A Genome-wide Association Study of Emphysema and Airway Quantitative Imaging Phenotypes

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Title

A Genome-wide Association Study of Emphysema and Airway Quantitative Imaging Phenotypes

Michael H. Cho[1,2], Peter J. Castaldi[1], Craig P. Hersh[1,2], Brian D. Hobbs[1,2], R. Graham Barr[4], Ruth Tal-Singer[5], Per Bakke[6], Amund Gulsvik[6], Raúl San José Estépar[3], Edwin Van Beek[7], Harvey O. Coxson[8], David A. Lynch[9], George R. Washko[2], Nan M. Laird[10], James D. Crapo[9], Terri H. Beaty[11], Edwin K. Silverman[1,2], on behalf of the NETT Genetics, ECLIPSE, and COPDGene Investigators

[1]Channing Division of Network Medicine, [2]Division of Pulmonary and Critical Care Medicine, and [3]Laboratory of Mathematics in Imaging, Department of Radiology, Brigham and Women's Hospital, Boston, MA; [4]Department of Medicine, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, [5]GlaxoSmithKline Research and Development, King Of Prussia, PA,[6]Department of Clinical Science, University of Bergen, Norway, [7]Queens Medical Research Institute, University of Edinburgh, Department of Radiology and Department of Biomedical Engineering, University of Iowa; [8]Department of Radiology, University of British Columbia, Vancouver, Canada [9]National Jewish Health, Denver, CO; [10]Harvard School of Public Health, Boston, MA; [11]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.

Corresponding author

Michael H. Cho (remhc@channing.harvard.edu), tel: 617-525-0897, fax: 888-487-1078.

Author Contributions: Study design: MHC, CPH, RGB, RTS, PB, AG, HOC, DAL, GRW, NL, JDC, THB, EKS. Phenotype acquisition and quality control: MHC, PJC, CPH, RGB, RTS, PB, AG, HOC, DAL, GRW, JDC, EKS. Genotype data acquisition and quality control: MHC, PJC, THB, EKS. Data analysis: MHC, PJC, BDH, NML, THB, EKS. Critical revision of the manuscript: all authors

Funding:

This work was supported by NHLBI R01 HL084323, P01 HL083069, P01 HL105339 and R01 HL089856 (E.K.S.); K08 HL097029 and R01 HL113264 (M.H.C.), and R01 HL089897 (J.D.C.); and the Alpha-1 Foundation (M.H.C.). The COPDGene study (NCT00608764) is also supported by the COPD Foundation through contributions made to an Industry Advisory Board comprised of AstraZeneca, Boehringer Ingelheim, Novartis, Pfizer, GlaxoSmithKline, Siemens and Sunovion. The National Emphysema Treatment Trial was supported by the NHLBI N01HR76101, N01HR76102, N01HR76103, N01HR76104, N01HR76105, N01HR76106, N01HR76107, N01HR76108, N01HR76109, N01HR76110, N01HR76111, N01HR76112, N01HR76113, N01HR76114, N01HR76115, N01HR76116, N01HR76118 and N01HR76119, the Centers for Medicare and Medicaid Services and the Agency for Healthcare Research and Quality. The Norway GenKOLS study (Genetics of Chronic Obstructive Lung Disease, GSK code RES11080), the ECLIPSE study (NCT00292552; GSK code SCO104960) were funded by GlaxoSmithKline.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, and Blood Institute or the National Institutes of Health.

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Running Head: GWAS of Quantitative Imaging Phenotypes

Descriptor number: 9.6 COPD: Epidemiology

Word count: 3288

At a Glance Commentary

Scientific Knowledge on the Subject

Chronic obstructive pulmonary disease is a complex and heterogeneous disease. Quantitative image analysis of chest CT scans can characterize this heterogeneity. Recent studies have identified genetic variants that increase susceptibility to emphysema or airway wall thickening, but have not examined both measurements in large populations of subjects with disease.

What This Study Adds to the Field

Our study confirms previously described associations and additionally identifies new genome-wide significant associations with emphysema near *SERPINA10* and *DLC1*. We also show that many loci previously identified in population-based studies of lung function are associated with emphysema or airway phenotypes. Genome-wide analysis of quantitative imaging may identify novel risk factors for COPD phenotypes, and also identify imaging features associated with previously identified genetic loci.

Abstract

Rationale: Chronic obstructive pulmonary disease (COPD) is defined by the presence of airflow limitation on spirometry, yet COPD subjects can have marked differences in CT imaging. These differences may be driven by genetic factors. We hypothesized that a genome-wide association study of quantitative imaging would identify loci not previously identified in analyses of COPD or spirometry. In addition, we sought to determine whether previously described genome-wide significant COPD and spirometric loci were associated with emphysema or airway phenotypes.

Objective: To identify genetic determinants of quantitative imaging phenotypes.

Methods: We performed a genome-wide association study on two quantitative emphysema and two quantitative airway imaging phenotypes in the COPDGene (non-Hispanic white and African-American), ECLIPSE, NETT, and GenKOLS studies; and on % gas trapping in COPDGene. We also examined specific loci reported as genome-wide significant for spirometric phenotypes related to airflow limitation or COPD.

Results: The total sample size across all cohorts was 12,031, of which 9,338 were from COPDGene. We identified five loci associated with emphysema-related phenotypes, one with airway-related phenotypes, and two with gas trapping. These loci included previously reported associations, including the *HHIP*, 15q25, and *AGER* loci, as well as novel associations near *SERPINA10* and *DLC1*. All previously reported COPD and a significant number of spirometric GWAS loci were at least nominally ($P < 0.05$) associated with either emphysema or airway phenotypes.

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Conclusions: Genome-wide analysis may identify novel risk factors for quantitative imaging characteristics in COPD, and also identify imaging features associated with previously identified lung function loci.

Introduction

Chronic obstructive pulmonary disease (COPD) is a highly prevalent and morbid disease, defined by a simple measurement - the presence of irreversible airflow limitation on spirometry. Despite this simple clinical definition, COPD is a complex and heterogeneous disease with marked differences in the presence of key components that contribute to airflow obstruction in COPD – emphysema and airways disease (1). With the advent of standardized quantitative measurements, chest CT scans have become the prevalent method of characterizing lung parenchyma and airways in COPD(2).

Over the past several years, advances in image generation and analysis have led to studies demonstrating clinical and pathophysiologic relevance of these imaging measures. These include associations with spirometry(3, 4), respiratory symptoms(5), susceptibility to osteoporosis(6) and lung cancer(7), exacerbations(8), and lung function decline(9, 10).

The development of COPD is strongly influenced by genetic factors(11). Genetic variation is also an important determinant of emphysema and airway disease. Emphysema or airway imaging characteristics appear to be separately heritable(12, 13). Obstruction on pulmonary function can be seen in diseases predominantly involving the airway (in cystic fibrosis), or in those that involve the parenchyma through emphysema (alpha-1 antitrypsin deficiency and cutis laxa)(14). Previous genome-wide studies have identified variants associated with emphysema(15–17) or airway disease(18), though generally in smaller sample sizes or predominantly population-based subjects.

We hypothesized quantitative imaging reflects component disease processes leading to airflow obstruction in COPD, and could have genetic determinants not discovered by analyses

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

using lung function alone. To address this hypothesis, we performed a genome-wide association study of quantitative emphysema and airway phenotypes in current and former cigarette smokers with and without COPD. We additionally hypothesized genetic loci associated with spirometry related to airflow obstruction in general population samples or with COPD affection status would demonstrate an association with imaging phenotypes. Some of these results have been previously presented as an abstract(19).

Methods

Imaging measurements were available in COPDGene (NCT00608764. www.copd.org) non-Hispanic white and African-Americans, the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE, SCO104960, NCT00292552, www.eclipse-copd.com), National Emphysema Treatment Trial (NETT), and GenKOLS (Genetics of COPD, Norway) study. Detailed descriptions including genotyping quality control, genotyping imputation, and quantitative imaging, have been previously published(5, 8, 20–27). All cohorts included only current or former smokers. COPDGene is a multicenter study including subjects of self-described non-Hispanic white or African-American ancestry and included subjects with and without COPD and with a range of spirometry. Subjects in the remaining studies were white. Controls had normal spirometry. Cases in the ECLIPSE and GenKOLS studies were at least GOLD spirometry grade 2 in severity. NETT cases had severe COPD (FEV_1 < 45% predicted) and were selected for the presence of emphysema.

Quantitative image analysis was performed on segmented CT chest images, using the number of voxels below -950 Hounsfield Units (%LAA-950) to estimate emphysema, and, alternatively, the Hounsfield Units at the 15th percentile of the density histogram (Perc15). The

airway wall area (Pi10) was the value for a hypothetical 10mm airway obtained by plotting a regression line of the square root of the airway wall area versus the airway internal perimeter(2). The wall area percent (WAP) was the percentage of the wall area compared to the total bronchial area for segmental and smaller airways (see Supplement). Percent gas trapping was measured at end-tidal exhalation and defined as the percent of lung voxels with < -856 HU(28).

We genotyped all subjects on Illumina platforms and imputed genotypes using MaCH and minimac(29) with 1000 Genomes Phase I v3 reference panels. We performed linear regression on each phenotype using residuals adjusted for age, sex, pack-years of smoking, current smoking status, and ancestry-based principal components. Imaging variables with marked non-normality were log-transformed (%LAA-950 and % gas trapping). COPDGene and ECLIPSE were additionally adjusted for CT scanner type. As airway measurements are not scaled to body size, we additionally adjusted for height. For gas trapping, a covariate for study center was also added to account for site-related technical variations in expiratory CT scans.

Results from all studies were combined into a meta-analysis. Given substantial heterogeneity within our studies, our primary analysis used a modified random-effects model(30). We also examined results using the standard fixed-effects model(31). As we hypothesized that emphysema and airway disease measured by quantitative CT may be causal for reduced lung function and COPD, our primary analyses included all subjects, with an additional analysis in cases only (including GOLD spirometry grade 1 for COPDGene subjects). To explore and control for the effect of ascertainment, we applied a method for analysis of secondary phenotype data within case-control association studies(32).

Additional methods are available in the Supplement.

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Results

Genome-wide association of five quantitative imaging phenotypes

Baseline characteristics of subjects in each cohort are shown in **Table 1**. The total sample size across all cohorts was 12,031. Genome-wide significant results from the modified random-effects meta-analysis are shown in **Table 2**. Loci with prior evidence of association with COPD, lung function, and / or emphysema – *HHIP*, *CHRNA3/5/IREB2*, and *AGER* – were the most significant associations with %LAA-950. We also identified additional associations at genome-wide significance ($P < 5x10^{-8}$) near *DLC1* and *SERPINA10*. An association near *CHRNA4* was just below genome-wide significance (rs183345681, $P = 1.8x10^{-7}$). An analysis of Perc15 also identified the *DLC1* and *HHIP* loci associations.

In our analysis of airway phenotypes, no association reached genome-wide significance for Pi10. One result for wall area percent yielded $P < 5x10^{-8}$ (rs142200419); however, this association was markedly attenuated in the fixed effects meta-analysis, due to effects in the opposite directions in one of the cohorts (Table S1). For the association analysis of gas trapping in COPDGene, the *AGER* and *LINC00310/KCNE2* loci achieved significance. No genome-wide significant results were identified in any of the case-only analyses (**Table S2**). For the regions yielding genome-wide significance in all subjects, we additionally examined results from an analysis accounting for ascertainment in COPDGene and GenKOLS, and including cases only from ECLIPSE (due to the small number of controls in this cohort). P-values obtained using this method(32) (**Table S1**) were generally only slightly less significant, with the possible exception of *HHIP* and *CHRNA3*, suggesting that overall our results were not simply driven by an association with case-control status. Results in cases and controls separately and, for loci not

previously described as genome-wide significant in COPD, a case-control analysis, are shown in **Tables S3** and **S4**.

The association with %LAA-950 near *SERPINA10* is also near *SERPINA1*, variants in which are the cause of alpha-1 antitrypsin deficiency. The most common form of severe alpha-1 antitrypsin deficiency is due to homozygosity for the Z allele, rs28929474. This variant was imputed with relatively high quality (Rsq > 0.9 in all white cohorts; 0.66 in COPDGene African-Americans). We examined the imputed rs28929474 in all cohorts, and did not find any ZZ subjects in NETT and GenKOLS; in COPDGene, seven non-Hispanic white ZZ subjects had been genotyped and subsequently excluded from analyses after *SERPINA1* genotyping (Foreman, In Preparation). All seven of these subjects were correctly identified with imputed genotypes. Linkage disequilibrium exists between our top associated SNP at this locus, rs45505795, and rs28929474 (D' 0.7, $r^2 = 0.295$). To determine if the association with rs45505795 could be accounted for by rs28929474, we performed a meta-analysis conditioned on rs28929474. The resulting P-value was 0.007, demonstrating that rs28929474 accounts for some, but not all, of the association signal. While known or identified ZZ homozygotes were excluded from COPDGene, NETT, and GenKOLS, ECLIPSE excluded only known alpha-1 deficient subjects. We identified six putative ZZ subjects in ECLIPSE. To determine whether the association signal in ECLIPSE was driven by the presence of these six subjects, we repeated the association analysis after dropping these subjects and found the P-value was slightly attenuated but remained significant (P = 0.0018), consistent overall with an increased risk of emphysema among MZ carriers.

