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Assessing pleiotropy and mediation in genetic loci associated with chronic obstructive pulmonary disease

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Abstract

Genetic association studies have increasingly recognized variant effects on multiple phenotypes. Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease with environmental and genetic causes. Multiple genetic variants have been associated with COPD, many of which show significant associations to additional phenotypes. However, it is unknown if these associations represent biological pleiotropy or if they exist through correlation of related phenotypes ("mediated pleiotropy"). Using 6,670 subjects from the COPDGene study, we describe the association of known COPD susceptibility loci with other COPD-related phenotypes and distinguish if these act directly on the phenotypes (i.e. biological pleiotropy) or if association is

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AUTHOR CONTRIBUTIONS

MMP, SML and MHC participated in study design, analysis of data, interpretation of results, manuscript drafting, and manuscript editing for intellectual content. BDH, MLM and RB participated in analysis of data and manuscript editing for intellectual content. EKS, THB, PJC, JEH participated in study design, interpretation of analysis results, and manuscript editing for intellectual content.

CONFLICTS OF INTEREST

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due to correlation (i.e. mediated pleiotropy). We identified additional associated phenotypes for 13 of 25 known COPD loci. Tests for pleiotropy between genotype and associated outcomes were significant for all loci. In cases of significant pleiotropy, we performed mediation analysis to test if SNPs had a direct association to phenotype. Most loci showed a mediated effect through the hypothesized causal pathway. However, many loci also had direct associations, suggesting causal explanations (i.e. emphysema leading to reduced lung function) are incomplete. Our results highlight the high degree of pleiotropy in complex disease-associated loci and provide novel insights into the mechanisms underlying COPD.

Keywords

Pleiotropy; Mediation; COPD; Emphysema; Causal Inference; GWAS

BACKGROUND

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of respiratory mortality worldwide (Soriano et al., 2017). COPD is diagnosed by a single test of post-bronchodilator airflow limitation (measured by spirometry), but the disease is highly heterogeneous. Clinicians have noted for decades that clinical manifestations of COPD including emphysema, chronic bronchitis, exercise capacity, and hypoxemia vary widely among patients with similar spirometric measurements (Burrows B, Fletcher CM, Heard BE, Jones NL, 1966). More recently, analysis of computed tomography (CT) images has led to the identification of measures, such as quantitative emphysema, airway wall thickening, and functional small airways disease, that are associated with COPD outcomes independent of spirometry (Galbán et al., 2012; Han et al., 2011; Nakano et al., 2005). Importantly, many of these CT measurements reflect physiological processes which lead to the phenomenon of airflow limitation (Hogg, 2004).

COPD is caused by both environmental (predominantly cigarette smoking) and genetic factors. The heritability of COPD case/control status has been estimated to be between 35–60% (Ingebrigtsen et al., 2010; Zhou et al., 2015), and evidence suggests that the components of COPD (i.e. emphysema and airway disease) may be independently heritable (Patel et al., 2008). To assess the genetic component of COPD, large genome-wide association studies (GWAS) studies have been performed, identifying more than 20 loci associated with COPD case/control status (Cho et al., 2014; Hobbs et al., 2017). Many of these loci also show significant associations with other COPD-related phenotypes (e.g. cigarette smoking (Consortium, 2010; Thorgeirsson et al., 2010), emphysema (Cho et al., 2015a)), a phenomenon known as genetic pleiotropy. There are multiple types of pleiotropy (Paaby & Rockman, 2013; Solovieff, Cotsapas, Lee, Purcell, & Smoller, 2013) and different types have different implications for the biological mechanism underlying disease. As defined in Solovieff et al (Solovieff et al., 2013), “biological pleiotropy” refers to a single locus that has independent effects on multiple phenotypes (i.e. locus $\rightarrow$ phenotype 1 and locus $\rightarrow$ phenotype 2). Alternatively, “mediated pleiotropy” refers to locus that has an effect on a phenotype only through its correlation to another phenotype (i.e. locus is associated with phenotype 2 because the causal mechanism is locus $\rightarrow$ phenotype 1 $\rightarrow$ phenotype 2).

Although biologically interesting, mediated pleiotropy implies that the locus-to-outcome association is only observed because it is preceded by a true association on the causal pathway, and therefore only biological pleiotropy is considered “genuine” pleiotropy.

