



Scientific Letter

COPD Patients Display Increased Peripheral Blood Somatic Mutations Which Associate With the Prevalence of Co-morbidities



To the Director,

Chronic obstructive pulmonary disease (COPD) is a severe and often progressive lung disease. It is currently the third leading cause of death, with 3.23 million deaths worldwide in 2019, according to the World Health Organization. COPD is caused by a combination of genetic predisposition and the chronic inhalation of smoke or other toxic particles. Over the years many studies have identified several gene variants that increase an individual's susceptibility for COPD.¹ Although, germline mutations have been extensively studied in COPD, very few studies have investigated somatic mutations. Unlike germline genetic variations, somatic mutations are acquired after conception and may not be passed on to the next generation. The number of somatic mutations increases during aging and can be induced by exposure to carcinogens. As early as 2003 it was postulated that somatic mutations can be involved in the pathophysiology of COPD, by e.g. amplifying pro-inflammatory signaling and impairing host defense systems to pathogens or damage-associated molecular patterns.^{2,3} Recently, an association was found between specific somatic mutations in hematopoietic stem cells that lead to clonal hematopoiesis, so-called clonal hematopoiesis of indeterminate potential (CHIP), and the prevalence of COPD.⁴ Specifically, in individuals with CHIP a strong association with COPD was identified, where COPD was found to be the co-morbidity with the highest association of all age-related morbidities examined.⁵ Previous attempts to perform a genome-wide study on the role of somatic mutations in COPD were unsuccessful as the sequencing depth was insufficient to provide the resolution required to detect somatic mutations.⁶ In the current study we optimized a method to identify somatic mutations in exome data and to differentiate them from germline mutations. Using this approach, we identified genes that carried functionally relevant somatic mutations in white blood cells. By comparing COPD patients to healthy controls, we assessed the prevalence of these somatic mutations and their relation to changes in lung function, emphysema severity, and the prevalence of COPD co-morbidities.

Whole-exome sequencing was performed on DNA extracted from full peripheral blood samples of severe COPD patients ($n = 165$; mean age 55.9 ± 8.4 ($p = 0.3220$), 28% male ($p = 0.0078$), 36 ± 18 packyears ($p = 0.1049$) and healthy controls ($n = 33$; mean age 57.6 ± 11.5 , 52% male, 44 ± 49 packyears), using the DNBSEQ™ Sequencing Technology, with the BGI V4 kit (PE100, 100× coverage) (BGI Genomics, Shenzhen, China). Both COPD patients as

well as the controls were all ex-smokers, with at least 20 pack-years of smoking history and having stopped smoking for at least 12 months. The study was approved by the medical ethical committee of the University Medical Center Groningen, and all subjects provided written informed consent. Data on COPD co-morbidities were obtained from self-reported co-morbidity surveys. Whole-exome sequencing was performed using the Illumina platform, and mapped against GRCh38 primary genome reference. Genetic variants obtained from DNA-sequencing data were compared to the variants observed within the same population using the GATK Mutect2 tool (using Genome of the Netherlands study; GoNL as population reference). To assess functionally relevant mutations, only mutations predicted to induce a dysfunctional or truncated protein were included in our study. Somatic mutations were selected as alternative alleles that were supported by at least two reads and constituting <15% of total reads overlapping the variant site.

Out of the 813 genes carrying functionally relevant somatic mutations, 500 genes were more often mutated in COPD patients compared to healthy controls (Fig. 1A). In order to make a selection for further analyses, genes with the largest difference in prevalence of somatic mutations between COPD patients and controls were selected. The top-10 differentially prevalent mutated genes were between 6 and 10% more often mutated in COPD patients compared to healthy controls (Fig. 1B). Subsequently, we focused on identifying the functional effects of these 10 differentially prevalent mutated genes.

Within the COPD group it was found that the prevalence of mutations in Zinc Finger Protein 880 (*ZNF880*) was significantly associated with better lung function (percent predicted forced expiration in 1 second (FEV₁pp), Spearman Rho = 0.195, $p = 0.011$ and percent predicted forced vital capacity (FVCpp), Spearman Rho = 0.17, $p = 0.025$) and lower levels of emphysema (percentage of voxels with less than 950 Hounsfield units (%-950 HU), Spearman Rho = -0.2, $p = 0.01$), while a higher prevalence of mutation of *IGHGP* was associated with a higher FVCpp (Spearman Rho = 0.15, $p = 0.048$). However, no association was found between the prevalence of mutations of the top-10 differentially mutated genes in COPD patients, and neutrophil, lymphocyte and monocyte counts in blood (*data not shown*). Of note, bronchial gene expression data for *OR2T2* were unavailable.