To further explore the potential functional consequences of individual loci described in this study, we searched for evidence of functional impact using existing data sources. Of the loci

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

described in this study not previously associated with COPD, one was a cis-eQTL in lung – rs55706246 near *LINC00310* was in modest LD ($r^2 = 0.24$) with rs2834438, an eQTL for *KCNE2* ($p = 3.1x10^{-7}$)(33). Using GWAS3D, the top-scoring variant at the *DLC1* locus was rs58863591, which had active enhancer marks (H3K4me1 and DNase hypersensitivity) and potential long-range interactions upstream of *DLC1* and near *SENP2*(34).

We also sought to determine whether the group of top (most significant) markers for each analysis ($P < 1x10^{-6}$) could yield to insights about cell types based on regulatory data ENCODE(35). In the emphysema analysis, cell type enhancer enrichment from analysis of %LAA-950 among all subjects included enhancers in umbilical vein endothelial cells (Huvec, $P = 6.0x10^{-4}$) and DNase I hypersensitivity sites in several types of endothelial cells ($P = 6.6x10^{-3}$ to 0.03 for pulmonary artery endothelial cells (HPAEC) and adult blood, adult lymphatic, and neonatal lymphatic microvascular endothelial cells (HMVEC)). We found similar findings for the Perc15 analysis, with the strongest DNase enrichment for pulmonary artery endothelial cells (P=0.017). For the airway phenotypes, we found modest evidence for enrichment for enhancers K562 (leukemia) and HSMM (skeletal muscle) cell lines (P = 0.02) and DNase enrichment in CD14+ monocytes (P = 0.04).

We also sought to determine whether our results were consistent with a set of genes more likely to act within a specific gene sets or pathways. Top-ranked results identified several individual potential pathways of interest, including the toll-like receptor and phosphoinositide 3-kinase pathways (iGSEA4GWAS(36)) and telomere maintenance (INRICH(37)) for the %LAA-950 analyses. Gene sets that appeared to overlap between top-ranked sets among different methods included regulation of apoptosis, isoprenoid biosynthetic process, nicotinic

158 acetylcholine channel activity, actin cytoskeleton, and B-cell receptor signaling for emphysema
159 GWAS; and for airway, WNT signaling and muscle contraction.

160 **Associations at loci previously identified in association with COPD or COPD-related**
161 **spirometric phenotypes**

162 Genome-wide association studies have identified multiple variants associated with
163 COPD(23–26, 38) or measures of lung function(39–41). We sought to determine whether there
164 was evidence these variants might have an effect on quantitative imaging phenotypes, even if
165 they did not reach genome-wide significance. After excluding loci previously associated in these
166 cohorts with COPD, we found a strong enrichment in nominally significant (P-value < 0.05) loci
167 among the two emphysema and two imaging phenotypes ($P = 4.9x10^{-9}$), suggesting many of
168 these variants may also affect quantitative imaging measurements. We further classified these
169 variants into those showing a stronger association (by one-sided P-value) with emphysema- or
170 airway-related phenotypes, assigning directionality such that the risk allele for COPD or reduced
171 lung function demonstrated greater emphysema or increased airway wall thickness (**Table 3**).
172 Enrichment for nominally significant P-values appeared to be greater among markers associated
173 with quantitative emphysema ($P = 1.9x10^{-6}$) versus those associated with airway wall thickness
174 ($P=1.3x10^{-3}$).

175 We next examined regulatory patterns using Haploreg(35) in variants classified as either
176 emphysema or airway-associated identified in **Tables 2 & 3**. 'Emphysema' variants were
177 modestly enriched for enhancers seen in hepatocellular carcinoma (HepG2, P=0.05), while those
178 more strongly associated with airway phenotypes were enriched for enhancers from lung
179 fibroblasts (NHLF) and epidermal keratinocytes (NHEK, P=0.03 to 0.04). Both analyses were

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

enriched for mammary epithelial cells (HMEC, P=2.5x10^{-4} to 1.6x10^{-3}) and umbilical vein endothelial cells (Huvec, P=0.02 to 0.03). The most significant DNase enrichment for emphysema-associated variants was lung-derived lymphatic microvascular endothelial cells (HMVEC-LLy; P 8x10^{-4}), while top results for airway-associated variants were embryonic lung fibroblasts (WI-38), mammary fibroblasts (HMF), and small airway epithelial cells (SAEC; P 3.6-6.6x10^{-4}). Emphysema-associated DNase results were not significant in the airway results, and vice versa.

Discussion

In a genome-wide association study of quantitative imaging phenotypes in smokers with and without COPD, we identified genome-wide significant associations with loci previously shown to be associated with COPD or with spirometric measures related to airflow limitation, including the 15q25, *HHIP*, and *AGER* loci, the latter also identified in in association with emphysema in a general population sample(15) and with emphysema and sRAGE levels in COPD(42). We also describe a genome-wide association with emphysema and variants near *SERPINA10*, and show that this association is in strong linkage disequilibrium with the Z-allele of *SERPINA1*, and not due the presence of PI ZZ individuals. This report is thus consistent with other reports showing an increased risk of airflow limitation for subjects with PI MZ(43, 44) and emphasizes the role of alpha-1 antitrypsin in the pathogenesis of COPD and emphysema in a broader group of patients.

One of our top associations with emphysema (both for %LAA-950 and Perc15) was a novel locus, located in the gene *DLC1* (deleted in liver cancer 1). *DLC1* frequently undergoes loss of heterozygosity or epigenetic silencing in solid cancers, including lung cancers(45). *DLC1*

appears to inhibit cell growth and increases apopotosis(46), and act as a tumor suppressor through the RhoGAP-dependent and RhoGAP-independent activity(47). *DLC1* is highly expressed in the lung(48, 49). In a study of regional emphysema, *DLC1* expression showed a trend towards decreased expression with an increase in the mean linear intercept(50) (nominal P-value, 0.04). Recently, a locus in *DLC1* was described in association with smoking behavior in African-Americans(51). We found a trend towards association with current smoking at this locus in COPDGene African-Americans (P = 0.06-0.07). However, we found no association with pack-years of smoking (P > 0.49). In addition, *DLC1* SNPs in this study are approximately 200kb away and not in linkage disequilibrium with our reported *DLC1* loci (r2 < 0.004 in COPDGene African-Americans), and we found no consistent evidence of effect on either pack-years or current smoking at either locus in other cohorts. We also note an additional association near *CHRNA4* just below genome-wide significance. Previous studies have identified associations with smoking behavior in this region(52, 53), though previously described variants do not appear to be in strong LD with our identified variant. Additional studies will be needed to confirm our associations and determine their relationship to cigarette smoking.

We also examined variants previously identified at genome-wide significance in association with COPD or spirometic measures related to airflow obstruction. Most of these loci were at least nominally significantly (P < 0.05) associated with one or more quantitative CT phenotypes. Many appeared to have stronger associations with either quantitative emphysema or airway phenotypes. These findings suggest that genetic determinants of lung function in the general population may influence emphysema or airway disease, and are consistent with the hypothesis that there may be variants affecting airflow obstruction in different ways detectable by quantitative imaging.

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

In addition to examining individual loci, our study also explores the relevance of groups of markers that may not reach genome-wide significance. An analysis of gene sets provides supportive evidence for biological mechanisms previously been implicated in COPD, including telomere maintenance(54–57), phosphoinositide-3-kinase(58, 59), actin organization, and B-cell receptor signaling(50). An exploratory analysis of regulatory regions from ENCODE identified enrichment for endothelial cells. In animal models, targeted disruption of endothelial cells through genetic or immune mechanisms leading to apoptosis can lead to emphysema(60–62). Endothelial cell apoptosis has been seen in emphysematous human tissue(60) and endothelial microparticles, a marker for apoptosis, were related to emphysema in the MESA study(63). In contrast to prior work(16), we did not see an enrichment for fibroblasts from our quantitative emphysema analyses, but did see such enrichment in our airway-related lung function analysis.

Emphysema and airway disease are important components of COPD. We used automated and standardized measurements, available on a large number of subjects and free of inter-reader variation. We performed an analysis including all subjects in an effort to maximize power, and applied a method to account for ascertainment based on case-control status. However, due to the high correlation of disease status with imaging characteristics, we cannot rule out a degree of confounding for some of our associations. Although we performed five association analyses, we reported unadjusted P-values as our phenotypes are correlated, and some of our findings are seen in multiple phenotypes. Quantitative imaging can be affected by factors not related to intrinsic lung pathology, such as degree of inflation, obesity, smoking, and characteristics of individual CT scanners(5, 64, 65). Our decision to adjust for specific covariates was based on a desire to maximize findings of genetic analysis by controlling for the influence of age, smoking, and effects of individual scanners, yet allowing for genetic effects

that may affect disease processes contributing to more than one characteristic (e.g., low BMI and emphysema(66)). Ultimately, our findings will require replication, ideally in additional large cohorts that include a range of severity of COPD.

Our analysis also included studies with different imaging protocols, proportions of severity of disease, and racial groups. Thus, despite our large sample size, these factors may have resulted in a reduction in statistical power. We attempted to at least partially address this issue by using a method(30) that can improve power in the setting of heterogeneity. While most of the P-values from this method were very similar to those using standard fixed-effects models, this method resulted in *AGER* reaching genome-wide significance, consistent with prior studies. Our study is unable to address several causes of potential heterogeneity. Genetic factors may be specific to racial / ethnic groups(15). Technical factors may be less likely to influence reads by radiologists or semi-supervised methods and may explain why we were unable to replicate previous findings based on these approaches(16, 17). These factors, as well as differing proportions of severity of disease, may also indicate why we were unable to replicate findings from a recently reported analysis of airway wall thickness(18). Chest CT scans contain a wealth of data, and current measures of overall lung density or airway wall measurements do not adequately represent all relevant features. Efforts to expand and standardize radiologist interpretation and novel computational and machine learning-based methods may improve the ability to detect genetic effects.

Our work also demonstrates that previously described genetic associations with lung function in the population appear to influence airway or emphysema phenotypes. Using data from the ENCODE project, we identified non-overlapping enrichment of regulatory regions for our two sets of analyses. Our results are consistent with the hypothesis that emphysema and

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

airway imaging characteristics may be driven by different pathogenic processes and genetic factors(12). However, lung function, disease status, and imaging features are all correlated, and the relationship between specific imaging features is potentially complex(67). Our relative preponderance of associations with quantitative emphysema compared to airway, for example, may reflect the stronger correlation between lung function and our quantitative emphysema measurements or technical factors that affect airway measurements(67, 68). Our sets, particularly for 'airway' were loosely defined, and included results not reaching a nominal level of significance. Additional analytic methods, such as causal modeling, may help clarify the relationships between genetic variants, lung function, and CT imaging. Ultimately, however, the specific effects of individual variants will need to be determined by careful functional studies.

Differences in susceptibility to and phenotypic heterogeneity in COPD remain poorly understood. Despite their limitations, genome-wide association studies are currently the most powerful method to identify novel genetic risk factors for this complex and heterogeneous disease. Our analysis reflects a coordinated effort across multiple studies and to our knowledge is the largest genome-wide analysis of quantitative pulmonary imaging reported to date, and the first to include a substantial number of subjects with COPD. Our work identifies several genetic loci that may influence specific imaging phenotypes and identifies potential functional pathways and cell types through which these loci may exert their phenotypic effects. It also describes CT imaging phenotype-specific associations for loci previously implicated in GWAS for COPD or spirometric phenotypes related to COPD. Additional insights will result from increasing power; thus we anticipate a critical role for combining existing and upcoming studies using improved imaging phenotypes, to help unravel the complexity of pulmonary pathology in COPD.

References

1. Burrows B, Fletcher CM, Heard BE, Jones NL, Wootliff JS. The emphysematous and bronchial types of chronic airways obstruction. *Lancet* 1966;1:830–835.

2. Nakano Y, Muro S, Sakai H, Hirai T, Chin K, Tsukino M, Nishimura K, Itoh H, Paré PD, Hogg JC, Mishima M. Computed tomographic measurements of airway dimensions and emphysema in smokers. Correlation with lung function. *Am J Respir Crit Care Med* 2000;162:1102–8.

3. Mets OM, Buckens CFM, Zanen P, Isgum I, van Ginneken B, Prokop M, Gietema HA, Lammers J-WJ, Vliegenthart R, Oudkerk M, van Klaveren RJ, de Koning HJ, Mali WPTM, de Jong PA. Identification of chronic obstructive pulmonary disease in lung cancer screening computed tomographic scans. *JAMA* 2011;306:1775–81.

