ORIGINAL ARTICLE

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

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Abstract

Rationale: Chronic obstructive pulmonary disease (COPD) is defined by the presence of airflow limitation on spirometry, yet subjects with COPD can have marked differences in computed tomography imaging. These differences may be driven by genetic factors. We hypothesized that a genome-wide association study (GWAS) 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.

Objectives: To identify genetic determinants of quantitative imaging phenotypes.

Methods: We performed a GWAS on two quantitative emphysema and two quantitative airway imaging phenotypes in the COPDGene (non-Hispanic white and African American), ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints), NETT (National Emphysema Treatment Trial), and GenKOLS (Genetics of COPD, Norway) studies and on percentage gas trapping in COPDGene. We also examined specific loci reported as genome-wide significant for spirometric phenotypes related to airflow limitation or COPD.

Measurements and Main Results: The total sample size across all cohorts was 12,031, of whom 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.

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.

Keywords: emphysema; airway; genetics; chronic obstructive pulmonary disease

(Received in original form January 24, 2015; accepted in final form May 28, 2015)

Author Contributions: Study design: M.H.C., C.P.H., R.G.B., R.T.-S., P.B., A.G., H.O.C., D.A.L., G.R.W., N.M.L., J.D.C., T.H.B., and E.K.S. Phenotype acquisition and quality control: M.H.C., P.J.C., C.P.H., R.G.B., R.T.-S., P.B., A.G., R.S.J.E., E.J.R.V.B., H.O.C., D.A.L., G.R.W., J.D.C., and E.K.S. Genotype data acquisition and quality control: M.H.C., P.J.C., T.H.B., and E.K.S. Data analysis: M.H.C., P.J.C., B.D.H., N.M.L., T.H.B., and E.K.S. Critical revision of the manuscript: all authors.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 192, Iss 5, pp 559–569, Sep 1, 2015

Originally Published in Press as DOI: 10.1164/rccm.201501-0148OC on June 1, 2015
Internet address: www.atsjournals.org

At a Glance Commentary

Scientific Knowledge on the Subject: Chronic obstructive pulmonary disease (COPD) is a complex and heterogeneous disease. Quantitative image analysis of chest computed tomography 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.

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 airway disease (1). With the advent of standardized quantitative measurements, chest computed tomography (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 monogenic diseases predominantly involving the airway (in cystic fibrosis) or in those that involve the parenchyma through emphysema (α_1-antitrypsin deficiency and cutis laxa) (14). Previous genome-wide studies have identified variants associated with emphysema (15–17) or airway disease (18), although generally in smaller sample sizes or predominantly population-based subjects.

We hypothesized that quantitative imaging reflects component disease processes leading to airflow obstruction in COPD and could have genetic determinants not discovered by analyses using lung function alone. To address this hypothesis, we performed a genome-wide association study (GWAS) of quantitative emphysema and airway phenotypes in current and former cigarette smokers with and without COPD. We additionally hypothesized that 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 American subjects, 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. Control subjects had normal spirometry. Cases in the ECLIPSE and GenKOLS studies were at least Global Initiative for Chronic Obstructive Lung Disease 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 percentage low attenuation area, using a threshold of −950 Hounsfield units (HU) (%LAA−950) to estimate emphysema, and, alternatively, the HU at the 15th percentile of the density histogram (Perc15). The airway wall area (Pi10) was the value for a hypothetical 10-mm internal perimeter 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 was the percentage of the wall area compared with the total bronchial area for segmental and smaller airways (*see* online supplement). Percent gas trapping was measured at end-exhalation and defined as

Supported by NHLBI grants 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 by 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 composed of AstraZeneca, Boehringer Ingelheim, Novartis, Pfizer, GlaxoSmithKline, Siemens, and Sunovion. The National Emphysema Treatment Trial was supported by NHLBI grants 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) and the ECLIPSE study (NCT00292552; GSK code SCO104960) were funded by GlaxoSmithKline.

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the percentage of lung voxels with density less than −856 HU (28).

We genotyped all subjects on Illumina platforms and imputed genotypes using MaCH and minimac (29) with 1,000 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 nonnormality were log-transformed (%LAA−950 and percentage 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 metaanalysis. 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 Global Initiative for Chronic Obstructive Lung Disease 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 online supplement.