The aims of this manuscript are two-fold: 1) describe the association of known COPD loci with other COPD- related phenotypes; and 2) distinguish if these loci act directly on a set of COPD-related traits (i.e. biological pleiotropy) or if the association is predominately due to correlation (i.e. mediated pleiotropy), with particular attention to the hypothesized causal pathways of emphysema and airway measurements on lung function (Burrows B, Fletcher CM, Heard BE, Jones NL, 1966; Galbán et al., 2012; Patel et al., 2008). To achieve this, we used 6,670 extensively phenotyped Non-Hispanic White subjects of the COPDGene study², a study of smokers with and without COPD.

METHODS

Study Design

The complete study protocol for COPDGene had been described in detail elsewhere (Cho et al., 2010; Regan et al., 2011), but briefly, we enrolled 10,192 self-identified Non-Hispanic Whites and African Americans between the ages of 45 and 80 years with a minimum of 10 pack-years lifetime smoking history. Participants were extensively phenotyped, including pre- and post- bronchodilator spirometry and computed tomography (CT) of the chest. The known-COPD loci we tested were originally identified in European-derived populations, and therefore, this analysis includes only Non-Hispanic White participants of COPDGene (n=6,670). Their clinical characteristics are presented in Table 1.

Genetic loci for assessment

All study participants were genotyped using the Illumina OmniExpress BeadChip imputed versus the 1000 Genomes phase 1 version 3 reference panel (Siva, 2008). We focused on loci that have been previously identified in large GWAS of COPD or emphysema as genome-wide significant ($P < 5 \times 10^{-8}$), including 22 COPD loci identified from a recent case-control analysis (Hobbs et al., 2017), rs626750 in *DLC1* (Cho et al., 2015a) (associated with emphysema), and rs75200691 in *MMP12* (Cho et al., 2014) (associated with severe COPD) and rs28929474 in *SERPINA1* (D L DeMeo, 2004; Foreman et al., 2017) (a known Mendelian locus for COPD). All loci had <1% missing values, were in Hardy-Weinberg equilibrium in control subjects ($P > 1 \times 10^{-6}$) and had a population frequency > 1% in Non-Hispanic Whites.

The primary confounder of single nucleotide polymorphism (SNP) to phenotype associations is genetic ancestry. To control for this, we estimated principal components (PCs) that summarize genetic ancestry to include as covariates in all analyses. PCs were estimated in the Eigenstrat program (AL Price, NJ Patterson, RM Plenge, ME Weinblatt, & NA Shadick, 2006; Alkes L Price, Zaitlen, Reich, & Patterson, 2010), using 32,033 uncorrelated SNPs with minor allele frequency > 0.05 as input, as previously described (Cho et al., 2014).

Phenotypes for assessment

Phenotypes collected in COPDGene include measures of spirometry, quantitative imaging, biomarkers, and questionnaire data. From these, we selected a subset to analyze based on: 1) minimal missingness (<10%); 2) strength of association with known COPD loci (in the top 5 associated phenotypes for at least one of the 25 SNPs); and 3) Pearson correlation < 0.7 with any other phenotype (calculated after transformations for non-normality). This resulted in 11 phenotypes for analysis, representing a broad range of clinically important metrics of COPD. These include: 1) the forced expiratory volume in one second over the forced vital capacity (FEV₁/FVC); 2) emphysema (measured as the percentage of low attenuation area less than -950 Hounsfield Units (HU)); 3) average cigarettes smoked per day; 4) the St. George's respiratory questionnaire score (SGRQ) that summarizes health-related quality of life; 5) bronchodilator responsiveness (measured as the absolute difference in FEV₁ before versus after bronchodilation); 6) body mass index (BMI); 7) the ratio of emphysema at -950 H (HU) in the upper third versus lower third of the lung; 8) total lung capacity as assessed on inspiratory chest CT; 9) chronic bronchitis (based on chronic cough and phlegm for at least three months per year for at least two years); 10) standardized airway wall thickness for an airway of size 10mm internal perimeter (pi10) and; 11) standardized airway wall thickness for an airway of size 15 mm internal perimeter (pi15). A detailed list of the 11 analyzed phenotypes and how they were collected is presented in Table 1.

Statistical analysis

We tested each of the 25 SNPs for association with each of the 11 phenotypes using linear or logistic regression. All outcomes were adjusted for age, sex, and principal components of genetic ancestry. Spirometry (FEV₁/FVC), imaging (emphysema, Pi10, Pi15, emphysema ratio, and total lung capacity), and symptom-related phenotypes (chronic bronchitis, SGRQ and bronchodilator responsiveness) were also adjusted for pack-years of smoking; Imaging, spirometry, and chronic bronchitis were also adjusted for current smoking status; bronchodilator response was also adjusted for baseline FEV₁; and imaging phenotypes were also adjusted for scanner type. Non-normally distributed phenotypes were either log transformed (BMI, emphysema) or inverse normal transformed (cigarettes smoked per day, emphysema ratio). We defined significant associations using a Bonferroni corrected P of 0.002 (0.05/25).