Lastly, we assessed whether the prevalence of gene mutations of the top-10 differentially mutated genes was related to the prevalence of three major COPD co-morbidities: osteoporosis, hypertension and myocardial infarction.⁷ The prevalence of major histocompatibility complex, class I, A (*HLA-A*) and Galectin 9C (*LGALS9C*) gene mutations were increased in COPD patients with osteoporosis as co-morbidity, compared to COPD patients without

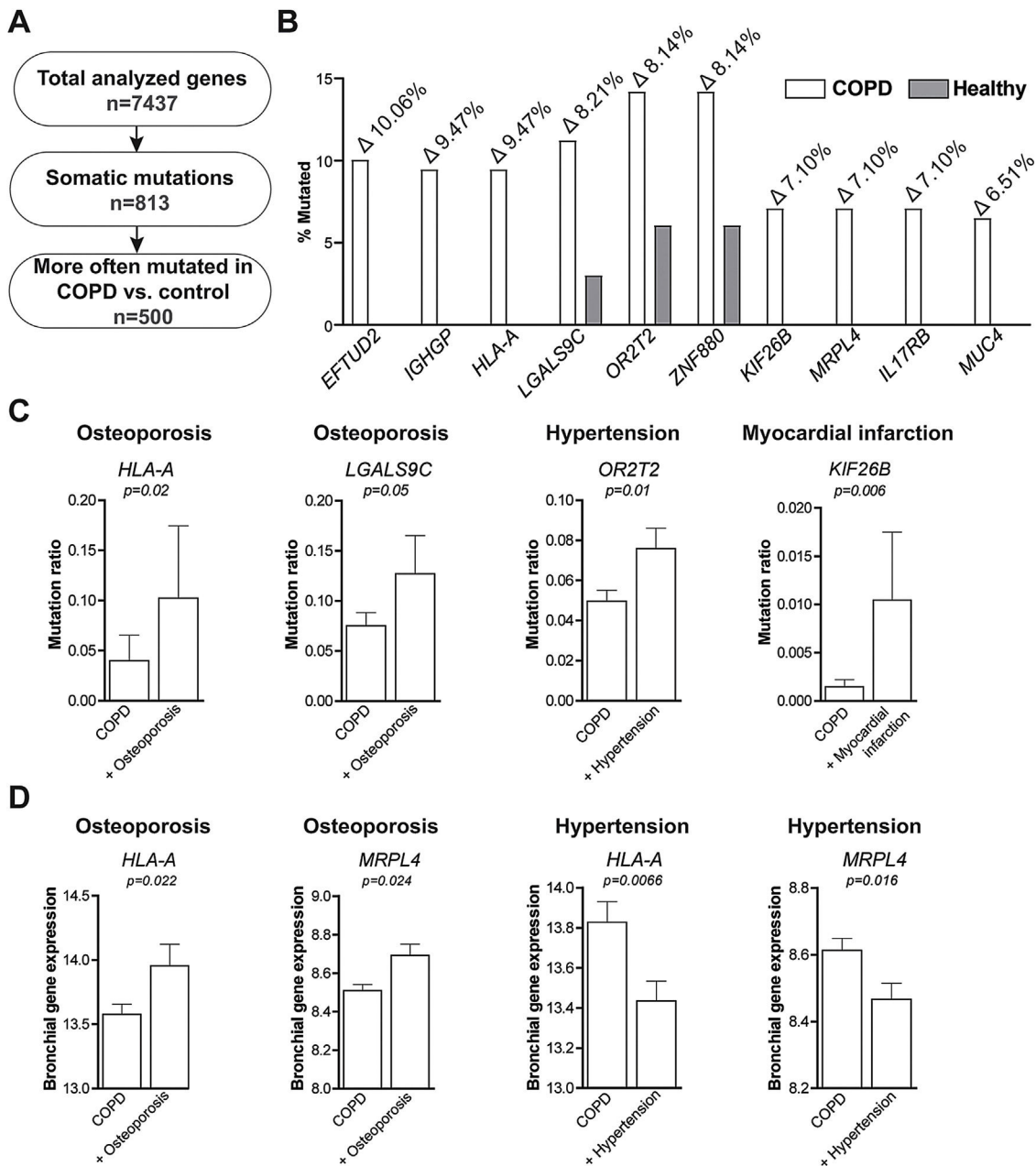


Fig. 1. Higher prevalence of somatic mutations in COPD patients compared to healthy controls and associations with COPD co-morbidities. (A) Flow chart of differentially prevalent mutated genes identified in our study. (B) The top-10 genes that displayed the highest differential prevalence of somatic mutations in COPD patients compared to healthy controls. The white bars represent the percentage of the COPD group that had a putative somatic mutation within the gene, the gray bars represent the percentage of mutations within the control group. The delta between percentage mutations in the COPD and control group is depicted above the white bars. (C) The prevalence of gene mutations of *HLA-A*, *LGALS9C*, *OR2T2* or *KIF26B* in COPD patients ($n = 165$) with or without osteoporosis ($n = 15$), hypertension ($n = 51$) or myocardial infarction ($n = 11$). (D) The gene expression levels of *HLA-A* and *MRPL4* in COPD patients ($n = 165$) with or without osteoporosis ($n = 15$) or hypertension ($n = 51$). The somatic mutation data was obtained from peripheral blood, while the gene expression data was obtained from bronchial biopsies. Statistical differences were tested using a Mann-Whitney U test, nominal p values are shown on top of the graph.

osteoporosis (Fig. 1C). Additionally, gene expression levels of *HLA-A* and mitochondrial ribosomal protein L4 (*MRPL4*) were increased in COPD patients with osteoporosis (Fig. 1D). The prevalence of kinesin family member 26B (*KIF26B*) gene mutations was increased in COPD patients with myocardial infarction and the prevalence of Olfactory Receptor Family 2 Subfamily T Member 2 (*OR2T2*) gene mutations was increased in COPD patients with hypertension (Fig. 1C). Additionally, the gene expression levels of *HLA-A* and *MRPL4* were lower in COPD patients with hypertension (Fig. 1D).

The genes that showed a higher prevalence of somatic mutations in individuals with COPD indicate the variety of cellular processes