4. Washko GR, Criner GJ, Mohsenifar Z, Sciurba FC, Sharafkhaneh A, Make BJ, Hoffman EA, Reilly JJ. Computed tomographic-based quantification of emphysema and correlation to pulmonary function and mechanics. *COPD*, 2008/06/24 ed. 2008;5:177–186.

5. Grydeland TB, Dirksen A, Coxson HO, Eagan TML, Thorsen E, Pillai SG, Sharma S, Eide GE, Gulsvik A, Bakke PS. Quantitative computed tomography measures of emphysema and airway wall thickness are related to respiratory symptoms. *Am J Respir Crit Care Med* 2010;181:353–9.

6. Bon J, Fuhrman CR, Weissfeld JL, Duncan SR, Branch RA, Chang C-CH, Zhang Y, Leader JK, Gur D, Greenspan SL, Sciurba FC. Radiographic Emphysema Predicts Low Bone Mineral Density in a Tobacco-exposed Cohort. *Am J Respir Crit Care Med* 2011;183:885–90.

7. Wilson DO, Weissfeld JL, Balkan A, Schragin JG, Fuhrman CR, Fisher SN, Wilson J, Leader JK, Siegfried JM, Shapiro SD, Sciurba FC. Association of radiographic emphysema and airflow obstruction with lung cancer. *Am J Respir Crit Care Med*, 2008/06/21 ed. 2008;178:738–744.

8. Han MK, Kazerooni EA, Lynch DA, Liu LX, Murray S, Curtis JL, Criner GJ, Kim V, Bowler RP, Hanania NA, Anzueto AR, Make BJ, Hokanson JE, Crapo JD, Silverman EK, Martinez FJ, Washko GR, The COPDGene Investigators F. Chronic obstructive pulmonary disease exacerbations in the COPDGene study: associated radiologic phenotypes. *Radiology* 2011;261:274–82.

9. Mohamed Hoesein FAA, de Hoop B, Zanen P, Gietema H, Kruitwagen CLJJ, van Ginneken B, Isgum I, Mol C, van Klaveren RJ, Dijkstra AE, Groen HJM, Boezen HM, Postma DS, Prokop M, Lammers J-WJ. CT-quantified emphysema in male heavy smokers: association with lung function decline. *Thorax* 2011;66:782–7.

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

10. Nishimura M, Makita H, Nagai K, Konno S, Nasuhara Y, Hasegawa M, Shimizu K, Betsuyaku T, Ito YM, Fuke S, Igarashi T, Akiyama Y, Ogura S. Annual change in pulmonary function and clinical phenotype in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2012;185:44–52.

11. Ingebrigtsen T, Thomsen SF, Vestbo J, van der Sluis S, Kyvik KO, Silverman EK, Svartengren M, Backer V. Genetic influences on Chronic Obstructive Pulmonary Disease - a twin study. *Respir Med* 2010;104:1890–5.

12. Patel BD, Coxson HO, Pillai SG, Agustí AGN, Calverley PMA, Donner CF, Make BJ, Müller NL, Rennard SI, Vestbo J, Wouters EFM, Hiorns MP, Nakano Y, Camp PG, Nasute Fauerbach P V, Screaton NJ, Campbell EJ, Anderson WH, Paré PD, Levy RD, Lake SL, Silverman EK, Lomas DA. Airway wall thickening and emphysema show independent familial aggregation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2008;178:500–5.

13. Zhou JJ, Cho MH, Castaldi PJ, Hersh CP, Silverman EK, Laird NM. Heritability of COPD and Related Phenotypes in Smokers. *Am J Respir Crit Care Med* 2013;doi:10.1164/rccm.201302-0263OC.

14. Urban Z, Gao J, Pope FM, Davis EC. Autosomal dominant cutis laxa with severe lung disease: synthesis and matrix deposition of mutant tropoelastin. 2005;124:1193–1199.

15. Manichaikul A, Hoffman EA, Smolonska J, Gao W, Cho MH, Baumhauer H, Budoff M, Austin JHM, Washko GR, Carr JJ, Kaufman JD, Pottinger T, Powell CA, Wijmenga C, Zanen P, Groen HJM, Postma DS, Wanner A, Rouhani FN, Brantly ML, Powell R, Smith BM, Rabinowitz D, Raffel LJ, Hinckley Stukovsky KD, Crapo JD, Beaty TH, Hokanson JE, Silverman EK, *et al.* Genome-wide study of percent emphysema on computed tomography in the general population. The Multi-Ethnic Study of Atherosclerosis Lung/SNP Health Association Resource Study. *Am J Respir Crit Care Med* 2014;189:408–18.

16. Castaldi PJ, Cho MH, Estépar RSJ, McDonald M-LN, Laird N, Beaty TH, Washko G, Crapo JD, Silverman EK. Genome-Wide Association Identifies Regulatory Loci Associated with Distinct Local Histogram Emphysema Patterns. *Am J Respir Crit Care Med* 2014;doi:10.1164/rccm.201403-0569OC.

17. Kong X, Cho MH, Anderson W, Coxson HO, Muller N, Washko G, Hoffman EA, Bakke P, Gulsvik A, Lomas DA, Silverman EK, Pillai SG. Genome-wide association study identifies BICD1 as a susceptibility gene for emphysema. *Am J Respir Crit Care Med* 2011;183:43–9.

18. Dijkstra AE, Postma DS, van Ginneken B, Wielpütz MO, Schmidt M, Becker N, Owsijewitsch M, Kauczor H-U, de Koning HJ, Lammers JW, Oudkerk M, Brandsma C-A, Bossé Y, Nickle DC, Sin DD, Hiemstra PS, Wijmenga C, Smolonska J, Zanen P, Vonk JM, van den Berge M, Boezen HM, Groen HJM. Novel Genes for Airway Wall Thickness

Identified with Combined Genome Wide Association and Expression Analyses. *Am J Respir Crit Care Med* 2014;doi:10.1164/rccm.201405-0840OC.

19. Cho MH, Castaldi P, Hersh CP, Barr RG, Tal-Singer R, Bakke P, Gulsvik A, Van Beek EJ, Coxson HO, Lynch DA, Washko GR, Laird N, Crapo J, Beaty T, Silverman EK. A Genome-Wide Association Study of Emphysema and Airway Quantitative Imaging Phenotypes. *Am Thorac Soc Int Conf* 2015. p. A2849.

20. Regan EA, Hokanson JE, Murphy JR, Make B, Lynch DA, Beaty TH, Curran-Everett D, Silverman EK, Crapo JD. Genetic epidemiology of COPD (COPDGene) study design. *COPD*, 2010/03/11 ed. 7:32–43.

21. Vestbo J, Anderson W, Coxson HO, Crim C, Dawber F, Edwards L, Hagan G, Knobil K, Lomas DA, MacNee W, Silverman EK, Tal-Singer R. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). *Eur Respir J* 2008;31:869–73.

22. Fishman A, Martinez F, Naunheim K, Piantadosi S, Wise R, Ries A, Weinmann G, Wood DE. A randomized trial comparing lung-volume-reduction surgery with medical therapy for severe emphysema. *N Engl J Med* 2003;348:2059–73.

23. Pillai SG, Ge D, Zhu G, Kong X, Shianna K V, Need AC, Feng S, Hersh CP, Bakke P, Gulsvik A, Ruppert A, Lodrup Carlsen KC, Roses A, Anderson W, Rennard SI, Lomas DA, Silverman EK, Goldstein DB. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet*, 2009/03/21 ed. 2009;5:e1000421.

24. Cho MH, Boutaoui N, Klanderman BJ, Sylvia JS, Ziniti JP, Hersh CP, DeMeo DL, Hunninghake GM, Litonjua AA, Sparrow D, Lange C, Won S, Murphy JR, Beaty TH, Regan EA, Make BJ, Hokanson JE, Crapo JD, Kong X, Anderson WH, Tal-Singer R, Lomas DA, Bakke P, Gulsvik A, Pillai SG, Silverman EK. Variants in FAM13A are associated with chronic obstructive pulmonary disease. *Nat Genet*, 2010/02/23 ed. 2010;42:200–202.

25. Cho MH, Castaldi PJ, Wan ES, Siedlinski M, Hersh CP, Demeo DL, Himes BE, Sylvia JS, Klanderman BJ, Ziniti JP, Lange C, Litonjua AA, Sparrow D, Regan EA, Make BJ, Hokanson JE, Murray T, Hetmanski JB, Pillai SG, Kong X, Anderson WH, Tal-Singer R, Lomas DA, Coxson HO, Edwards LD, MacNee W, Vestbo J, Yates JC, Agusti A, *et al.* A genome-wide association study of COPD identifies a susceptibility locus on chromosome 19q13. *Hum Mol Genet* 2012;21:947–57.

26. Cho MH, McDonald M-LN, Zhou X, Mattheisen M, Castaldi PJ, Hersh CP, Demeo DL, Sylvia JS, Ziniti J, Laird NM, Lange C, Litonjua AA, Sparrow D, Casaburi R, Barr RG, Regan EA, Make BJ, Hokanson JE, Lutz S, Dudenkov TM, Farzadegan H, Hetmanski JB, Tal-Singer R, Lomas DA, Bakke P, Gulsvik A, Crapo JD, Silverman EK, Beaty TH. Risk

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

loci for chronic obstructive pulmonary disease: a genome-wide association study and meta-analysis. *lancet Respir Med* 2014;2:214–25.

27. DeMeo DL, Hersh CP, Hoffman EA, Litonjua AA, Lazarus R, Sparrow D, Benditt JO, Criner G, Make B, Martinez FJ, Scanlon PD, Sciurba FC, Utz JP, Reilly JJ, Silverman EK. Genetic determinants of emphysema distribution in the national emphysema treatment trial. *Am J Respir Crit Care Med* 2007;176:42–48.

28. Schroeder JD, McKenzie AS, Zach JA, Wilson CG, Curran-Everett D, Stinson DS, Newell JD, Lynch DA. Relationships between airflow obstruction and quantitative CT measurements of emphysema, air trapping, and airways in subjects with and without chronic obstructive pulmonary disease. *AJR Am J Roentgenol* 2013;201:W460–70.

29. Howie B, Fuchsberger C, Stephens M, Marchini J, Abecasis GR. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nat Genet* 2012;44:955–9.

30. Han B, Eskin E. Random-Effects Model Aimed at Discovering Associations in Meta-Analysis of Genome-wide Association Studies. *Am J Hum Genet* 2011;88:586–98.

31. De Bakker PIW, Ferreira M a R, Jia X, Neale BM, Raychaudhuri S, Voight BF. Practical aspects of imputation-driven meta-analysis of genome-wide association studies. *Hum Mol Genet* 2008;17:R122–8.

32. Lin DY, Zeng D. Proper analysis of secondary phenotype data in case-control association studies. *Genet Epidemiol* 2009;33:256–65.

33. Hao K, Bossé Y, Nickle DC, Paré PD, Postma DS, Laviolette M, Sandford A, Hackett TL, Daley D, Hogg JC, Elliott WM, Couture C, Lamontagne M, Brandsma C-A, van den Berge M, Koppelman G, Reicin AS, Nicholson DW, Malkov V, Derry JM, Suver C, Tsou JA, Kulkarni A, Zhang C, Vessey R, Opiteck GJ, Curtis SP, Timens W, Sin DD. Lung eQTLs to help reveal the molecular underpinnings of asthma. *PLoS Genet* 2012;8:e1003029.

34. Li MJ, Wang LY, Xia Z, Sham PC, Wang J. GWAS3D: Detecting human regulatory variants by integrative analysis of genome-wide associations, chromosome interactions and histone modifications. *Nucleic Acids Res* 2013;41:W150–8.

35. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 2012;40:D930–4.

36. Zhang K, Cui S, Chang S, Zhang L, Wang J. i-GSEA4GWAS: a web server for identification of pathways/gene sets associated with traits by applying an improved gene set enrichment analysis to genome-wide association study. *Nucleic Acids Res*, 2010/05/04 ed. 2010;38:W90–5.

37. Lee PH, O'Dushlaine C, Thomas B, Purcell SM. INRICH: interval-based enrichment analysis for genome-wide association studies. *Bioinformatics* 2012;28:1797–9.

38. Wilk JB, Shrine NRG, Loehr LR, Zhao JH, Manichaikul A, Lopez LM, Smith AV, Heckbert SR, Smolonska J, Tang W, Loth DW, Curjuric I, Hui J, Cho MH, Latourelle JC, Henry AP, Aldrich M, Bakke P, Beaty TH, Bentley AR, Borecki IB, Brusselle GG, Burkart KM, Chen T, Couper D, Crapo JD, Davies G, Dupuis J, Franceschini N, *et al.* Genome-wide association studies identify CHRNA5/3 and HTR4 in the development of airflow obstruction. *Am J Respir Crit Care Med* 2012;186:622–32.

