A mechanistic insight into severe COPD: the nose as a surrogate for the airways

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A severe COPD signature in bronchial and nasal epithelial cells reflects reduced tissue repair and ECM regulation https://bit.ly/476S3PJ

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Almost 400 million people worldwide suffer from the chronic inflammatory airway disease COPD [1–3]. It currently constitutes the third greatest cause of death globally and this is expected to rise in the near future due to the continued impact of cigarette smoking, environmental pollution and the use of vapes [2].

Acute exposure to cigarette smoke and pollution causes irritation and inflammation including mucus hypersecretion; development of COPD is a consequence of increased duration and amount of exposure [4]. However, there is a clear individual susceptibility to COPD as some subjects develop a much more severe disease or develop COPD at an earlier age [5]. Patients with severe COPD have a greater healthcare and societal burden than those with moderate disease [2]. It is therefore important to identify these subjects and determine whether these patients with severe irreversible airflow limitation, mucus hypersecretion with extensive emphysema or small-airway disease represent a distinct COPD phenotype with the potential for specific targeted interventions.

In a first step to addressing this question, VAN NIJNATTEN *et al.* [6] undertook bulk RNA sequencing of bronchial brushings obtained from 123 patients with severe COPD (Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages 3 and 4), 23 patients with mild–moderate COPD (GOLD 1 and 2) and 23 non-COPD controls who were enrolled in the SHERLOCk (An Integrative Genomic Approach to Solve the Puzzle of Severe Early-Onset COPD) cohort (www.clinicaltrials.gov identifier numbers NCT04263961 and NCT04023409). Importantly, patients had stopped smoking for ⩾2 months and had no evidence of an exacerbation or lung infection 4 weeks before bronchoscopy. They initially identified differentially expressed genes (DEGs) between severe COPD and non-COPD controls (false discovery rate <0.05, fold-change >|2|) correcting for age and smoking. They then removed the genes that were associated with mild–moderate disease only and confirmed that these were different in a direct comparison between severe and mild–moderate disease. Next they undertook cellular deconvolution to identify genes that may reflect changes in cell composition in COPD and removed these form the list of DEGs. Finally, they removed genes that represented a response to inhaled corticosteroids (ICS) to leave a group of genes that represented a signature for severe COPD in bronchial brushings (figure 1).

In detail, the study identified 435 DEGs (213 up-regulated and 222 down-regulated) between severe COPD and non-COPD controls. To account for the different numbers of subjects in each group, 1000 bootstrapping iterations were performed on the original 435 severe COPD DEGs, which indicated that >60% of the 435 genes were replicated. In addition, there were 123 DEGs between mild–moderate COPD and non-COPD controls. These 123 genes were removed from the 435 severe COPD gene list and 285 of the resulting 312 genes were confirmed as being DEGs by directly comparing severe and mild–moderate COPD samples. Of these 285 genes, 118 genes were up-regulated and 167 genes were down-regulated in severe COPD.