Tests for Pleiotropy

SNPs with greater than 1 associated phenotype were tested for pleiotropy via the R package pleiotropy (Lutz, Fingerlin, Hokanson, & Lange, 2016), which formally tests whether multiple phenotypes are associated with a single locus of interest using a permutation-based framework. For each SNP associated with multiple outcomes, we test for pleiotropy using the "pleiotropySNP" function with 10,000 permutations and included covariates defined in Supplemental Table 1. The method permutes the 2 (or more) phenotypes of interest and compares observed P -values of association to a set of permuted P -values. We used the "cutoff" approach to evaluate this comparison, as defined in Lutz et al. (Lutz et al., 2016). A multiple-comparison corrected P -value of $P < 0.0038$ was considered significant pleiotropy.

Test for Mediation

The pleiotropy method tests whether a SNP is associated with multiple outcomes. However, it cannot distinguish between biological and mediated pleiotropy, and significant results may be due to correlation between phenotypes. Therefore, we performed mediation analysis using the causal inference approach to mediation analysis (Pearl, 2001; Vanderweele & Vansteelandt, 2009), which allows us to assess if a SNP has a direct effect on the outcome of interest or if the SNP only acts on the phenotype of interest through a secondary phenotype (i.e. a mediated effect). Differentiating between these two scenarios is necessary to understand the biological mechanism through which a locus affects multiple phenotypes.

We used the R package Mediation (Imai, Keele, & Tingley, 2010), which uses a three-step procedure to estimate the average causal mediation effects (ACME) and the average causal direct effect (ADE). The first step is to estimate the distribution of the mediator for each genotype, using a model of the mediator as a function of genotype (e.g. $\text{emphysema} \sim \text{genotype} + \text{PC1} + \text{PC2} + \text{PC3} + \text{PC4} + \text{PC5}$). The next step is to estimate the phenotype's potential outcomes for each of the genotype and mediator values. This is achieved by fitting a model of the phenotype as a function of the genotype, mediator and covariates (e.g. $\text{FEV}_1/\text{FVC} \sim \text{emphysema} + \text{genotype} + \text{covariates}$). The final step combines the fitted models with the "mediate()" function, providing estimates and p-values of direct and indirect effects of genotype on outcome. All outcomes and mediators in our analysis were continuous measurements, so linear regressions were used to estimate inputs to the mediate function. The first five PCs summarizing genetic ancestry were included as covariates of the SNP to mediator relationship, as we expect the primary confounder of this relationship to be genetic ancestry. Confounders defined in Supplemental Table 1 were included as covariates of the mediator to outcome relationships.

Mediation analysis requires that the causal model (i.e. the temporal sequence of phenotypes) is pre-specified. We based causal models on current knowledge of COPD pathophysiology in order to determine the mediator and the outcome in each mediation analysis. In cases where associations included both emphysema and FEV_1/FVC , emphysema was assumed to be the mediator and FEV_1/FVC was assumed to be the outcome (Burrows B, Fletcher CM, Heard BE, Jones NL, 1966; Galbán et al., 2012). In cases where cigarette smoking was associated with a SNP, it was assumed to be the mediator when using other outcomes (e.g. FEV_1/FVC) (Consortium, 2010; Thorgeirsson et al., 2010). In the case where a SNP was associated with BMI and emphysema, emphysema was assumed to be the mediator and BMI was assumed to be the outcome (Coxson et al., 2004). In all other cases FEV_1/FVC was assumed to be the mediator for outcomes.

RESULTS

We analyzed 11 clinically important COPD phenotypes for their association with 25 genetic loci known to affect disease susceptibility. To distinguish independent signals, we chose to assess phenotypes that were not highly correlated (Pearson correlation < 0.7) (Supplementary Figure 1). Of the 25 previously identified COPD loci, 13 were significantly associated with more than one phenotype at $P < 0.002$ in our dataset (Figure 1, Table 2). The outcome associated with the greatest number of known COPD susceptibility loci was FEV_1/FVC .

FVC, followed by emphysema and cigarette smoking (Supplementary Figure 2). Additional associations that did not reach significance across multiple phenotypes are presented in Supplementary Table 2, including an association of the *SERPINA1* z-allele variant (rs28929474) and emphysema.