39. Soler Artigas M, Loth DW, Wain L V, Gharib SA, Obeidat M, Tang W, Zhai G, Zhao JH, Smith AV, Huffman JE, Albrecht E, Jackson CM, Evans DM, Cadby G, Fornage M, Manichaikul A, Lopez LM, Johnson T, Aldrich MC, Aspelund T, Barroso I, Campbell H, Cassano PA, Couper DJ, Eiriksdottir G, Franceschini N, Garcia M, Gieger C, Gislason GK, *et al.* Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nat Genet* 2011;43:1082–90.

40. Hancock DB, Artigas MS, Gharib SA, Henry A, Manichaikul A, Ramasamy A, Loth DW, Imboden M, Koch B, McArdle WL, Smith A V, Smolonska J, Sood A, Tang W, Wilk JB, Zhai G, Zhao JH, Aschard H, Burkart KM, Curjuric I, Eijgelsheim M, Elliott P, Gu X, Harris TB, Janson C, Homuth G, Hysi PG, Liu JZ, Loehr LR, *et al.* Genome-wide joint meta-analysis of SNP and SNP-by-smoking interaction identifies novel loci for pulmonary function. *PLoS Genet* 2012;8:e1003098.

41. Repapi E, Sayers I, Wain L V, Burton PR, Johnson T, Obeidat M, Zhao JH, Ramasamy A, Zhai G, Vitart V, Huffman JE, Igl W, Albrecht E, Deloukas P, Henderson J, Granell R, McArdle WL, Rudnicka AR, Barroso I, Loos RJ, Wareham NJ, Mustelin L, Rantanen T, Surakka I, Imboden M, Wichmann HE, Grkovic I, Jankovic S, Zgaga L, *et al.* Genome-wide association study identifies five loci associated with lung function. *Nat Genet*, 2009/12/17 ed. 2009;42:36–44.

42. Cheng DT, Kim DK, Cockayne DA, Belousov A, Bitter H, Cho MH, Duvoix A, Edwards LD, Lomas DA, Miller BE, Reynaert N, Tal-Singer R, Wouters EFM, Agustí A, Fabbri LM, Rames A, Visvanathan S, Rennard SI, Jones P, Parmar H, MacNee W, Wolff G, Silverman EK, Mayer RJ, Pillai SG. Systemic soluble receptor for advanced glycation endproducts is a biomarker of emphysema and associated with AGER genetic variants in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2013;188:948–57.

43. Molloy K, Hersh CP, Morris VB, Carroll TP, O'Connor CA, Lasky-Su JA, Greene CM, O'Neill SJ, Silverman EK, McElvaney NG. Clarification of the risk of chronic obstructive pulmonary disease in α1-antitrypsin deficiency PiMZ heterozygotes. *Am J Respir Crit Care Med* 2014;189:419–27.

44. Sørheim I-C, Bakke P, Gulsvik A, Pillai SG, Johannessen A, Gaarder PI, Campbell EJ, Agustí A, Calverley PMA, Donner CF, Make BJ, Rennard SI, Vestbo J, Wouters EFM,

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Paré PD, Levy RD, Coxson HO, Lomas DA, Hersh CP, Silverman EK. α_1-Antitrypsin protease inhibitor MZ heterozygosity is associated with airflow obstruction in two large cohorts. *Chest* 2010;138:1125–32.

45. Liao Y-C, Lo SH. Deleted in liver cancer-1 (DLC-1): a tumor suppressor not just for liver. *Int J Biochem Cell Biol* 2008;40:843–7.

46. Zhou X, Thorgeirsson SS, Popescu NC. Restoration of DLC-1 gene expression induces apoptosis and inhibits both cell growth and tumorigenicity in human hepatocellular carcinoma cells. *Oncogene* 2004;23:1308–13.

47. Healy KD, Hodgson L, Kim T-Y, Shutes A, Maddileti S, Juliano RL, Hahn KM, Harden TK, Bang Y-J, Der CJ. DLC-1 suppresses non-small cell lung cancer growth and invasion by RhoGAP-dependent and independent mechanisms. *Mol Carcinog* 2008;47:326–37.

48. Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, Zwahlen M, Kampf C, Wester K, Hober S, Wernerus H, Björling L, Ponten F. Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol* 2010;28:1248–50.

49. Lonsdale J, Thomas J, Salvatore M, Phillips R, Lo E, Shad S, Hasz R, Walters G, Garcia F, Young N, Foster B, Moser M, Karasik E, Gillard B, Ramsey K, Sullivan S, Bridge J, Magazine H, Syron J, Fleming J, Siminoff L, Traino H, Mosavel M, Barker L, Jewell S, Rohrer D, Maxim D, Filkins D, Harbach P, *et al.* The Genotype-Tissue Expression (GTEx) project. *Nat Genet*, 2013/05/30 ed. 2013;45:580–585.

50. Campbell JD, McDonough JE, Zeskind JE, Hackett TL, Pechkovsky D V, Brandsma C-A, Suzuki M, Gosselink J V, Liu G, Alekseyev YO, Xiao J, Zhang X, Hayashi S, Cooper JD, Timens W, Postma DS, Knight DA, Lenburg ME, Hogg JC, Spira A. A gene expression signature of emphysema-related lung destruction and its reversal by the tripeptide GHK. *Genome Med* 2012;4:67.

51. Gelernter J, Kranzler HR, Sherva R, Almasy L, Herman AI, Koesterer R, Zhao H, Farrer LA. Genome-wide association study of nicotine dependence in American populations: identification of novel risk loci in both African-Americans and European-Americans. *Biol Psychiatry* 2015;77:493–503.

52. Lazary J, Dome P, Csala I, Kovacs G, Faludi G, Kaunisto M, Dome B. Massive withdrawal symptoms and affective vulnerability are associated with variants of the CHRNA4 gene in a subgroup of smokers. In: Chen L, editor. *PLoS One* 2014;9:e87141.

53. Han S, Yang B-Z, Kranzler HR, Oslin D, Anton R, Gelernter J. Association of CHRNA4 polymorphisms with smoking behavior in two populations. *Am J Med Genet B Neuropsychiatr Genet* 2011;156B:421–9.

54. Savale L, Chaouat A, Bastuji-Garin S, Marcos E, Boyer L, Maitre B, Sarni M, Housset B, Weitzenblum E, Matrat M, Le Corvoisier P, Rideau D, Boczkowski J, Dubois-Randé J-L,

Chouaid C, Adnot S. Shortened telomeres in circulating leukocytes of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2009;179:566–71.

55. Tsuji T, Aoshiba K, Nagai A. Alveolar cell senescence in patients with pulmonary emphysema. *Am J Respir Crit Care Med* 2006;174:886–93.

56. Alder JK, Guo N, Kembou F, Parry EM, Anderson CJ, Gorgy AI, Walsh MF, Sussan T, Biswal S, Mitzner W, Tuder RM, Armanios M. Telomere Length is a Determinant of Emphysema Susceptibility. *Am J Respir Crit Care Med* 2011;doi:10.1164/rccm.201103-0520OC.

57. Nunes H, Monnet I, Kannengiesser C, Uzunhan Y, Valeyre D, Kambouchner M, Naccache J-M. Is telomeropathy the explanation for combined pulmonary fibrosis and emphysema syndrome?: report of a family with TERT mutation. *Am J Respir Crit Care Med* 2014;189:753–4.

58. Barnes PJ. Emerging pharmacotherapies for COPD. *Chest*, 2008/12/09 ed. 2008;134:1278–1286.

59. Fang X, Li K, Tao X, Chen C, Wang X, Wang L, Wang DC, Zhang Y, Bai C, Wang X. Effects of phosphoinositide 3-kinase on protease-induced acute and chronic lung inflammation, remodeling, and emphysema in rats. *Chest* 2013;143:1025–35.

60. Kasahara Y, Tuder RM, Taraseviciene-Stewart L, Le Cras TD, Abman S, Hirth PK, Waltenberger J, Voelkel NF. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. 2000;106:1311–1319.

61. Taraseviciene-Stewart L, Scerbavicius R, Choe K-H, Moore M, Sullivan A, Nicolls MR, Fontenot AP, Tuder RM, Voelkel NF. An animal model of autoimmune emphysema. *Am J Respir Crit Care Med* 2005;171:734–42.

62. Giordano RJ, Lahdenranta J, Zhen L, Chukwueke U, Petrache I, Langley RR, Fidler IJ, Pasqualini R, Tuder RM, Arap W. Targeted induction of lung endothelial cell apoptosis causes emphysema-like changes in the mouse. *J Biol Chem* 2008;283:29447–60.

63. Thomashow MA, Shimbo D, Parikh MA, Hoffman EA, Vogel-Claussen J, Hueper K, Fu J, Liu C-Y, Bluemke DA, Ventetuolo CE, Doyle MF, Barr RG. Endothelial microparticles in mild chronic obstructive pulmonary disease and emphysema. The Multi-Ethnic Study of Atherosclerosis Chronic Obstructive Pulmonary Disease study. *Am J Respir Crit Care Med* 2013;188:60–8.

64. Stratelis G, Fransson SG, Schmekel B, Jakobsson P, Molstad S. High prevalence of emphysema and its association with BMI: A study of smokers with normal spirometry. 2008/10/11 ed. 2008;1–7.doi:903492403 [pii] 10.1080/02813430802452732.

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

549 65. Hoffman EA, Ahmed FS, Baumhauer H, Budoff M, Carr JJ, Kronmal R, Reddy S, Barr
550 RG. Variation in the percent of emphysema-like lung in a healthy, nonsmoking
551 multiethnic sample. The MESA lung study. *Ann Am Thorac Soc* 2014;11:898–907.

552 66. Wan ES, Cho MH, Boutaoui N, Klanderman BJ, Sylvia JS, Ziniti JP, Won S, Lange C,
553 Pillai SG, Anderson WH, Kong X, Lomas DA, Bakke PS, Gulsvik A, Regan EA, Murphy
554 JR, Make BJ, Crapo JD, Wouters EF, Celli BR, Silverman EK, Demeo DL. Genome-Wide
555 Association Analysis of Body Mass in Chronic Obstructive Pulmonary Disease. *Am J*
556 *Respir Cell Mol Biol* 2010;doi:10.1165/rcmb.2010-0294OC.

557 67. Diaz AA, Come CE, Ross JC, San José Estépar R, Han MK, Loring SH, Silverman EK,
558 Washko GR. Association between airway caliber changes with lung inflation and
559 emphysema assessed by volumetric CT scan in subjects with COPD. *Chest* 2012;141:736–
560 44.

561 68. Smith BM, Hoffman EA, Rabinowitz D, Bleecker E, Christenson S, Couper D, Donohue
562 KM, Han MK, Hansel NN, Kanner RE, Kleerup E, Rennard S, Barr RG. Comparison of
563 spatially matched airways reveals thinner airway walls in COPD. The Multi-Ethnic Study
564 of Atherosclerosis (MESA) COPD Study and the Subpopulations and Intermediate
565 Outcomes in COPD Study (SPIROMICS). *Thorax* 2014;doi:10.1136/thoraxjnl-2014-
566 205160.

567 69. Repapi E, Sayers I, Wain L V, Burton PR, Johnson T, Obeidat M, Zhao JH, Ramasamy A,
568 Zhai G, Vitart V, Huffman JE, Igl W, Albrecht E, Deloukas P, Henderson J, Granell R,
569 McArdle WL, Rudnicka AR, Barroso I, Loos RJF, Wareham NJ, Mustelin L, Rantanen T,
570 Surakka I, Imboden M, Wichmann HE, Grkovic I, Jankovic S, Zgaga L, *et al.* Genome-
571 wide association study identifies five loci associated with lung function. *Nat Genet*
572 2010;42:36–44.

573 70. Wilk JB, Chen TH, Gottlieb DJ, Walter RE, Nagle MW, Brandler BJ, Myers RH, Borecki
574 IB, Silverman EK, Weiss ST, O'Connor GT. A genome-wide association study of
575 pulmonary function measures in the Framingham Heart Study. *Am J Respir Crit Care*
576 *Med*, 2009/03/21 ed. 2009;5:e1000429.

577 71. Hancock DB, Eijgelsheim M, Wilk JB, Gharib SA, Loehr LR, Marciante KD,
578 Franceschini N, van Durme YMTA, Chen T-HH, Barr RG, Schabath MB, Couper DJ,
579 Brusselle GG, Psaty BM, van Duijn CM, Rotter JI, Uitterlinden AG, Hofman A, Punjabi
580 NM, Rivadeneira F, Morrison AC, Enright PL, North KE, Heckbert SR, Lumley T,
581 Stricker BHC, O'Connor GT, London SJ. Meta-analyses of genome-wide association
582 studies identify multiple loci associated with pulmonary function. *Nat Genet*, 2009/12/17
583 ed. 2009;42:45–52.