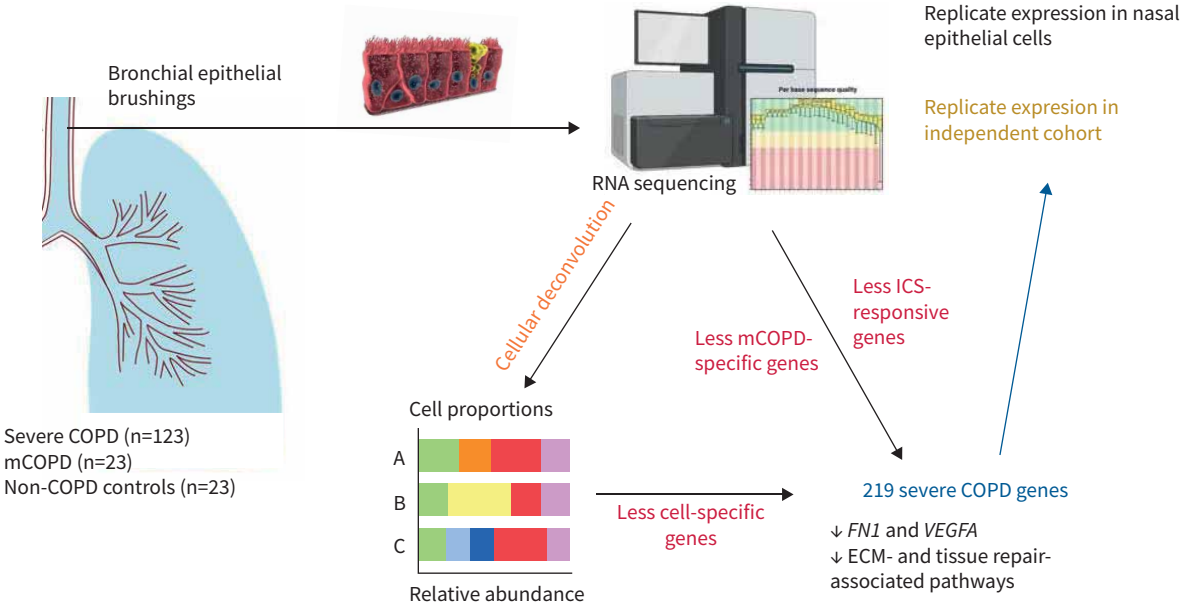

FIGURE 1 Overview of the study protocol. Bronchial brushings were obtained from patients with severe (n=123) and mild–moderate (n=23) COPD and 23 non-COPD control subjects and RNA sequencing performed. After identifying a set of differentially expressed genes between severe COPD and non-COPD subjects, genes within the dataset relating to mild–moderate COPD, specific cell subtypes and inhaled corticosteroid (ICS) use were removed to give a 219 gene severe COPD bronchial epithelial cell signature. Key down-regulated genes included fibronectin-1 (*FN1*) and vascular endothelial growth factor α (*VEGFA*) which were linked to suppression of extracellular matrix (ECM)- and tissue repair-associated pathways. The reduced expression of these genes and pathways were replicated in paired nasal bronchial brushings and in nasal epithelial cells from a separate cohort of severe COPD patients and non-COPD controls. mCOPD: moderate COPD.

Cellular deconvolution identified a greater proportion of goblet cells and a lower proportion of basal and ciliated cells in severe COPD compared with non-COPD controls. Adjusting for this difference in cellular makeup, 23 genes were removed from the 285 severe COPD DEGs to leave 262 genes that were unaffected by cell proportions. The Groningen group had previously identified a set of 2691 genes that represented the clinical response to ICS [7], of which 43 were present in the 262 severe COPD gene set, removal of these 43 genes left a signature for severe COPD consisting of 219 genes (104 genes being up-regulated and 115 being down-regulated).

The top 10 up-regulated genes in severe COPD bronchial brushings were *MEX3D*, *LINC00857*, *CEACAM5*, *TMC7*, *FNDC10*, *TPRXL*, *NETO2*, *SERPINB5*, *CALML3* and *MUC12* whilst the top 10 down-regulated genes were *FXYD6*, *GGTA1P*, *GEM*, *CPED1*, *KCNJ5*, *VEGFA*, *JAKMIP2*, *DOK2*, *KMO* and *GPR174*. No pathways were significantly associated with up-regulated genes in severe COPD. However, protein–protein interaction analysis of the 219 severe COPD DEGs using StringDB analysis showed significant associations of extracellular matrix (ECM)-related pathways which correlated with the reduced numbers of basal and ciliated epithelial cells. Two key regulatory genes with >25 connections were identified as fibronectin (*FN1*) and vascular endothelial growth factor α (*VEGFA*) which are involved in tissue development. *FN1* is highly expressed in fibroblasts, macrophages, and endothelial and smooth muscle cells, whilst *VEGFA* is mostly expressed in airway basal cells and goblet cells [8]. The two hub genes were associated with 15 connecting genes (*SPARC*, *TWIST1*, *LIF*, *SEMA3E*, *FOS*, *PTHLH*, *PECAM1*, *ABCB1*, *BDNF*, *CEACAM5*, *CX3CR1*, *CYR61*, *DCN*, *DKK1* and *EGR1*) which are linked to the regulation of developmental processes, tissue development and cell division. Other regulatory genes included *FOS*, *EGR1*, *FCGR3A*, *BDNF* and *NR4A1*.