Tests of Pleiotropy

We tested for pleiotropy in the 13 loci associated with more than one phenotype via the R package pleiotropy (Lutz et al., 2016), and all tests were significant at $P < 0.0038$ (0.05/13 tests) (Table 2). Many of the loci associated with multiple phenotypes were associated with both FEV₁/FVC and emphysema (*TGFB2*, *RARB*, *TET2*, *HHIP*, *DSP*, *AGER*, *RIN3*, and *CHRNA5*). In all cases, the direction of effect across phenotypes was consistent with expectations (i.e. loci associated with a decrease in FEV₁/FVC were also associated with an increase in emphysema). We also found loci associated with both SGRQ scores and FEV₁/FVC (*HHIP*, *FAM13A*, *MMP12*), and loci associated with both total lung capacity and emphysema (*AGER*, *CYP2A6*). We identified 2 loci where one of the identified phenotypes was cigarette smoking (*CHRNA5*, *CYP2A6*), both of which have been previously described as smoking-related loci (Bierut, 2009; Consortium, 2010). Lastly, we found an emphysema-associated locus near *DLC1* was also associated with BMI.

Mediation Analyses

To distinguish biological pleiotropy from mediated pleiotropy, we performed mediation analysis (Salinas, Wang, & DeWan, 2017). An example causal diagram used to conceptualize the COPD disease process is presented in Figure 2. All loci showed a mediated effect through the hypothesized causal pathways consistent with mediated pleiotropy, with one exception, a locus previously associated with cigarette smoking, *CYP2A6* (Table 3). This locus's effect on total lung capacity was not significantly mediated through cigarettes per day ($P = 0.1$). However, there was a significant mediated effect of this locus (*CYP2A6*) through smoking of on the related trait of emphysema ($P < 0.0001$). Both *CYP2A6* and the other smoking-associated locus, *CHRNA5*, showed evidence of direct effects on COPD-related outcomes (i.e. not entirely mediated through cigarette smoking); Assuming no unmeasured confounding, these findings suggest these genetic variants also contribute to disease independent of smoking.

Additionally, mediation analysis revealed the *AGER* locus had both a mediated and direct effect on total lung capacity ($P < 0.0001$ and $P = 0.004$, respectively), suggesting this SNP influences both phenotypes (emphysema and total lung capacity). As expected, the association of *FAM13A* and *MMP12* with St. Georges Respiratory Questionnaire score was mediated though FEV₁/FVC without a direct effect ($P = 0.33$ and $P = 0.13$, respectively). Lastly, the association of *DLC1* with emphysema was mediated through BMI but the locus also had a direct effect on emphysema ($P = 0.001$).

Unsurprisingly, many of the loci ($n=8$) showed associations with both FEV₁/FVC and emphysema, two of the most important components of COPD. Although there are strong correlations clinically between these phenotypes (Thomsen et al., 2015), it remains unclear what genetic mechanisms underlie these relationships. We found variable evidence of direct

effects at these loci. The *TGFB2*, *HHIP* and *AGER* loci showed a significant mediated effect on FEV₁/FVC through emphysema, however there was not a significant direct effect, suggesting that SNPs affect spirometric ratio only through their association with emphysema. In contrast, we found evidence for both direct and mediated effects for *RARB*, *TET2*, *DSP*, *RIN3*, and *CHRNA5* on FEV₁/FVC.

Parametric response mapping (PRM) phenotypes

To further assess the relationship of genotypes to the pathophysiologic processes that result in airflow limitation (emphysema and small airways disease), we incorporated a recently developed measurement, parametric response mapping (PRM). This measurement matches inspiratory and expiratory lung scans to produce measurements of both small airway disease and emphysema (Galbán et al., 2012). These more specific measurements strongly associate with COPD severity (Pompe et al., 2015, 2017) and are hypothesized to better capture components of disease pathophysiology. As such, we performed mediation analysis using these measurements as mediators on the 8 loci associated with both FEV₁/FVC and emphysema. Causal models used either PRM defined emphysema (PRMemph) or PRM defined airways disease (PRMair) as the mediator and FEV₁/FVC as the outcome.

In most cases, the results of this analysis agreed with the conclusions of the mediation analyses using FEV₁/FVC and the standard measure of emphysema (i.e. for the *TGFB2* and *AGER* loci, there is no direct effect of the SNP on FEV₁/FVC and for the *RARB*, *DSP*, and *RIN3* loci there is both a direct and mediated effect of SNP on FEV₁/FVC) (Supplemental Table 3). However, for the *TET2* locus, our data support the opposite conclusion (no direct effect on FEV₁/FVC). Finally, for the *HHIP* and *CHRNA5* loci conclusions about direct effects on FEV₁/FVC depend on the mediators chosen (either PRMemph or PRMair). For example, all of the association of *HHIP* on FEV₁/FVC is through PRMemph when it is included as a mediator, however this is not true when PRMair is included in the model instead. This suggests that the PRM measurements may capture different aspects of the disease than either emphysema or FEV₁/FVC alone.