584

Tables

Table 1: Baseline characteristics of subjects with quantitative imaging phenotypes. Cases = GOLD Grade 2 or more severe (e.g. NETT) cases; Controls = GOLD 0 smoking controls; Non-cases: includes GOLD 0, 1, and PRISm subjects.

	COPDGene non-Hispanic Whites		COPDGene African Americans		ECLIPSE		NETT	GenKOLS (Norway)	
	Non-cases	Cases	Non-cases	Cases	Controls	Cases	Cases	Controls	Cases
n	3062	3243	2132	901	145	1393	332	406	417
Age	59.7 (8.6)	64.4 (8.3)	53 (6)	58.6 (8.1)	57.3 (9.4)	63.4 (7)	67.4 (5.9)	55.6 (9.4)	64.2 (9.3)
Pack-years	39.7 (21.5)	54.4 (27.5)	36.6 (20.5)	42 (23.1)	31.8 (26.6)	49.8 (26.7)	65.8 (30.8)	19.8 (14.1)	31 (18.2)
Sex (%Male)	1462 (47.7%)	1832 (56.5%)	1209 (56.7%)	497 (55.2%)	85 (58.6%)	911 (65.4%)	212 (63.9%)	216 (53.2%)	263 (63.1%)
Current smokers	1263 (41.2%)	1199 (37%)	1838 (86.2%)	595 (66%)	58 (40%)	480 (34.5%)	0	164 (40.4%)	210 (50.4%)
FEV_1, % predicted	91.3 (14.8)	57.4 (23)	92.2 (16.5)	59.5 (22)	108.6 (13.4)	47.4 (15.5)	28.2 (7.3)	94.9 (9.2)	52.5 (16.9)
%LAA-950	1.2 (0-26.9)	7.5 (0-61.9)	0.7 (0-35.8)	4.6 (0-61.2)	2.3 (0.1-14.2)	16.3 (0.1-58.7)	15 (0.3-49.9)	0.5 (0-34.4)	7 (0-53.2)
Perc15, HU	-909.9 (22.8)	-938.1 (26.8)	-893.4 (28.1)	-926.5 (32)	-906.2 (25.9)	-950.9 (25.9)	-949.7 (17.8)	-891.6 (26.3)	-932.8 (30.2)
Pi10, mm	3.64 (0.11)	3.69 (0.14)	3.69 (0.13)	3.73 (0.15)	4.34 (0.15)	4.41 (0.20)	4.58 (0.49)	4.76 (0.29)	4.94 (0.34)
Wall area percent (WAP)	60.2 (2.8)	62.3 (3.1)	61.2 (3.3)	62.9 (3.3)	63.2 (3.7)	65.6 (4.1)	73.2 (3.8)	74.8 (2.9)	76.1 (3)
Gas trapping, %	9.3 (0-83.4)	34 (0.1-87.8)	7.2 (0-70.5)	29.3 (0.2-85.2)					

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Table 2: Genome-wide significant associations. %LAA-950: percent of low attenuation area less than -950 Hounsfield units; Perc15 - Hounsfield Units at the 15th percentile of the density histogram; WAP percentage of the wall area compared to the total bronchial area.

Phenotype	Chr	Marker Name	Closest Gene	Effect Allele	Allele Frequency		Modified Random Effects			Fixed Effects		
Emphysema					Nhw	Aa	P value	Beta	SE	P value	Beta	Se
%LAA-950	4	rs13141641	*HHIP*	T	0.59	0.89	**1.7×10^{-12}**	0.12	0.023	8.4×10^{-13}	0.12	0.018
	15	rs55676755	*CHRNA3*	C	0.63	0.84	**2.4×10^{-9}**	-0.11	0.017	1.4×10^{-9}	-0.11	0.017
	6	rs2070600	*AGER*	T	0.04	0.01	**4.6×10^{-9}**	-0.14	0.11	6.5×10^{-8}	-0.24	0.044
	8	rs75200691	*DLC1*	T	0.88	0.92	**9.7×10^{-9}**	0.15	0.026	5.7×10^{-9}	0.15	0.026
	14	rs45505795	*SERPINA10*	C	0.04	0.008	**1.4×10^{-8}**	-0.31	0.08	9.8×10^{-9}	-0.31	0.053
Perc 15	8	rs74834049	*DLC1*	A	0.12	0.08	**6.0×10^{-10}**	-3.4	0.54	3.3×10^{-10}	-3.4	0.54
	4	rs13141641	*HHIP*	T	0.59	0.89	**8.4×10^{-10}**	-2.2	0.39	4.7×10^{-10}	-2.2	0.36
Airway												
WAP	4	rs142200419	*MIR2054*	T	0.98	N/A	**4.6×10^{-9}**	0.24	1	8.8×10^{-5}	0.9	0.23
Gas trapping												
%	6	rs2070600	*AGER*	T	0.04	0.01	**3.5×10^{-9}**	-0.23	0.039	2.4×10^{-9}	-0.23	0.039
	21	rs55706246	*LINC00310*	A	0.11	0.03	**1.3×10^{-8}**	0.28	0.18	2.1×10^{-7}	0.15	0.029

Table 3: P-values for genetic variants previously reported in genome-wide association analyses(23–26, 39, 40, 69–71)**.** The risk allele for spirometric phenotypes denotes the allele associated with a lower FEV_1 or FEV_1/FVC ratio, and thus would be expected to increase risk for COPD. The sign associated with the P-values denotes whether the direction of association is consistent with the direction for COPD (increase in %LAA-950, Pi10, wall area percent, or gas trapping; decrease in Perc15). In Table 3b, results are grouped by whether the smaller directional P-value was found in emphysema phenotypes (top) or airway-related phenotypes (bottom). Genome-wide significant loci from **Table 2** (e.g. *HHIP*) are not included here. All refers to all subjects, case refers to all cases (GOLD 1-4 or 2-4).

Table 3a: Variants from GWAS of moderate-to-severe or severe COPD

SNP	Chr	Locus	Risk Allele	Emphysema				Airway				Gas Trapping	
				%LAA-950		Perc15		Pi10		Wall Area Percent			
				All	Case	All	Case	All	Case	All	Case	All	Case
rs626750	11	*MMP12*	G	2x10-5	**4x10-7**	6x10-6	7x10-7	-0.1	-0	0.2	-0.1	0.008	0.1
rs4846480	1	*TGFB2*	A	**2x10-6**	3x10-5	1x10-4	5x10-4	-0.7	-0.4	0.2	-0.9	3x10-4	0.009
rs7937	19	*RAB4B*	T	**2x10-6**	0.03	6x10-5	0.03	0.9	-0.08	0.4	-0.04	9x10-4	0.2
rs754388	14	*RIN3*	C	**3x10-5**	0.1	5x10-5	0.04	0.4	-0.5	0.04	-0.6	0.003	0.1
rs7671167	4	*FAM13A*	T	3x10-4	0.3	**2x10-4**	0.07	0.6	-0.8	0.1	-0.5	9x10-5	0.6

Table 3b: Variants from GWAS of lung function

SNP	Chr	Locus	Risk Allele	Emphysema				Airway				Gas Trapping	
				%LAA-950		Perc15		Pi10		Wall Area Percent			
				All	Case	All	Case	All	Case	All	Case	All	Case
rs153916	5	*SPATA9-RHOBTB3*	T	0.001	0.02	**2x10-5**	0.02	-0.2	-0.3	0.9	-0.7	0.002	0.1
rs1529672	3	*RARB*	C	8x10-4	0.06	**2x10-4**	0.08	0.5	-1	0.1	0.9	2x10-4	0.03
rs2284746	1	*MFAP2*	G	0.002	0.2	**0.002**	0.1	-0.06	-0.5	0.9	1	8x10-4	0.07
rs12899618	15	*THSD4*	A	**0.003**	0.2	0.02	0.3	0.7	0.4	0.02	0.3	0.003	0.6
rs7765379	6	*HLA-DQB1*	T	**0.004**	0.05	0.04	0.08	-0.4	-0.5	-0.4	-0.2	0.2	0.9
rs9978142	21	*KCNE2-LINC00310*	T	**0.005**	0.06	0.04	0.07	-0.01	-0.05	-0.5	-0.9	0.04	0.004

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

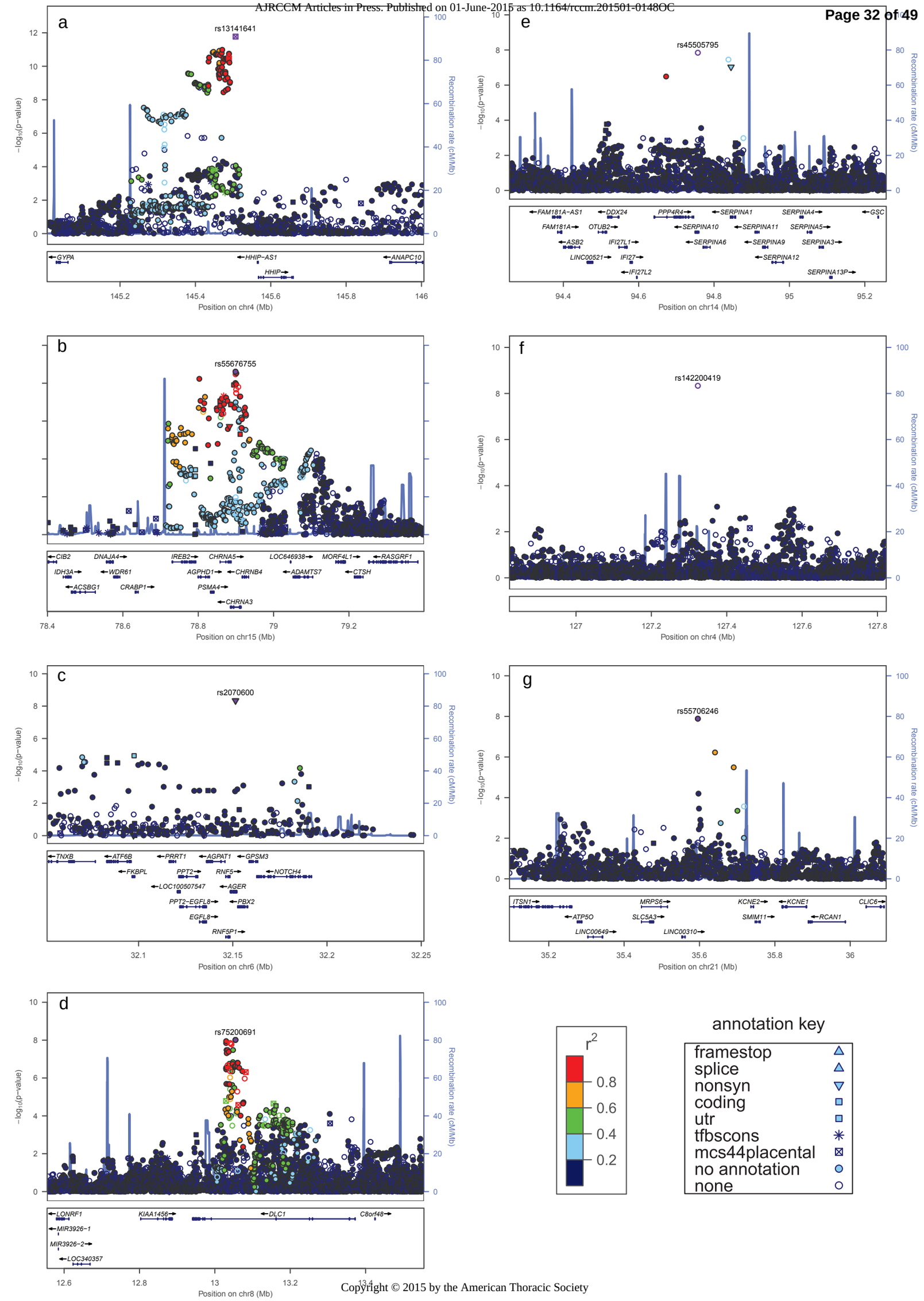
rs3817928	6	*GPR126*	A	**0.01**	0.5	0.01	0.8	-0.1	-0.3	0.4	0.4	0.006	0.2
rs1036429	12	*CCDC38*	C	0.04	0.03	**0.01**	0.06	-0.5	-0.5	0.1	0.5	0.04	0.4
rs11134779	5	*ADAM19*	G	0.02	0.1	**0.01**	0.2	0.5	0.3	0.5	-0.7	0.04	0.08
rs11172113	12	*LRP1*	T	**0.04**	-0.9	0.2	-0.6	0.4	0.6	0.5	0.09	9x10-5	0.2
rs993925	1	*TGFB2-LYPLAL1*	C	0.2	-0.3	**0.1**	-0.1	-0.8	-0.6	-1	-0.4	0.004	0.9
rs7594321	2	*DNER*	C	0.2	0.6	**0.1**	0.8	-0.4	0.3	-0.5	-1	0.07	0.2
rs2798641	6	*ARMC2*	T	0.5	0.3	0.6	-0.4	0.1	0.03	**8x10-4**	0.004	0.06	-0.7
rs10516526	4	*GSTCD/INTS12/NPNT*	A	0.4	-0.3	0.4	-0.2	0.04	0.009	**0.001**	0.003	0.006	0.3
rs11168048	5	*HTR4*	T	0.05	0.5	0.09	0.8	0.06	0.2	**0.002**	0.07	0.3	-0.5
rs2865531	16	*CFDP1*	A	-1	-0.7	-0.9	-0.8	0.08	0.4	**0.007**	0.07	0.3	-0.3
rs2571445	2	*TNS1*	A	0.4	0.2	-0.3	0.4	1	-0.5	**0.008**	0.1	-0.2	-0.7
rs11654749	17	*KCNJ2*	T	-0.1	-0.05	-0.09	-0.04	0.4	-0.5	**0.02**	1	-0.5	-0.3
rs1344555	3	*MECOM*	T	-0.8	-1	-0.5	-0.8	0.5	0.7	0.3	**0.05**	-0.1	0.9
rs2857595	6	*NCR3-AIF1*	A	0.9	0.6	0.7	0.3	0.3	0.09	0.3	**0.06**	-0.6	0.6
rs11001819	10	*C10orf11*	G	-0.04	-0.01	-0.02	-0	0.7	0.8	**0.07**	0.1	-1	-0.1
rs16909898	9	*PTCH1*	G	-1	-0.1	0.7	-0.2	0.5	-0.8	**0.2**	-0.9	0.1	-0.9
rs12447804	16	*MMP15*	T	-0.2	-0.3	-0.3	-0.3	0.6	0.5	0.7	**0.2**	-0.6	-0.6
rs7068966	10	*CDC123*	C	-0.5	-0.5	0.8	-1	-0.1	-0.1	**0.2**	0.9	0.8	-0.7
rs6903823	6	*ZKSCAN3*	G	-0.7	0.9	1	0.8	0.7	0.9	0.9	-0.9	-0.4	-0.7
rs12477314	2	*HDAC4-FLJ43879*	C	-0.3	-0.08	-0.3	-0.1	-0.01	-0.05	-0.6	-0.5	-0.7	-0.1