involved in the disease. Zinc finger proteins are DNA-binding transcription factors that can regulate the expression of specific genes.⁸ Little is known about the genes affected by *ZNF880* specifically, nor its role in COPD. However, *ZNF880* has been described as a frequently-mutated oncogene of which the expression is increased in smokers. In the current study we showed that *ZNF880* is more often mutated in COPD patients but that the prevalence of *ZNF880* mutations is associated with higher lung function and reduced emphysema, suggesting a positive effect of *ZNF880* mutations. *MRPL4* is a nuclear gene that aids the protein synthesis within mitochondria, has been described as a prognostic gene for the outcome

of squamous cell carcinoma in COPD patients.⁹ The increased susceptibility of COPD patients to develop somatic mutations in this gene demonstrated in this study, could be a common consequence of smoke exposure but it may be worthwhile to explore whether this may represent a causal association with squamous cell carcinoma. Likewise, KIF26B has a functional role in the development of non-small cell lung cancer.¹⁰ We showed that the prevalence of *HLA-A* gene mutations was higher in COPD patients that also have osteoporosis compared to COPD patients without osteoporosis. This is in agreement with a previous study showing that *HLA-A* gene polymorphisms associate with increased susceptibility to develop osteoporosis.¹¹ Moreover, the number of males was higher in the control group compared to the COPD group, potentially influencing our results. In summary, COPD patients harbor more putative somatic mutations in their peripheral blood cells. The prevalence of gene mutations of several genes displayed a weak but significant association with improved lung function. Additionally, we identified that specific genes were more often mutated in COPD patients with a co-morbidity which may be an indication that the increased number of somatic mutations contributes to the development of co-morbidities in COPD patients. However, future studies should be aimed at identifying the role of somatic mutations in the development of COPD co-morbidities.

Ethical Approval

The study was approved by the medical ethical committee of the University Medical Center Groningen, and all subjects provided written informed consent. Severe COPD patients are derived from ClinicalTrials.gov Identifier: NCT04263961 and healthy controls are derived from ClinicalTrials.gov Identifier: NCT00848406. Data from these study cohorts can be accessed through collaboration by contacting Maarten van den Berge (m.van.den.berge@umcg.nl).

Authors' Contributions

Concept and design: AF, VRW, JBS, SDP. *Data analysis:* AF, VG, SDP. *Patient inclusion/data collection:* CAB, WT, MvdB, DJS. *Project supervision:* JKB, DJS, SDP. *Manuscript preparation:* VRW, SDP. *Manuscript revision:* AF, VRW, JBS, CAB, WT, JKB, MvdB, DJS, VG, SDP.

Conflict of Interest

None declared.

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