Sensitivity Analyses

There are several assumptions to mediation analysis. First, one must account for a SNP-by-mediator interaction on the outcome of interest (should it exist). We considered the possibility of a SNP-by-mediator interaction in all tested models by including an interaction term in the mediation analysis. However, we found no evidence of a significant interaction using this method (Supplemental Figure 3). Additionally, we used the Imai method (Imai, Keele, & Tingley, 2010) to assess how sensitive our results were to unmeasured confounding of the mediator-to-outcome relationship. We did not find any evidence that results were unduly influenced by unmeasured confounding (Supplemental Figure 4). Lastly, we used the Umediation package (Lutz et al., 2017) to test how sensitive our results were to unmeasured confounding of the SNP-mediator relationship and the SNP-outcome relationship. We found limited evidence that an unknown confounder affected our results (Supplemental Figure 5).

DISCUSSION

Large genome-wide association studies have established multiple loci associated with COPD (Hobbs et al., 2017) and related phenotypes. However, a comprehensive assessment of pleiotropy and causal modeling of the relationships between genetic variants and these phenotypes has not been adequately described. Identifying these relationships may give insights into the biological mechanisms of disease and the utility of specific phenotypes in COPD as mediators of disease outcome. Using the well-phenotyped COPD Gene study population, we identified multiple additional associated phenotypes for 13 known loci, all of which showed significance in a formal test of pleiotropy. To distinguish between biological pleiotropy and mediated pleiotropy, we performed mediation analysis. Nearly all loci showed a mediated effect through the hypothesized causal pathway, consistent with mediated pleiotropy. However, many (although not all) loci also had direct associations, indicating that causal explanations using existing phenotypes (or endophenotypes) are incomplete.

Cigarette smoking is the most important environmental risk factor for COPD. While the association of the 15q25.1 locus (which includes genes coding for nicotinic acetylcholine receptors) with nicotine dependence is indisputable, evidence for its independent association with airflow obstruction is variable. Our results suggest that genotype at rs17486278 in *CHRNA5* has an independent effect on FEV₁/FVC that is not completely mediated through cigarette smoking ($p < 1 \times 10^{-5}$). There are 2 possible explanations for this finding: 1) the smoking measurement used (average cigarettes smoked per day) does not accurately capture the pathological effects of smoking and the observed association of rs17486278 to FEV₁/FVC is a result of residual confounding; or 2) independent of smoking, genotype at rs17486278 affects airflow limitation. Previous studies have presented contradictory evidence of these explanations (Obeidat et al., 2018; Siedlinski et al., 2013; Vanderweele et al., 2012; Wilk et al., 2012; Zhang et al., 2017). While there is evidence of association between *CHRNA5* and lung function in never-smokers (Wilk et al., 2012), suggesting an independent effect on lung function, recent analyses have found no association of this region with lung function or lung cancer in never-smokers (Obeidat et al., 2018; Wang, Broderick, Matakidou, Eisen, & Houlston, 2011). Likewise, previous mediation analyses in this region have found both a direct effect (Siedlinski et al., 2013) and no direct effect (Vanderweele et al., 2012) of *CHRNA5* genotype on airflow obstruction. It remains unclear if this locus acts on COPD in addition to the effects of smoking.

While whether or not there is a direct effect of genetic variants in the 15q25 locus is still controversial, there is some biological plausibility to the independent association of this locus. In the GTEx project, rs17486278 is a lung eQTL for both *CHRNA5* and *RP11-650212.2*. *CHRNA5* is expressed in lung tissue, airway smooth muscle, and bronchial epithelial cells (Wilk et al., 2000). Additionally, a *CHRNA5* variant was found to be associated with bronchial hyper-responsiveness in children (Torjussen et al., 2012). A previous study that silenced *CHRNA5* in bronchial epithelial cells found reduced expression of adhesion molecules and increased cell motility (Krais et al., 2011) that may influence the repair and remodeling processes that are important to COPD development. 15q25 also harbors several other genes, including *PSMA4* (Sakornsakolpat et al., 2018) and *IREB2*.