Figures

Figure 1: Local association plots for genome-wide significant loci. a-e**)** %LAA-950, f) wall area percent, g) % gas trapping.

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Additional data are available in the Supplement.



AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Title

A Genome-wide Association Study of Emphysema and Airway Quantitative Imaging Phenotypes

Michael H. Cho[1,2], Peter J. Castaldi[1], Craig P. Hersh[1,2], Brian D. Hobbs[1,2], R. Graham Barr[4], Ruth Tal-Singer[5], Per Bakke[6], Amund Gulsvik[6], Raúl San José Estépar[3], Edwin Van Beek[7], Harvey O. Coxson[8], David A. Lynch[9], George R. Washko[2], Nan M. Laird[10], James D. Crapo[9], Terri H. Beaty[11], Edwin K. Silverman[1,2], on behalf of the NETT Genetics, ECLIPSE, and COPDGene Investigators

[1]Channing Division of Network Medicine, [2]Division of Pulmonary and Critical Care Medicine, and [3]Laboratory of Mathematics in Imaging, Department of Radiology, Brigham and Women's Hospital, Boston, MA; [4]Department of Medicine, College of Physicians and Surgeons, and Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, [5]GlaxoSmithKline Research and Development, King Of Prussia, PA,[6]Department of Clinical Science, University of Bergen, Norway, [7]Queens Medical Research Institute, University of Edinburgh, Department of Radiology and Department of Biomedical Engineering, University of Iowa; [8]Department of Radiology, University of British Columbia, Vancouver, Canada [9]National Jewish Health, Denver, CO; [10]Harvard School of Public Health, Boston, MA; [11]Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD.

Supplemental Data

Supplemental Methods

Study Populations

Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE; SCO104960, NCT00292552, www.eclipse-copd.com): ECLIPSE cases and controls were aged 40-75 with at least a 10 pack-year smoking history without other respiratory diseases and without known alpha-1 antitrypsin deficiency. Cases were GOLD Grade 2 and above (post-bronchodilator forced expiratory volume in 1 second (FEV_1) < 80% predicted and FEV_1/forced vital capacity (FVC) < 0.7); controls had no evidence of obstruction and FEV_1 > 85% predicted. Details of the ECLIPSE study have been previously published(1).

Genotyping was performed using the Illumina HumanHap 550 V3 (Illumina, San Diego, CA), and BeadStudio quality control, including reclustering on project samples was performed following Illumina guidelines. Quality control was performed using Python (www.python.org) and R (www.r-project.org) scripts in conjunction with plink (v1.05). Subjects and markers with a call rate of < 95% were excluded. Population stratification exclusion and adjustment on self-reported white subjects was performed using EIGENSOFT Version 2.0. Details of the genotyping and previous genome-wide association have been published(2). Imputation was updated using MaCH and minimac with the 1000 Genomes Phase I v3 EUR reference panel as previously described(3), resulting in a total of 11,040,911 variants with Rsq > 0.3.

Low-dose (120kVp and 40mAs) CT scans were performed at baseline, 1 year, and 3 year time points; baseline scans were used for the current analysis. All scans were performed using multidetector CT scans (GE Healthcare, Milwaukee, Wis. or Siemens Healthcare, Erlangen,

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Germany) and images were reconstructed using 1.0mm (Siemens) or 1.25mm (GE) contiguous slices and an intermediate spatial frequency reconstruction algorithm. CT scanners were calibrated regularly using standard water calibration phantoms. All CT scans were analyzed at the University of British Columbia using Pulmonary Workstation 2.0 software (VIDA Diagnostics, Coralville, IA, U.S.A.). Airways were segmented using a region growing algorithm using the third (segmental) to fifth generation airways(4, 5). Wall area percent was calculated using the mean value of measurements for selected segmental airways (the same as used for COPDGene below) across all lobes.

National Emphysema Treatment Trial (NETT, www.nhlbi.nih.gov/health/prof/lung/nett/): NETT subjects had severe airflow obstruction by post-bronchodilator spirometry (FEV1 < 45% predicted) and evidence of emphysema on computed tomography (CT). Subjects with significant sputum production or bronchiectasis were excluded. Details of the NETT trial have been published(6).

For the NETT Genetics Ancillary Study, we genotyped a subset of 382 self-reported white subjects without severe alpha-1 antitrypsin deficiency with available blood for genotyping who provided written consent. Genotyping was performed using the Illumina Quad 610 array (Illumina, San Diego, CA), with quality control, population stratification adjustment, and imputation procedures as previously described previously(2). A separate set of principal components was calculated for the NETT cases. Imputation was updated using MaCH and minimac with the 1000 Genomes Phase I v3 EUR reference panel(3) , resulting in a total of 10,659,967 variants with Rsq > 0.3.

NETT CT scans were performed on one of three types of scanners (General Electric, Fairfield, CT; Siemens, Malvern, PA; or Picker International, Toronto, ON, Canada) with a range of 2- to 8-mm slice thickness, with 75% of the scan data from 4 to 5 mm. Densitometric measures were performed with the Pulmonary Analysis Software Suite (PASS, Iowa City, IA). Airway measurements were obtained using 3D Slicer (www.Slicer.org) and Airway Inspector (www.airwayinspector.org) at Brigham and Women's Hospital. The full width at half-maximum (FWHM) method was used to measure the wall thickness and wall area of each airway.

Norway (GenKOLS, Genetics of Chronic Obstructive Lung Disease, GSK code RES11080): GenKOLS cases and controls had at least a > 2.5 pack year smoking history. Cases had post-bronchodilator FEV1 < 80% predicted and FEV1/FVC < 0.7, while controls had normal spirometry. Subjects with severe alpha-1 antitrypsin deficiency and other lung diseases (aside from asthma) were excluded. Details of the GenKOLS study have been previously published(7).

Genotyping was performed using Illumina HumanHap 550 arrays (Illumina, San Diego, CA), with quality control, population stratification adjustment, and imputation procedures as described previously. A separate set of principal components was calculated for the subset of subjects with CT imaging data. Imputation was updated using MaCH and minimac with the 1000 Genomes Phase I v3 EUR reference panel(3), resulting in a total of 10,657,975 variants with Rsq > 0.3.

High-resolution CT chest scans were performed on a subset of the cohort using a GE LightSpeed Ultra. A low spatial frequency reconstruction algorithm was used for density measurements, and a high spatial frequency algorithm (bone) for airway measurements. Images were analyzed at the James Hogg iCAPTURE Centre (Vancouver, BC, Canada). Emphysema extent was assessed on lung images segmented using a modified boarder tracing algorithm with prior position

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

knowledge, and the extent of emphysema was assessed using the percentage of lung voxels with attenuation values less than -950 Hounsfield units (HU). Airways with an internal perimeter > 6mm were identified on the CT scans and measured using the Full Width at Half Maximum algorithm. Details on the imaging techniques in GenKOLS have been previously described(8).

COPDGene (NCT00608764, www.copdgene.org). COPDGene subjects were of non-Hispanic white or African-American ancestry, aged 45-80 years old, with a minimum of 10 pack-years of smoking, and without a history of lung disease other than asthma. Subjects found to have evidence of other lung disease on CT, such as significant bronchiectasis or interstitial lung disease, were excluded from the current analysis. Genotyping was performed by Illumina (San Diego, CA) on the HumanOmniExpress array, with quality control and imputation as previously described(3), resulting in a total of 11,437,352 variants for non-Hispanic whites and 22,904,273 for African-Americans with Rsq > 0.3.

CT chest imaging was performed on all subjects using a standardized protocol(9). Quantitative analysis utilized the lower-spatial-resolution smooth reconstruction algorithm. Analysis of emphysema severity was performed on segmented lung images by using the Slicer software package (http://www.slicer.org/). Emphysema percentage was defined as all lung voxels with a CT attenuation value of less than −950 HU. Airway analysis was performed by using the VIDA Pulmonary Workstation, version 2.0 (Vida Diagnostics, Coralville, Iowa, http://www.vidadiagnostics.com/). Measurements were obtained along the center line of the lumen, in the middle third of the airway segment, for one segmental airway of each lung lobe including the lingula; the mean value across all lobes was used for analysis. Details of the imaging techniques have been described previously(10).

Additional Genetic Analysis Methods

Imputed genotypes were included for analysis if they had an Rsq of 0.3 or greater. Individual genetic variants were included in the meta-analysis if they were missing in no more than one study (except for gas trapping, where the variant was required to be present in both COPDGene populations); variants with minor allele frequency < 1% overall or < 0.5% in individual studies were excluded, resulting in 6.9 (gas trapping) to 7.6 million (all other phenotypes) total analyzed variants. All variants were oriented to the '+' strand of the hg19 reference assembly. P-values were not adjusted for multiple comparisons.

Our primary analyses were performed in all subjects, with a method used to specifically address ascertainment. We additionally assessed the impact of each of the top variants in cases and non-cases separately using the same methods as for the overall meta-analysis. For results in the *SERPINA10* locus, we performed a meta-analysis conditioning on the *SERPINA1* Z allele by performing a linear regression including this SNP as a covariate in the model, and performing a meta-analysis on the target SNP.

To determine whether loci previously described in association with lung function were enriched for nominally significant ($P < 0.05$) associations in our quantitative imaging, we performed a Fisher's exact test. To determine whether any of the variants that we identified in this analysis were expression quantitative trait loci in lung, we searched the published dataset of Hao et al (11) and data from the GTeX consortium. Since Hao et al report only significant genotyped loci, we searched for variants in linkage disequilibrium with our top-reported variants using plink. VEGAS version 0.8.27 (12) gene-based analysis was performed using the CEU reference

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

haplotypes and including the top 20 percent of SNPs for a given gene. For the GRAIL (http://www.broadinstitute.org/mpg/grail/grail.php)(13) analysis, HapMap2 CEU variants were pruned for linkage disequilibrium using plink(14) with 250kb windows and an r^2 of 0.1. Results with overall P-value < $1x10^{-4}$ were input as seed and query regions, including text from PubMed articles up to May 2012. For the analysis using iGSEA4GWAS(15), default settings of 500kb up and downstream boundaries and canonical pathways was used. For DEPICT(16) (http://www.broadinstitute.org/mpg/depict/), SNPs were pruned to 500kb boundaries with an r2 of 0.05. For INRICH(17), input files were pruned using an r2 of 0.05 using a range of 20kb up and downstream with 10,000 replicates. MAGENTA(18) was run using version July 2011, under default settings. Overlap between results from these analyses was examined using an FDR < 0.05 for iGSEA4GWAS, P < 0.005 for DEPICT, P < 0.05 for INRICH, and nominal GSEA 75^{th} percentile P < 0.05, to allow similar number of results in each dataset.