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 metaanalysis are shown in Table 2 and Figure 1. 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 ($P < 5 \times 10^{-8}$) near *DLC1* and *SERPINA10*. An association near *CHRNA4* was just below genome-wide significance (rs183345681, $P = 1.8 \times 10^{-7}$). 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 $P < 5 \times 10^{-8}$ (rs142200419); however, this association was markedly attenuated in the fixed effects metaanalysis, due to effects in the opposite direction in one of the cohorts (*see* Table E1 in the online supplement). 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 E2). 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 control subjects in this cohort). *P* values obtained using this method (32) (Table E1) 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 control subjects separately and, for loci not previously described as genome-wide significant in COPD, a case–control analysis are shown in Tables E3 and E4.

The association with %LAA−950 near *SERPINA10* is also near *SERPINA1*, variants in which are the cause of α_1-antitrypsin deficiency. The most common form of severe α_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 American subjects). 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. All seven of these subjects were correctly identified with imputed genotypes. Linkage disequilibrium exists between our top associated single-nucleotide polymorphism 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 metaanalysis conditioned on rs28929474. The resulting *P* value was 0.007, demonstrating that rs28929474 accounts for some, but not all, of the association signal. Although known or identified ZZ homozygotes were excluded from COPDGene, NETT, and GenKOLS, ECLIPSE excluded only known α_1-antitrypsin–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 that the ECLIPSE *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 described in this study not previously associated with COPD, one was a *cis*-expression quantitative trait locus in lung: rs55706246 near *LINC00310* was in modest linkage disequilibrium ($r^2 = 0.24$) with rs2834438, an expression quantitative trait locus for *KCNE2* ($P = 3.1 \times 10^{-7}$) (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 ($P < 1 \times 10^{-6}$) could yield insights about cell types based on regulatory data in 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, $P = 6.0 \times 10^{-4}$) and DNase I hypersensitivity sites in several types of endothelial cells ($P = 6.6 \times 10^{-3}$ 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 ($P = 0.04$).

Table 1. Baseline Characteristics of Subjects with Quantitative Imaging Phenotypes

	COPDGene Non-Hispanic White Subjects		COPDGene African American Subjects		ECLIPSE		NETT	GenKOLS (Norway)	
	Noncases	Cases	Noncases	Cases	Control Subjects	Cases	Cases	Control Subjects	Cases
n	3,062	3,243	2,132	901	145	1,393	332	406	417
Age, yr	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)
Men, n (%)	1,462 (47.7)	1,832 (56.5)	1,209 (56.7)	497 (55.2)	85 (58.6)	911 (65.4)	212 (63.9)	216 (53.2)	263 (63.1)
Current smokers, n (%)	1,263 (41.2)	1,199 (37)	1,838 (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	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)	—	—	—	—	—

Definition of abbreviations: %LAA−950 = percentage low attenuation area, using a threshold of −950 HU; COPD = chronic obstructive pulmonary disease; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; GenKOLS = Genetics of COPD, Norway; GOLD = Global Initiative for Chronic Obstructive Lung Disease; HU = Hounsfield units; NETT = National Emphysema Treatment Trial; Perc15 = HU at the 15th percentile of the density histogram; Pi10 = airway wall area: the value for a hypothetical 10-mm airway obtained by plotting a regression line of the square root of the airway wall area versus the airway internal perimeter.

Values given as n (%), mean (SD) or, for nonnormal variables, median (range). Cases are GOLD grade 1 or more severe (e.g., NETT) cases; control subjects are GOLD grade 0 smoking control subjects; noncases include GOLD 0 and PRISm (preserved ratio, impaired spirometry) subjects.

Table 2. Genome-Wide Significant Associations

Phenotype	Chr	Marker Name	Closest Gene	Effect Allele	Allele Frequency		Modified Random Effects			Fixed Effects		
					Nhw	Aa	*P* Value	β	SE	*P* Value	β	SE
Emphysema												
%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
Perc15	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

Definition of abbreviations: %LAA−950 = percentage low attenuation area, using a threshold of −950 HU; Aa = African American; Chr = chromosome; HU = Hounsfield units; N/A = not applicable; Nhw = non-Hispanic white; Perc15 = HU at the 15th percentile of the density histogram; WAP = percentage of the wall area compared to the total bronchial area.

We also sought to determine whether our results were consistent with a set of genes more likely to act within 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 acetylcholine channel activity, actin cytoskeleton, and B-cell receptor signaling for emphysema GWAS, and for airway, WNT signaling and muscle contraction.