DeMeo et al found differential expression of *IREB2* mRNA and protein in lung tissue samples from COPD cases as compared to healthy controls (DeMeo et al., 2009), and *IREB2* plays a key role in iron homeostasis and COPD (Cloonan et al., 2016), suggesting it may be the underlying COPD susceptibility gene.

Our results also highlight the important relationship between lung function and imaging measurements, particularly emphysema. Lung parenchymal destruction leads to reduced lung elastic recoil and airflow limitation (Burrows B, Fletcher CM, Heard BE, Jones NL, 1966). Because the pathophysiology of emphysema is a specific disease process, emphysema might be expected to act as an endophenotype. We found variable evidence of this conclusion. A few loci appeared to be associated exclusively with emphysema and not with lung function (e.g. *DLC1* and *SERPINA1*). By contrast, other loci had effects that were entirely mediated through measured emphysema (e.g. *TGFB2*, *HHIP* and *AGER*). Lastly, there were loci that showed evidence of both a mediated effect through emphysema and a direct effect of genotype on airflow limitation. This suggests that different loci could act through different biological mechanisms, and highlights the need for better phenotypes and biomarkers in COPD to dissect causal pathways.

We used mediation analysis to help elucidate the biological mechanism through which a SNP affects multiple phenotypes. This method relies on important assumptions, including that the causal pathway is correctly specified, that there is no unmeasured confounding, and that the mediator is accurately and precisely measured. We specified causal models *a priori* based on pathophysiological knowledge of COPD. We believe we accounted for known confounders, however it is possible that results are due to an unknown or unmeasured confounder. To assess the robustness of our results to this possibility, we performed 2 sensitivity analyses to test these assumptions (Imai, Keele, & Yamamoto, 2010; Lutz et al., 2017, Supplement). Based on these analyses, we do not believe unmeasured confounders unduly influence our results. However, strong unmeasured confounding could have significant effect, particularly on our estimates of causal mediated effects. Additionally, this manuscript focuses on the effect of a genetic locus on an outcome through a single mediator. We acknowledge the possibility of multiple mediators given that COPD is a complex disease with multiple underlying mechanisms. Specifically, at the *CHRNA5* and *CYP2A* loci, genotype may act through both smoking and emphysema on outcome (FEV₁/FVC and total lung capacity, respectively). Future work should test more complex causal models to better understand the biological mechanisms underlying COPD.

We chose the COPDGene study sample for its extensive phenotyping, collected using standardized protocols, including imaging measurements that are hypothesized to better assess disease processes. However, there are a few limitations of this dataset. Smoking behaviors (including the average number of cigarettes smoked per day measurement used in our analysis) are based on self-report. Participants may not have reported changes in smoking behaviors over their lifetime, potentially resulting in residual confounding that could bias reported results. Moreover, available measures of smoking behaviors (cigarettes smoked per day and current smoking status) may not completely capture the toxic effects of tobacco inhalation and recent work suggests objective biomarkers of smoking (e.g. exhaled carbon monoxide or cotinine) can more accurately capture smoking behavior (Obeidat et al.,

2018). These measures were not available in the COPDGene study. Additionally, because the COPDGene cohort was ascertained based on case/control status, analyzing secondary quantitative phenotypes can be biased due to this ascertainment condition. Associations with these secondary phenotypes and genetic regions of interest have been identified in previous studies where secondary analyses correcting for ascertainment was applied (Cho et al., 2015b). However, these methods were not applied in our data. And lastly, the reduction in sample size as compared to previously published large GWASs (Hobbs et al., 2017) affected our ability to detect possibly significant phenotype associations and pleiotropy.

We wish to distinguish our mediation analysis from the Mendelian randomization method (Smith et al., 2005; Smith & Ebrahim, 2005), which aims to establish causality of an exposure on an outcome through instrumental variable analysis (e.g. where SNP is the instrument for the exposure of interest). While the methods have similar experimental designs and causal models, mediation analysis tests the mechanism by which an exposure is related to an outcome (direct relationship and/or indirect relationship) via the inclusion of a mediator variable. Unlike Mendelian randomization, we do not assume that all of the effect of the exposure (i.e. the SNP) is through the mediator, and we do not assume the exposure cannot be pleiotropic. In fact, the latter is exactly the hypothesis we wish to test.

We did not find substantial evidence for pleiotropy for non-respiratory related phenotypes in COPDGene. However, larger studies have found association with other phenotypes (such as pulmonary fibrosis, blood cell counts, and anthropomorphic measures). The smaller sample size as compared to previously published large GWASs (Hobbs et al., 2017) may have affected our ability to detect possibly significant phenotype associations and pleiotropy. Thus, pleiotropy and mediation by other phenotypes may require further study.

