 2.29 (0.26) | 0.002   | 2.23 (0.27) | 0.005   | 1.95 (0.27) | 0.01   | 1.59 (0.29) | 0.1    |
| AD              | 2.49 (0.23) | <0.0001 | 2.54 (0.23) | <0.0001 | 1.98 (0.24) | 0.004  | 1.5 (0.26)  | 0.1    |
| SE              | 3.15 (0.23) | <0.0001 | 2.30 (0.24) | 0.0006  | 0.98 (0.30) | 1.0    | 0.81 (0.35) | 0.5    |
| Cancer mortality |          |          |          |          |          |          |          |          |
| ULE             | 2.00 (0.20) | <0.0001 | 1.81 (0.20) | 0.003   | 1.62 (0.20) | 0.02   | 1.44 (0.22) | 0.1    |
| AD              | 1.18 (0.19) | 0.4     | 1.28 (0.20) | 0.2     | 0.96 (0.20) | 0.9    | 0.83 (0.22) | 0.4    |
| SE              | 3.28 (0.17) | <0.0001 | 1.99 (0.17) | <0.0001 | 0.95 (0.22) | 0.8    | 1.03 (0.26) | 0.9    |
| Other mortality  |          |          |          |          |          |          |          |          |
| ULE             | 1.27 (0.21) | 0.3     | 1.20 (0.22) | 0.4     | 1.09 (0.22) | 0.7    | 1.09 (0.23) | 0.7    |
| AD              | 1.37 (0.17) | 0.07    | 1.41 (0.18) | 0.06    | 1.03 (0.18) | 0.9    | 1.01 (0.20) | 0.9    |
| SE              | 2.3 (0.17)  | <0.0001 | 2.06 (0.18) | <0.0001 | 0.95 (0.23) | 0.8    | 1.35 (0.28) | 0.3    |

Univariable Cox-proportional hazard models included only visit 1 k-means cluster assignment. Multivariable model 1 also included adjustment for age, sex, race, BMI and smoking pack-years. In multivariable model 2, the body mass index, airflow obstruction, dyspnea, and exercise capacity (BODE) index was also added as a covariate. In multivariable model 3, the Global Initiative for Chronic Obstructive Lung Disease (GOLD) grade was added to the list of covariates used in model 2. The reference group was the relatively resistant smokers (RRS) cluster.

Log-rank tests were used to compare clusters and multiple comparisons were corrected with the Benjamini-Hochberg method. The log-rank tests revealed that the SE cluster had higher risks of all-cause, COPD respiratory, cancer and other-causes related mortalities than the RRS, ULE, and AD clusters (P-values < 0.05). The ULE cluster had higher risks of all-cause, COPD respiratory, CVD, and cancer mortalities than the RRS cluster (P-values < 0.05). The AD cluster had higher risks of all-cause, COPD respiratory and CVD mortalities than the RRS cluster (P-values < 0.05). The AD cluster had higher risks of all-cause, COPD respiratory and CVD mortalities than the RRS cluster, and higher cancer mortality than the ULE cluster (P-values < 0.05).

Abbreviations: Std Err = standard error. ULE = Upper lobe predominant emphysema; AD = Airway-predominant disease; SE = Severe emphysema; CVD = Cardiovascular disease; HR = Hazard ratio; SE = Standard error.

P-values < 0.05 are italicized.
Table S7 (enclosed in a separate Excel document). Differential gene expression between k-means clusters (covariates: k-means cluster assignment, age, race, sex, and current smoking status, white blood cell count proportions, and library prep batch). We corrected for multiple comparisons with the Benjamini-Hochberg method. In this file, there are 6 sheets for each pairwise comparison between the four k-means clusters. The cluster following the “vs.” is the reference group. Abbreviations: RRS = Relatively resistant smokers; ULE = Upper lobe predominant emphysema; AD = Airway predominant disease; SE = Severe emphysema.

Table S8 (enclosed in a separate Excel document). Significantly enriched gene ontology (GO) terms between k-means clusters (weighted Fisher P-values < 0.005 and number of significant genes ≥ 3). In this file, there are 6 sheets for each pairwise comparisons between the four k-means clusters. The cluster following the “vs.” is the reference group. Abbreviations: RRS = Relatively resistant smokers; ULE = Upper lobe predominant emphysema; AD = Airway predominant disease; SE = Severe emphysema.