Associations at Loci Previously Identified in Association with COPD or COPD-related Spirometric Phenotypes

GWASs have identified multiple variants associated with COPD (23–26, 38) or measures of lung function (39–41). We sought to determine whether there was evidence these variants might have an effect on quantitative imaging phenotypes, even if they did not reach genome-wide significance. After excluding loci previously associated in these cohorts with COPD, we found a strong enrichment in nominally significant ($P < 0.05$) loci among the two emphysema and two imaging phenotypes ($P = 4.9 \times 10^{-9}$), suggesting many of these variants may also affect quantitative imaging measurements. We further classified these variants into those showing a stronger association (by one-sided *P* value) with emphysema- or airway-related phenotypes, assigning directionality such that the risk allele for COPD or reduced lung function demonstrated greater emphysema or increased airway wall thickness (Tables 3 and 4). Enrichment for nominally significant *P* values appeared to be greater among markers associated

Table 3. Variants from Genome-Wide Association Studies of Moderate to Severe or Severe Chronic Obstructive Pulmonary Disease

Marker Name	Chr	Closest Gene	Risk Allele	Emphysema				Airway					
				%LAA−950		Perc15		Pi10		Wall Area Percent		Gas Trapping	
				All	Case	All	Case	All	Case	All	Case	All	Case
rs626750	11	*MMP12*	G	2×10^{-5}	4×10^{-7}	6×10^{-6}	7×10^{-7}	−0.1	−0	0.2	−0.1	0.008	0.1
rs4846480	1	*TGFB2*	A	2×10^{-6}	3×10^{-5}	1×10^{-4}	5×10^{-4}	−0.7	−0.4	0.2	−0.9	3×10^{-4}	0.009
rs7937	19	*RAB4B*	T	2×10^{-6}	0.03	6×10^{-5}	0.03	0.9	−0.08	0.4	−0.04	9×10^{-4}	0.2
rs754388	14	*RIN3*	C	3×10^{-5}	0.1	5×10^{-5}	0.04	0.4	−0.5	0.04	−0.6	0.003	0.1
rs7671167	4	*FAM13A*	T	3×10^{-4}	0.3	2×10^{-4}	0.07	0.6	−0.8	0.1	−0.5	9×10^{-5}	0.6

Definition of abbreviations: %LAA−950 = percentage low attenuation area, using a threshold of −950 HU; Chr = chromosome; COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease; HU = Hounsfield units; Perc15 = HU at the 15th percentile of the density histogram; Pi10 = airway wall area: the value for a hypothetical 10-mm airway obtained by plotting a regression line of the square root of the airway wall area versus the airway internal perimeter.

P values for genetic variants previously reported in genome-wide association analyses (23–26, 39, 40, 41, 69, 70). 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). Genome-wide significant loci from Table 2 (e.g., *HHIP*) are not included here. "All" refers to all subjects, and "Case" refers to all cases (GOLD 1–4 or 2–4).

Table 4. Variants from Genome-Wide Association Studies of Lung Function

Marker Name	Chr	Closest Gene(s)	Risk Allele	Emphysema				Airway					
				%LAA−950		Perc15		Pi10		Wall Area Percent		Gas Trapping	
				All	Case	All	Case	All	Case	All	Case	All	Case
rs153916	5	*SPATA9/RHOBTB3*	T	0.001	0.02	2×10^{-5}	0.02	−0.2	−0.3	0.9	−0.7	0.002	0.1
rs1529672	3	*RARB*	C	8×10^{-4}	0.06	2×10^{-4}	0.08	0.5	−1	0.1	0.9	2×10^{-4}	0.03
rs2284746	1	*MFAP2*	G	0.002	0.2	0.002	0.1	−0.06	−0.5	0.9	1	8×10^{-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
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	9×10^{-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	8×10^{-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

Definition of abbreviations: %LAA−950 = percentage low attenuation area, using a threshold of −950 Hounsfield units; Chr = chromosome; COPD = chronic obstructive pulmonary disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease; Perc15 = Hounsfield units at the 15th percentile of the density histogram; Pi10 = airway wall area: the value for a hypothetical 10-mm airway obtained by plotting a regression line of the square root of the airway wall area versus the airway internal perimeter.