Identifying the genetic causes of COPD and its related phenotypes is essential to the understanding of disease etiology and to the identification of disease subtypes. Our work provides a systematic assessment of pleiotropy and mediation in a complex disease, one of the most common diseases and causes of death in the U.S. and worldwide, and novel insights into the biological mechanisms underlying COPD. We have identified genetic loci affecting multiple COPD-related phenotypes and used causal inference to show that 1) there is a high degree of pleiotropy in COPD phenotypes, and 2) while most of these cases appear to be results of mediated pleiotropy, in many cases, we find evidence of direct effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Association of 25 COPD-related SNPs with 11 tested phenotypes. Association was assessed using linear or logistic regression controlling for age, sex, 5 principal components of genetic ancestry, and additional relevant covariates (delineated in the Methods section).

Figure 2.

Example directed acyclic graph (DAG) used for mediation analysis. Genetic ancestry was summarized by the first 5 principal components.

Table 1.

Complete list of 11 tested phenotypes and covariates, study population mean and standard deviation and measurement method.

Phenotypes	N	Mean (SD)	Description
Age	6670	62.09 (8.84)	
Sex (% Male)	6670	52.40%	
Pack-years of lifetime smoking	6670	47.31 (26.02)	1 pack-year = 1 pack of cigarettes smoked daily for 1 year
Current smokers	6670	39.20%	
FEV ₁	6651	2.21 (0.95)	Lung function: Forced expiratory volume in 1 second
FEV ₁ /FVC	6651	0.64 (0.17)	Lung function: FEV ₁ / Forced vital capacity
Emphysema	6298	0.97 (1.60)	Quantitative emphysema, using log of -950 Hounsfield Units
Cigarettes per day	6670	25.84 (11.44)	Cigarettes smoked per day, averaged over lifetime
SGRQ	6669	26.59 (22.65)	Symptoms: St George's Respiratory Questionnaire
BDR (% FEV ₁ change)	6590	6.32 (9.50)	Pre/Post-bronchodilator FEV ₁ % change
BMI	6670	28.68 (6.06)	Body Mass Index
Emphysema ratio	6192	1.81 (7.5)	Ratio of emphysema in upper third to lower third from inspiratory chest CT
Total lung capacity (%P)	6304	98.09 (16.22)	Total lung capacity, percent of predicted value
Chronic Bronchitis	6670	20.94%	Chronic cough and phlegm for at least three months per year for at least two years
Pi10	6163	3.67 (0.14)	Airway thickness: square root of wall area for bronchus with 10mm lumen perimeter
Pi15	6163	5.14 (0.19)	Airway thickness: square root of wall area for bronchus with 15mm lumen perimeter

FEV₁ = forced expiratory volume in 1 second, FVC = forced vital capacity, SGRQ= St. George's Respiratory Questionnaire, BMI = body mass index, BDR = bronchodilator response, Pi10, Pi15 = standardized airway wall thickness (square root of wall area) for an airway of size 10mm and 15mm, CT= computed tomography, %P = percent predicted.

Table 2.

COPD loci associated with more than one phenotype at $P < 0.002$ ($n=13$) in linear or logistic regression. All tests for pleiotropy of SNP and associated outcomes were significant ($P < 0.00038$).

SNP	Chr	Gene	Phenotypes associated at $p < 0.002$ (effect direction)	Pleiotropy P-value
rs10429950	1	<i>TGFB2</i>	FEV ₁ /FVC (–), emphysema (+)	0.0013
rs1529672	3	<i>RARB</i>	FEV ₁ /FVC (+), emphysema (–)	0.0007
rs2047409	4	<i>TET2</i>	FEV ₁ /FVC (+), emphysema (–)	0.0009
rs6837671	4	<i>FAM13A</i>	FEV ₁ /FVC (+), SGRQ (–)	0.0008
rs13141641	4	<i>HHIP</i>	FEV ₁ /FVC (–), emphysema (+), emphysema ratio (+), SGRQ (+)	0.0001
rs2076295	6	<i>DSP</i>	FEV ₁ /FVC (+), emphysema (–)	0.0004
rs2070600	6	<i>AGER</i>	FEV ₁ /FVC (+), emphysema (–), total lung capacity (–)	<0.0001
rs75200691	8	<i>DLC1</i>	emphysema (–), BMI (+)	0.0004
rs626750	11	<i>MMP12</i>	FEV ₁ /FVC (–), SGRQ (+)	0.0004
rs754388	14	<i>RIN3</i>	FEV ₁ /FVC (–), emphysema (+), emphysema ratio (+)	0.0001
rs1441358	15	<i>THSD4</i>	FEV ₁ /FVC (+), emphysema ratio (–)	0.0024
rs17486278	15	<i>CHRNA5</i>	FEV ₁ /FVC (+), emphysema (–), emphysema ratio (–), cigarettes per day (–)	<0.0001
rs12459249	19	<i>CYP2A6</i>	emphysema (–), total lung capacity (–), cigarettes per day (–)	0.0001