For the analysis of enhancer and promoter enrichment in ENCODE data, we used Haploreg v2(19), using SNPs with GWAS P-values of < $1x10^{-6}$ for the top GWAS results, an r^2 of 0.8 and using 1000 Genomes EUR Pilot data as background for enrichment. Briefly, Haploreg calculates enrichment using the background set of variants to determine the level of overlap of specifically annotated regions from the ENCODE project, and calculates an uncorrected binomial P-value.

Linkage disequilibrium between SNPs was estimated using the 1000 Genomes reference data in SNAP(20), the 1000 Genomes EUR reference data, or (for the calculation with the reported *DLC1* variant) the imputed genotypes in the African-American COPDGene samples, and calculated using plink. All chromosomal positions are given using the NCBI37/hg19 assembly, and alleles are referenced to the + strand.

Supplemental Results

Genome-wide Association Quality Control

None of the individual genome-wide association results for each cohort and phenotype demonstrated evidence of substantial inflation of p-values (λ_{GC} range 1.0 – 1.02). For the meta-analyses, the fixed effects analysis for Pi10 in all subjects demonstrated minimal evidence of inflation (λ_{GC} =1.06, λ_{GC1000} =1.01), the remainder of both fixed and modified random effects studies did not show evidence of inflation (λ_{GC} = 1.02).

Supplemental Tables

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Table S1: Detailed results for the top genome-wide association results. Results given for each cohort. For the analyses involving all subjects, the second line shows the P-values from the SPREG(21) analysis (for COPDGene and Norway) or for cases only (ECLIPSE).

Phenotype	Cohort	Closest Gene	Marker Name	COPDGene non-Hispanic Whites			COPDGene African-Americans			ECLIPSE			NETT			Norway		
				Beta	SE	P-value	Beta	SE	P-value	Beta	SE	P-value	Beta	SE	P-value	Beta	SE	P-value
%LAA-950	All	*HHIP*	rs13141641	0.16	0.024	7.6x10-11	0.11	0.056	0.059	0.11	0.038	0.0031	0.004	0.067	0.95	0.12	0.076	0.12
						5.5x10-9			0.082			0.12						0.15
		CHRNA3	rs55676755	-0.13	0.025	9.4x10-8	-0.094	0.045	0.037	-0.092	0.039	0.018	-0.02	0.066	0.76	-0.097	0.077	0.21
						3.8x10-6			0.1			0.015						0.47
		AGER	rs2070600	-0.35	0.058	1.6x10-9	-0.22	0.18	0.21	-0.22	0.1	0.029	0.26	0.14	0.065	-0.1	0.18	0.56
						1.1x10-8			0.2			0.042						0.41
		DLC1	rs75200691	0.16	0.037	2.6x10-5	0.18	0.063	0.0042	0.13	0.057	0.027	0.11	0.097	0.25	0.17	0.11	0.11
						3.5x10-5			0.0057			0.082						0.1
		SERPINA10	rs45505795	-0.28	0.074	1.7x10-4	-0.56	0.23	0.013	-0.39	0.1	0.00011	-0.064	0.16	0.7	-0.64	0.21	0.0024
						4.4x10-4			0.033			0.0011						0.0069
Perc15, HU	All	*DLC1*	rs74834049	-3.3	0.7	3.0x10-6	-3.6	1.4	0.011	-3	1.5	0.052	-3.5	2.1	0.095	-5.3	2.3	0.02
						3.8x10-6			0.015			0.15						0.017
		HHIP	rs13141641	-2.5	0.45	1.7x10-8	-1	1.2	0.42	-3	0.99	0.0022	-0.25	1.4	0.86	-1.8	1.5	0.23
						9.3x10-7			0.52			0.041						0.3
WAP, %	All	*MIR2054*	rs142200419	1.3	0.27	1.1x10-6				1.8	0.67	0.0093	0.56	1.4	0.7	-2.8	0.71	6.9x10-5
						8.0x10-6						0.0016						7.7x10-5
Gas trapping, %	All	*AGER*	rs2070600	-0.24	0.042	1.4x10-8	-0.13	0.15	0.39									
						2.0x10-8			0.2									
		LINC00310	rs55706246	0.11	0.03	2.3x10-4	0.45	0.099	4.7x10-6									
						1.0x10-4			3.2x10-7									

Table S2: Additional results from each genome-wide study. Results with P < 1x10-6 in either the modified random effects or fixed effects analysis are shown.

Phenotype	Group	Chr	Marker Name	Closest Gene	Effect Allele	Allele Frequency		Modified Random Effrects			Fixed Effects		
Emphysema						Nhw	Aa	P value	Beta	SE	P value	Nhw	Aa
%LAA-950, %	All	9	rs3919995	*ZNF462*	A	0.59	0.5	1.3x10-7	-0.081	0.023	8.1x10-8	-0.088	0.016
		20	rs183345681	*CHRNA4*	A	0.23	0.18	1.8x10-7	-0.12	0.023	1.1x10-7	-0.12	0.023
		14	rs117167774	*LOC100506433*	T	0.013	0.013	1.8x10-7	0.47	0.23	0.00013	0.33	0.086
		2	rs360488	*FAM84A*	A	0.23	0.082	3.7x10-7	0.09	0.038	3.0x10-7	0.11	0.021
		1	rs7512679	*TGFB2*	T	0.24	0.47	4.5x10-7	0.092	0.018	2.9x10-7	0.092	0.018
		8	rs7823498	*NRG1*	T	0.79	0.73	4.6x10-7	-0.098	0.019	3.1x10-7	-0.098	0.019
		11	rs7947523	*MIR4300*	C	0.68	0.44	4.9x10-7	-0.086	0.048	0.00014	-0.064	0.017
		20	rs2070755	*PCK1*	C	0.49	0.4	5.3x10-7	0.11	0.047	0.00041	0.058	0.016
		8	rs10109725	*CSMD1*	T	0.03	0.0069	6.3x10-7	0.28	0.14	8.6x10-6	0.25	0.055
		5	rs924633	*DNAH5*	A	0.95	0.92	9.2x10-7	0.18	0.092	8.1x10-5	0.14	0.036
		4	rs62343714	*LOC401164*	T	0.092	0.16	1.2x10-6	0.12	0.036	8.8x10-7	0.13	0.026
		19	rs7937	*MIA-RAB4B*	T	0.57	0.3	1.5x10-6	-0.08	0.016	9.7x10-7	-0.08	0.016
	Cases	11	rs608194	*MMP12*	T	0.18	0.33	1.4x10-7	0.05	0.074	2.9x10-5	0.11	0.027
		6	rs72971709	*GRIK2*	A	0.013	0.0029	2.6x10-7	0.38	0.31	2.6x10-5	0.44	0.1
		18	rs12605822	*ANKRD12*	A	0.13	0.11	3.6x10-7	0.17	0.072	3.0x10-6	0.15	0.031
		14	rs3811345	*LINC00617*	A	0.87	0.86	4.4x10-7	0.16	0.03	2.8x10-7	0.16	0.03
		15	rs9788721	*AGPHD1*	T	0.62	0.62	5.5x10-7	-0.1	0.025	3.5x10-7	-0.11	0.021
		1	rs72482608	*PRRX1*	A	0.62	0.52	7.6x10-7	-0.11	0.021	4.8x10-7	-0.11	0.021
		5	rs13184316	*ARL15*	A	0.23	0.05	8.2x10-7	0.07	0.1	0.78	-0.0073	0.027
Perc15, HU	All	1	rs72637224	*XCL2*	T	0.05	0.14	3.3x10-7	3.6	1.2	2.1x10-7	3.5	0.68
		16	rs9933712	*ERCC4*	A	0.021	0.38	4.2x10-7	5.2	1.8	2.6x10-7	3.7	0.72
		20	rs183345681	*CHRNA4*	A	0.23	0.18	4.7x10-7	2.4	0.47	3.0x10-7	2.4	0.47
		12	rs75751297	*FLJ31485*	A	0.47	0.36	6.6x10-7	2.4	0.48	4.2x10-7	2.4	0.48
		11	rs7125940	*MIR4300*	T	0.34	0.58	6.9x10-7	-1.9	1	8.3x10-5	-1.4	0.35
		15	rs144442299	*UNC13C*	T	0.018	0.0051	7.8x10-7	-5.4	2.9	5.4x10-7	-7.4	1.5
		20	rs2070755	*PCK1*	C	0.49	0.4	8.5x10-7	-2.5	1.1	0.0092	-0.88	0.34

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

		3	rs111646341	*LSAMP*	A	0.97	0.98	9.0x10-7	5.8	1.8	5.7x10-7	5.6	1.1
		14	rs45505795	*SERPINA10*	C	0.038	0.0076	9.5x10-7	6.4	2.7	2.6x10-6	5.2	1.1
		4	rs10016562	*TRPC3*	T	0.62	0.73	1.0x10-6	1.6	0.5	6.4x10-7	1.7	0.35
		8	rs7823498	*NRG1*	T	0.79	0.73	1.0x10-6	2	0.4	6.4x10-7	2	0.4
		15	rs9788721	*AGPHD1*	T	0.62	0.62	1.1x10-6	1.7	0.35	6.7x10-7	1.7	0.35
		6	rs2647050	*HLA-DQB1*	T	0.65	0.65	1.2x10-6	1.6	0.48	7.7x10-7	1.7	0.35
		20	rs6080212	*KIF16B*	A	0.16	0.15	1.4x10-6	-2.2	0.45	8.8x10-7	-2.2	0.45
	Cases	10	rs139326003	*MBL2*	A	0.12	0.089	1.6x10-7	4.2	0.95	1.2x10-7	3.9	0.74
		11	rs185888204	*OR8B3*	A	0.11	0.11	1.9x10-7	-7.1	3	2.5x10-6	-6.1	1.3
		15	rs503464	*CHRNA5*	A	0.22	0.27	2.5x10-7	-3.2	0.6	1.5x10-7	-3.2	0.6
		18	rs12605822	*ANKRD12*	A	0.13	0.11	4.0x10-7	-3.3	1.4	5.3x10-7	-3.6	0.71
		1	rs72482608	*PRRX1*	A	0.62	0.52	5.0x10-7	2.5	0.48	3.2x10-7	2.5	0.48
		11	rs654600	*MMP12*	A	0.83	0.72	5.2x10-7	-1.7	1.6	5.1x10-5	-2.5	0.63
		4	rs13140744	*TRPC3*	T	0.38	0.26	8.9x10-7	-2.2	0.64	5.7x10-7	-2.4	0.48
		1	rs75565482	*XCL2*	A	0.95	0.91	1.1x10-6	5.1	1.6	7.1x10-7	5.2	1.1
		14	rs3811345	*LINC00617*	A	0.87	0.86	1.5x10-6	-3.4	0.7	9.5x10-7	-3.4	0.7
Airway													
Pi10	All	8	rs13281609	*CSMD3*	T	0.047	0.0079	3.2x10-7	-0.044	0.01	2.2x10-7	-0.043	0.0082
		11	rs113835537	*CTSF*	A	0.84	0.83	8.5x10-7	0.012	0.0023	5.4x10-7	0.012	0.0023
		1	rs654950	*HIVEP3*	C	0.42	0.12	8.6x10-7	-0.011	0.0055	3.5x10-6	-0.0089	0.0019
	Cases	3	rs168302	*GRM7*	T	0.66	0.87	9.8x10-8	-0.016	0.004	6.0x10-8	-0.017	0.0032
		9	rs4877691	*FAM75D1*	A	0.24	0.38	6.6x10-7	-0.017	0.0078	2.0x10-6	-0.016	0.0034
		2	rs115089939	*LOC647012*	T	0.99	1	1.1x10-6	-0.086	0.017	7.2x10-7	-0.086	0.017
		5	rs79581221	*ATG10*	T	0.014	0.0017	1.1x10-6	-0.077	0.016	7.4x10-7	-0.077	0.016
WAP	All	1	rs12724666	*PDZK1P1*	A	0.033	0.0092	8.7x10-8	1.1	0.2	5.9x10-8	1.1	0.2
		8	rs2513900	*AZIN1*	C	0.51	0.74	2.6x10-7	0.23	0.043	1.7x10-7	0.23	0.043
		17	rs3826538	*RPA1*	T	0.072	0.27	1.5x10-6	-0.35	0.071	9.3x10-7	-0.35	0.071
	Cases	3	rs76493322	*GRM7*	A	0.46	0.45	3.1x10-7	-0.36	0.069	2.0x10-7	-0.36	0.069
		2	rs10932600	*ATIC*	A	0.62	0.73	1.3x10-6	-0.32	0.065	8.4x10-7	-0.32	0.065
		1	rs61797053	*KIAA1324*	A	0.067	0.019	1.5x10-6	0.67	0.14	9.5x10-7	0.67	0.14
Gas Trapping													