P values for genetic variants previously reported in genome-wide association analyses (23–26, 39, 40, 41, 69, 70). 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). Genome-wide significant loci from Table 2 (e.g., *HHIP*) are not included here. "All" refers to all subjects, and "Case" refers to all cases (GOLD 1–4 or 2–4). Results are grouped by whether the smaller directional *P* value was found in emphysema phenotypes (top) or airway-related phenotypes (bottom). Additional data are available in the online supplement.

with quantitative emphysema ($P = 1.9 \times 10^{-6}$) versus those associated with airway wall thickness ($P = 1.3 \times 10^{-3}$).

We next examined regulatory patterns using Haploreg (35) in variants classified as either emphysema or airway associated identified in Tables 2–4. "Emphysema" variants were modestly enriched for enhancers seen in hepatocellular carcinoma (HepG2, $P = 0.05$), whereas those more strongly associated with airway phenotypes were enriched for enhancers from lung fibroblasts (NHLF) and epidermal keratinocytes (NHEK, $P = 0.03$–0.04). Both analyses were enriched for mammary epithelial cells (HMEC, $P = 2.5 \times 10^{-4}$ to 1.6×10^{-3}) and umbilical vein endothelial cells (HUVEC, $P = 0.02$–0.03). The most significant DNase enrichment for emphysema-associated variants was lung-derived lymphatic microvascular endothelial cells (HMVEC-LLy; $P = 8 \times 10^{-4}$), whereas top results for airway-associated variants were embryonic lung fibroblasts (WI-38), mammary fibroblasts (HMF), and small airway epithelial cells (SAEC; $P = 3.6$–6.6×10^{-4}). Emphysema-associated DNase results were not significant in the airway results, and vice versa.

Discussion

In a GWAS 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 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