Since obtaining and analysing bronchial brushings is difficult and potentially risky for patients with severe COPD, VAN NIJNATTEN *et al.* [6] examined whether this severe COPD signature in bronchial brushings tracked out to nasal brushings in the same patients isolated at the same time. Using a means of comparing signatures across groups known as gene set variation analysis, there was a significant enrichment of the down-regulated bronchial gene signature in the nasal brushes from severe COPD compared to mild–moderate COPD and non-COPD controls but no enrichment of the up-regulated bronchial brushing severe

COPD gene signature. Importantly, this enrichment of the down-regulated signature in severe COPD compared with non-COPD controls was replicated in an independent nasal gene expression dataset. Again, there was no replication of the up-regulated signature (figure 1).

Finally, the authors performed a meta-analysis using the 219 unique severe COPD genes in the paired nasal brushings and the independent nasal brushings datasets. This identified 83 genes (42 down-regulated and 41 up-regulated) that were significantly associated with severe COPD in the same direction. StringDB analysis of these 83 genes delineated a similar *FN1* and *VEGFA*-associated network as seen in the bronchial brushings together with enrichment of pathways involved in the ECM, collagen binding, cell adhesion and cell signalling.

In summary, the paper by VAN NIJNATTEN *et al.* [6] has identified a 219 gene severe COPD bronchial epithelial gene signature that is distinct from that for mild–moderate COPD. This supports their hypothesis that severe COPD is a distinct phenotype. Pathway analyses demonstrated that severe COPD-associated genes are mainly involved in immune response, developmental processes and ECM binding. Protein interaction networks indicate *VEGFA* and *FN1* as potential key drivers in severe COPD. Additionally, the gene signature that was lower in severe COPD bronchial brushes was also represented in matched nasal brushings as well as nasal samples from an independent severe COPD cohort. Of interest, the signature-related gene set that was present in both nasal cohorts was again centred around *VEGFA* and *FN1* (figure 1).

The reduced expression of the two hub genes *FN1* and *VEGFA* may indicate the loss of important repair processes in severe COPD. The reduced *VEGFA* expression shown by VAN NIJNATTEN *et al.* [6], together with the previously reported reduction of its receptor vascular endothelial growth factor receptor 2 (*VEGFR2*) in endothelial cells of COPD patients [9], is important in the formation of pulmonary capillaries and may result in the greater levels of emphysema seen in patients with severe disease. The effect of reduced *VEGFA* expression in the nose and bronchial epithelial cell subtypes of severe COPD patients requires further investigation.

FN1 is a glycoprotein expressed by many lung structural and immune cells such as fibroblasts, monocytes, and endothelial and smooth muscle cells during development but is poorly expressed in adult healthy lungs except when required for tissue repair [10]. Together, this suggests that severe COPD is associated with a defect in epithelial and endothelial cell repair mechanisms that result in the development of emphysema and small-airway remodelling. The reduction in ECM deposition and regulatory pathways also indicates a key role of the airway and potentially the lung matrix in severe COPD, which can affect structural cell function [11].

The study has several limitations, however, including the disparity in group sizes, which was partially mitigated by the use of bootstrapping, showing that 63% of the severe COPD bronchial epithelial cell DEGs could be replicated but ideally, future studies should be better matched for cohort group sizes. It is important to formally assess ICS dose in future studies as this will vary according to severity [1, 12] and according to sex [13] which was also significantly different in the study of VAN NIJNATTEN *et al* [6]. As a result, the use of a generic ICS-response signature may have removed important genes from the severe COPD signature.

It will be important to investigate whether the changes in gene expression reported here will track out to changes in protein expression and function. The demonstration that the down-regulated DEGs in bronchial brushings in severe COPD were also replicated in the nose indicate a noninvasive way of investigating whether protein expression of FN1 and VEGFA also alters with COPD severity. The study also highlights the importance of looking at both up- and down-regulated gene expression patterns. As a community, we often focus on genes and proteins that are increased in disease whereas the results here suggest that a loss of pathways may be more important in severe COPD.

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