All	4	rs1512281	*HHIP-AS1*	A	0.59	0.88	2.3x10-7	0.082	0.016	1.9x10-7	0.082	0.016
	8	rs74834049	*DLC1*	A	0.11	0.082	6.1x10-7	0.12	0.024	5.0x10-7	0.12	0.024
	1	rs6669119	*PAX7*	T	0.1	0.12	9.90E-07	-0.14	0.062	1.60E-06	-0.11	0.024
	8	rs2844036	*ANKRD46*	A	0.78	0.88	1.10E-06	-0.11	0.022	8.60E-07	-0.11	0.022
	10	rs655766	*BAMBI*	T	0.28	0.22	1.20E-06	0.08	0.016	9.90E-07	0.08	0.016
Cases	12	rs10875912	*MLL2*	T	0.66	0.67	8.30E-08	-0.091	0.017	7.10E-08	-0.091	0.017
	20	rs430086	*MACROD2*	A	0.98	0.86	2.50E-07	0.16	0.16	9.80E-06	0.19	0.044
	2	rs72822868	*SNAR-H*	T	0.91	0.98	5.20E-07	0.23	0.046	4.30E-07	0.23	0.046
	12	rs2460882	*SP1*	T	0.84	0.38	6.40E-07	0.11	0.022	5.30E-07	0.11	0.022
	11	rs1789001	*OR9G4*	A	0.57	0.43	6.80E-07	0.079	0.024	5.60E-07	0.084	0.017
	6	rs12527942	*MRPL14*	T	0.03	0.048	9.40E-07	0.32	0.34	0.033	0.1	0.047
	8	rs13259853	*CSMD1*	A	0.44	0.099	1.00E-06	-0.088	0.022	8.30E-07	-0.09	0.018
	17	rs12449664	*NTN1*	A	0.14	0.084	1.10E-06	0.15	0.03	9.10E-07	0.15	0.03

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

Table S3: Lookup of top quantitative CT association results in all subjects within separate analyses in COPD cases and non-cases.

Phenotype	Chr	Marker Name	Closest Gene	Effect Allele	Cases						Non-cases					
					Modified Random Effects			Fixed Effects			Modified Random Effects			Fixed Effects		
					P value	Beta	SE	P value	Beta	Se	P value	Beta	SE	P value	Beta	Se
%LAA-950	4	rs13141641	*HHIP*	T	**4.4×10^{-5}**	0.09	0.030	3.6×10^{-5}	0.09	0.021	**2.0×10^{-2}**	0.05	0.021	1.5×10^{-2}	0.05	0.021
	15	rs55676755	*CHRNA3*	C	**3.2×10^{-6}**	-0.08	0.034	3.1×10^{-6}	-0.09	0.021	**5.1×10^{-1}**	0.02	0.022	4.4×10^{-1}	0.02	0.022
	6	rs2070600	*AGER*	T	**1.9×10^{-2}**	-0.08	0.095	3.1×10^{-2}	-0.12	0.054	**1.9×10^{-3}**	-0.11	0.126	4.4×10^{-3}	-0.18	0.073
	8	rs75200691	*DLC1*	T	**6.3×10^{-3}**	0.09	0.032	4.4×10^{-3}	0.09	0.032	**1.4×10^{-4}**	0.12	0.032	9.4×10^{-5}	0.12	0.032
	14	rs45505795	*SERPINA10*	C	**3.9×10^{-4}**	-0.21	0.056	2.6×10^{-4}	-0.21	0.056	**1.8×10^{-2}**	-0.18	0.073	1.4×10^{-2}	-0.18	0.073
Perc 15	8	rs74834049	*DLC1*	A	**7.5×10^{-4}**	-2.6	0.74	5.1×10^{-4}	-2.6	0.74	**2.7×10^{-4}**	-0.27	0.81	1.9×10^{-4}	-2.4	0.064
	4	rs13141641	*HHIP*	T	**8.3×10^{-5}**	-2.0	0.49	5.5×10^{-5}	-2.0	0.49	**2.7×10^{-1}**	-0.53	0.43	2.2×10^{-1}	-0.52	0.43
Airway																
WAP	4	rs142200419	*MIR2054*	T	**3.1×10^{-4}**	0.30	1	3.9×10^{-2}	0.7	0.34	**3.7×10^{-3}**	-0.29	1.23	1.7×10^{-2}	0.71	0.30
Gas trapping																
%	6	rs2070600	*AGER*	T	**5.2×10^{-3}**	-0.15	0.083	4.4×10^{-3}	-0.13	0.047	**5.1×10^{-4}**	-0.18	0.05	4.3×10^{-4}	-0.18	0.050
	21	rs55706246	*LINC00310*	A	**3.7×10^{-3}**	0.15	0.099	3.2×10^{-3}	0.09	0.032	**2.2×10^{-2}**	0.19	0.159	3.9×10^{-2}	0.08	0.038

Table S4: Top overall quantitative CT loci not previously reported in case-control association analyses for moderate-to-severe and severe COPD in COPDGene, ECLIPSE, GenKOLS, and NETT/NAS(3)

Chr	Marker Name	Closest Gene	Effect Allele	Moderate-To-Severe COPD		Severe COPD	
				P-value	Beta	P-value	Beta
6	rs2070600	*AGER*	T	2.9×10^{-4}	-0.35	1.4×10^{-5}	-0.45
8	rs75200691	*DLC1*	T	0.35	0.05	0.21	0.08
8	rs74834049	*DLC1*	A	0.39	-0.04	0.20	-0.08
14	rs45505795	*SERPINA10*	C	3.4×10^{-5}	0.42	1.6×10^{-5}	0.51
4	rs142200419	*MIR2054*	T	0.25	0.19	0.47	0.14

21	rs55706246	*LINC00310*	A	6.5×10^{-3}	-0.16	1.2×10^{-2}	-0.18

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

References

1. Vestbo J, Anderson W, Coxson HO, Crim C, Dawber F, Edwards L, Hagan G, Knobil K, Lomas DA, MacNee W, Silverman EK, Tal-Singer R. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). *Eur Respir J* 2008;31:869–73.

2. Cho MH, Boutaoui N, Klanderman BJ, Sylvia JS, Ziniti JP, Hersh CP, DeMeo DL, Hunninghake GM, Litonjua AA, Sparrow D, Lange C, Won S, Murphy JR, Beaty TH, Regan EA, Make BJ, Hokanson JE, Crapo JD, Kong X, Anderson WH, Tal-Singer R, Lomas DA, Bakke P, Gulsvik A, Pillai SG, Silverman EK. Variants in FAM13A are associated with chronic obstructive pulmonary disease. *Nat Genet*, 2010/02/23 ed. 2010;42:200–202.

3. Cho MH, McDonald M-LN, Zhou X, Mattheisen M, Castaldi PJ, Hersh CP, Demeo DL, Sylvia JS, Ziniti J, Laird NM, Lange C, Litonjua AA, Sparrow D, Casaburi R, Barr RG, Regan EA, Make BJ, Hokanson JE, Lutz S, Dudenkov TM, Farzadegan H, Hetmanski JB, Tal-Singer R, Lomas DA, Bakke P, Gulsvik A, Crapo JD, Silverman EK, Beaty TH. Risk loci for chronic obstructive pulmonary disease: a genome-wide association study and meta-analysis. *lancet Respir Med* 2014;2:214–25.

4. Coxson HO, Dirksen A, Edwards LD, Yates JC, Agusti A, Bakke P, Calverley PM, Celli B, Crim C, Duvoix A, Fauerbach PN, Lomas DA, Macnee W, Mayer RJ, Miller BE, Müller NL, Rennard SI, Silverman EK, Tal-Singer R, Wouters EF, Vestbo J. The presence and progression of emphysema in COPD as determined by CT scanning and biomarker expression: a prospective analysis from the ECLIPSE study. *lancet Respir Med* 2013;1:129–36.

5. Gietema HA, Müller NL, Fauerbach PVN, Sharma S, Edwards LD, Camp PG, Coxson HO. Quantifying the extent of emphysema: factors associated with radiologists' estimations and quantitative indices of emphysema severity using the ECLIPSE cohort. *Acad Radiol* 2011;18:661–71.

6. Fishman A, Martinez F, Naunheim K, Piantadosi S, Wise R, Ries A, Weinmann G, Wood DE. A randomized trial comparing lung-volume-reduction surgery with medical therapy for severe emphysema. *N Engl J Med* 2003;348:2059–73.

7. Pillai SG, Ge D, Zhu G, Kong X, Shianna K V, Need AC, Feng S, Hersh CP, Bakke P, Gulsvik A, Ruppert A, Lodrup Carlsen KC, Roses A, Anderson W, Rennard SI, Lomas DA, Silverman EK, Goldstein DB. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet*, 2009/03/21 ed. 2009;5:e1000421.

8. Grydeland TB, Dirksen A, Coxson HO, Eagan TML, Thorsen E, Pillai SG, Sharma S, Eide GE, Gulsvik A, Bakke PS. Quantitative computed tomography measures of

emphysema and airway wall thickness are related to respiratory symptoms. *Am J Respir Crit Care Med* 2010;181:353–9.

9. Regan EA, Hokanson JE, Murphy JR, Make B, Lynch DA, Beaty TH, Curran-Everett D, Silverman EK, Crapo JD. Genetic epidemiology of COPD (COPDGene) study design. *COPD*, 2010/03/11 ed. 7:32–43.

10. Han MK, Kazerooni EA, Lynch DA, Liu LX, Murray S, Curtis JL, Criner GJ, Kim V, Bowler RP, Hanania NA, Anzueto AR, Make BJ, Hokanson JE, Crapo JD, Silverman EK, Martinez FJ, Washko GR, The COPDGene Investigators F. Chronic obstructive pulmonary disease exacerbations in the COPDGene study: associated radiologic phenotypes. *Radiology* 2011;261:274–82.

11. Hao K, Bossé Y, Nickle DC, Paré PD, Postma DS, Laviolette M, Sandford A, Hackett TL, Daley D, Hogg JC, Elliott WM, Couture C, Lamontagne M, Brandsma C-A, van den Berge M, Koppelman G, Reicin AS, Nicholson DW, Malkov V, Derry JM, Suver C, Tsou JA, Kulkarni A, Zhang C, Vessey R, Opiteck GJ, Curtis SP, Timens W, Sin DD. Lung eQTLs to help reveal the molecular underpinnings of asthma. *PLoS Genet* 2012;8:e1003029.

12. Liu JZ, Mcrae AF, Nyholt DR, Medland SE, Wray NR, Brown KM, Investigators A, Hayward NK, Montgomery GW, Visscher PM. A Versatile Gene-Based Test for Genome-wide Association Studies. *Am J Hum Genet* 2010;87:139–45.

13. Raychaudhuri S, Plenge RM, Rossin EJ, Ng ACY, Purcell SM, Sklar P, Scolnick EM, Xavier RJ, Altshuler D, Daly MJ. Identifying relationships among genomic disease regions: predicting genes at pathogenic SNP associations and rare deletions. *PLoS Genet* 2009;5:e1000534.

14. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, De Bakker PIW, Daly MJ, others. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559–575.

15. Zhang K, Cui S, Chang S, Zhang L, Wang J. i-GSEA4GWAS: a web server for identification of pathways/gene sets associated with traits by applying an improved gene set enrichment analysis to genome-wide association study. *Nucleic Acids Res*, 2010/05/04 ed. 2010;38:W90–5.

16. Wood AR, Esko T, Yang J, Vedantam S, Pers TH, Gustafsson S, Chu AY, Estrada K, Luan J, Kutalik Z, Amin N, Buchkovich ML, Croteau-Chonka DC, Day FR, Duan Y, Fall T, Fehrmann R, Ferreira T, Jackson AU, Karjalainen J, Lo KS, Locke AE, Mägi R, Mihailov E, Porcu E, Randall JC, Scherag A, Vinkhuyzen AAE, Westra H-J, *et al.* Defining the role of common variation in the genomic and biological architecture of adult human height. *Nat Genet* 2014;advance on:1173–86.

AJRCCM Articles in Press. Published on 01-June-2015 as 10.1164/rccm.201501-0148OC

17. Lee PH, O'Dushlaine C, Thomas B, Purcell SM. INRICH: interval-based enrichment analysis for genome-wide association studies. *Bioinformatics* 2012;28:1797–9.

18. Segre A V, Groop L, Mootha VK, Daly MJ, Altshuler D. Common inherited variation in mitochondrial genes is not enriched for associations with type 2 diabetes or related glycemic traits. *PLoS Genet*, 2010/08/18 ed. 2010;6.:

19. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 2012;40:D930–4.

20. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PIW. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 2008;24:2938–9.

21. Lin DY, Zeng D. Proper analysis of secondary phenotype data in case-control association studies. *Genet Epidemiol* 2009;33:256–65.