Mediated and direct effects estimated from mediation analysis for loci associated with great than 1 phenotype. Causal models used in mediation analysis were pre-specified based on current knowledge of COPD pathophysiology. The first five PCs summarizing genetic ancestry were included as confounders of the SNP to mediator relationship. Confounders defined in Supplemental Table 1 were included as covariates of the mediator to outcome relationships. Estimates that are significant after correction for multiple comparisons ($P < 0.00227$) are indicated by an asterisk(*).

Table 3.

SNP	Gene	Mediator	Outcome	Direct effect	Direct effect P	Mediated effect	Mediated effect P
rs10429950	<i>TGFB2</i>	emphysema	FEV ₁ /FVC	-0.0030	0.1703	-0.0098	0.0002*
rs1529672	<i>RARB</i>	emphysema	FEV ₁ /FVC	0.0067	0.0091	0.0106	0.0003*
rs2047409	<i>TET2</i>	emphysema	FEV ₁ /FVC	0.0045	0.0242	0.0087	0.0001*
rs6837671	<i>FAM13A</i>	FEV ₁ /FVC	SGRQ	-0.2840	0.3847	-0.9940	<1×10 ⁻⁵ *
rs13141641	<i>HHIP</i>	emphysema	FEV ₁ /FVC	-0.0032	0.1100	-0.0122	<1×10 ⁻⁵ *
rs13141641	<i>HHIP</i>	FEV ₁ /FVC	SGRQ	0.0507	0.0130	0.0993	<1×10 ⁻⁵ *
rs13141641	<i>HHIP</i>	emphysema ratio	FEV ₁ /FVC	-0.0087	0.0011*	-0.0064	<1×10 ⁻⁵ *
rs2076295	<i>DSP</i>	emphysema	FEV ₁ /FVC	0.0067	0.0020*	0.0073	0.0020*
rs2070600	<i>AGER</i>	emphysema	FEV ₁ /FVC	0.0039	0.4100	0.0312	<1×10 ⁻⁵ *
rs2070600	<i>AGER</i>	FEV ₁ /FVC	total lung capacity	-1.7390	0.0049	-1.9900	<1×10 ⁻⁵ *
rs75200691	<i>DLC1</i>	emphysema	BMI	0.0057	0.0160	0.0032	0.0004*
rs626750	<i>MMP12</i>	FEV ₁ /FVC	SGRQ	0.6600	0.1100	1.2390	<1×10 ⁻⁵ *
rs754388	<i>RIN3</i>	emphysema	FEV ₁ /FVC	-0.0058	0.0220	-0.0101	0.0020*
rs754388	<i>RIN3</i>	emphysema ratio	FEV ₁ /FVC	-0.0122	0.0028	-0.0036	0.0003*
rs1441358	<i>THSD4</i>	emphysema ratio	FEV ₁ /FVC	0.0064	0.0217	0.0027	0.0005*
rs17486278	<i>CHRNA5</i>	emphysema	FEV ₁ /FVC	0.0057	0.0044	0.0125	<1×10 ⁻⁵ *
rs17486278	<i>CHRNA5</i>	cig per day	FEV ₁ /FVC	0.0168	<1×10 ⁻⁵ *	0.0024	<1×10 ⁻⁵ *
rs17486278	<i>CHRNA5</i>	cig per day	emphysema	-0.1404	<1×10 ⁻⁵ *	-0.0184	<1×10 ⁻⁵ *
rs17486278	<i>CHRNA5</i>	cig per day	emphysema ratio	-0.1235	<1×10 ⁻⁵ *	-0.0156	<1×10 ⁻⁵ *
rs17486278	<i>CHRNA5</i>	emphysema ratio	FEV ₁ /FVC	0.0105	<1×10 ⁻⁴ *	0.0059	<1×10 ⁻⁵ *
rs12459249	<i>CYP2A6</i>	cig per day	emphysema	0.0962	0.0011*	0.0136	2×10 ⁻⁵ *
rs12459249	<i>CYP2A6</i>	cig per day	total lung capacity	1.4021	4×10 ⁻⁵ *	0.0320	0.1000