Table S9 (enclosed in a separate Excel document). SOMAscan plasma proteins associated to k-means cluster membership (univariable linear regression models on the left and multivariable linear regression models on the right). Covariates: k-means cluster assignment, age, sex, race, and current smoking status. In this file, there are three sheets for ULE vs. RRS, AD vs. RRS, and SE vs RRS comparisons and one sheet where we are listing the significant proteins (FDR 10%). The cluster following the “vs.” is the reference group. Abbreviations: RRS = Relatively resistant smokers; ULE = Upper lobe predominant emphysema; AD = Airway-predominant disease; SE = Severe emphysema.
Table S10 (enclosed in a separate Excel document). Significant (FDR 10%) SOMAscan proteomic associations to absolute and relative adjusted Perc15 density (emphysema) changes within subjects in the SE cluster at Visit 1 who remained in this cluster at Visit 2. Proteins were associated to emphysema changes between Visits 1 and 2 using Cox-proportional hazards. Covariates were age, sex, race, and pack-years of smoking. FDR values < 0.1 are italicized. Significantly associated proteins to both absolute and relative emphysema changes that were shared between both analyses are also included (indicated as "Shared proteins").
Table S11. Top 5 significant (FDR 10%) SOMAscan plasma proteins associated to k-means clusters transitions between Visits 1 and 2

| Protein name          | Protein ID | Beta coefficient (standard error) | FDR     |
|-----------------------|------------|-----------------------------------|---------|
| **RRS to ULE vs RRS to RRS** |            |                                   |         |
| 14-3-3 protein theta  | P63104     | 3.6 (0.18)                        | < 0.0001|
| IL-17 receptor D      | Q8NFM7     | 1.3 (0.21)                        | < 0.0001|
| IL-20 receptor A      | Q13261     | 1.4 (0.24)                        | < 0.0001|
| Protein FAM107B       | Q9H098     | 1.6 (0.36)                        | < 0.0001|
| PGRP-S                | O75594     | 2.6 (0.64)                        | < 0.0001|
| **RRS to AD vs RRS to RRS** |            |                                   |         |
| IMDH2                 | P12268     | 0.15 (0.04)                       | 0.02    |
| GI24                  | Q9H7M9     | 0.13 (0.03)                       | 0.02    |
| MIP-3a                | P78556     | 0.2 (0.05)                        | 0.02    |
| NACA                  | Q13765     | 0.3 (0.08)                        | 0.02    |
| Amphiregulin          | P15514     | 0.32 (0.09)                       | 0.02    |

The proteomic characteristics of subjects who transitioned to upper lobe predominant emphysema (ULE), airway predominant (AD) or severe emphysema (SE) clusters at Visit 2 from the relatively resistant (RRS) cluster at Visit 1 were compared to subjects who were assigned to the RRS cluster at Visit 1 and remained in the RRS cluster at Visit 2. Univariate models contain only the cluster transitions, while multivariable models include adjustments for age, sex, race, and current smoking status. False discovery rate (FDR) values < 0.1 are italicized. Abbreviations: PGRP-S = Peptidoglycan recognition protein; IMDH2 = Inosine-5'-monophosphate dehydrogenase 2; GI24 = V-type immunoglobulin domain-containing suppressor of T-cell activation; MIP-3a = C-C motif chemokine 20; NACA = Nascent polypeptide-associated complex subunit alpha.
Table S12 (enclosed in a separate Excel document), SOMAscan plasma proteins associated to k-means clusters transitions between Visits 1 and 2. The proteomic characteristics of subjects who transitioned to the upper lobe predominant emphysema, airway predominant or severe emphysema (RRS to other subgroups) clusters at Visit 2 from the relatively resistant (RRS) cluster at Visit 1 were compared to subjects who were assigned to the RRS cluster at Visit 1 and remained in the RRS cluster at Visit 2 (RRS to RRS). Univariate models contain only the cluster transitions as a covariate. Multivariable models include adjustments for age, sex, race, and current smoking status. False discovery rate (FDR) values < 0.1 are italicized.