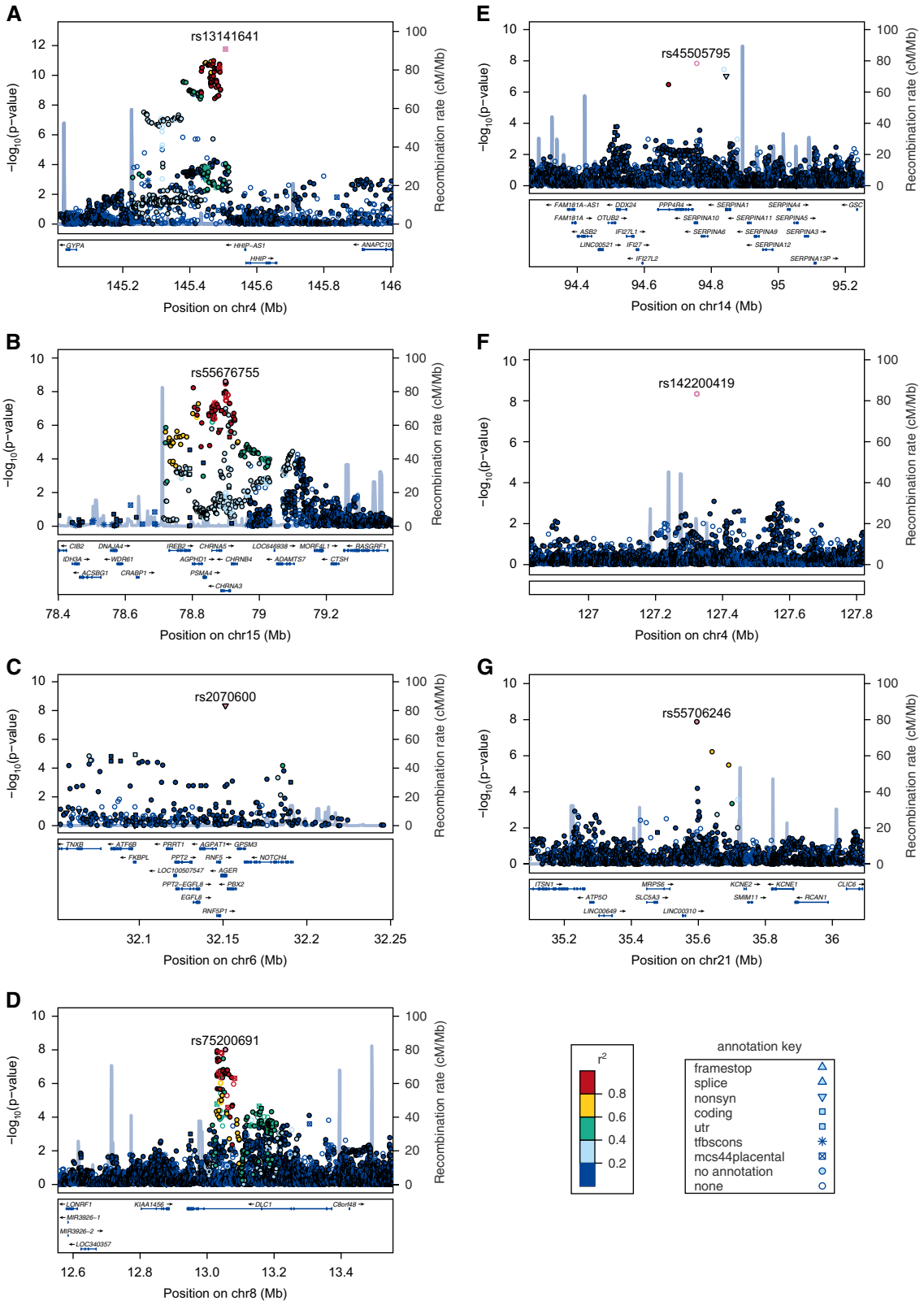

Figure 1. Local association plots for genome-wide significant loci. (*A–E*) Percentage low attenuation area, using a threshold of −950 Hounsfield units; (*F*) wall area percent; (*G*) percentage gas trapping. mcs44 = in a region highly conserved in placental mammals; tfbscons = in a conserved region predicted to be a transcription factor binding site; utr = untranslated region.

emphasizes the role of α_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 apoptosis (46) and acts as a tumor suppressor through RhoGAP-dependent and RhoGAP-independent activity (47). *DLC1* is highly expressed in the lung (48, 49). In a study of regional emphysema, *DLC1* showed a trend toward 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 American subjects (51). We found a trend toward association with current smoking at this locus in COPDGene African American subjects ($P = 0.06$–0.07). However, we found no association with pack-years of smoking ($P > 0.49$). In addition, *DLC1* single-nucleotide polymorphisms in this smoking behavior study are approximately 200 kb away and not in linkage disequilibrium with our reported *DLC1* loci ($r^2 < 0.004$ in COPDGene African American subjects), 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), although 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 spirometric 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.

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 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 (Multiethnic Study of Atherosclerosis) 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 interreader 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 body mass index 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. Although 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 semisupervised 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 nonoverlapping enrichment of regulatory regions for our two sets of analyses. Our results are consistent with the hypothesis that emphysema and 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 with 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, GWASs 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 GWASs 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. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Burrows B, Fletcher CM, Heard BE, Jones NL, Wootliff JS. The emphysematous and bronchial types of chronic airways obstruction: a clinicopathological study of patients in London and Chicago. *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, *et al*. Computed tomographic measurements of airway dimensions and emphysema in smokers. Correlation with lung function. *Am J Respir Crit Care Med* 2000;162:1102–1108.
3. Mets OM, Buckens CFM, Zanen P, Isgum I, van Ginneken B, Prokop M, Gietema HA, Lammers J-WJ, Vliegenthart R, Oudkerk M, *et al*. Identification of chronic obstructive pulmonary disease in lung cancer screening computed tomographic scans. *JAMA* 2011;306: 1775–1781.
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;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: 353–359.
6. Bon J, Fuhrman CR, Weissfeld JL, Duncan SR, Branch RA, Chang C-CH, Zhang Y, Leader JK, Gur D, Greenspan SL, *et al*. Radiographic emphysema predicts low bone mineral density in a tobacco-exposed cohort. *Am J Respir Crit Care Med* 2011;183:885–890.
7. Wilson DO, Weissfeld JL, Balkan A, Schragin JG, Fuhrman CR, Fisher SN, Wilson J, Leader JK, Siegfried JM, Shapiro SD, *et al*. Association of radiographic emphysema and airflow obstruction with lung cancer. *Am J Respir Crit Care Med* 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, *et al*.; COPDGene Investigators. Chronic obstructive pulmonary disease exacerbations in the COPDGene study: associated radiologic phenotypes. *Radiology* 2011;261:274–282.
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, *et al*. CT-quantified emphysema in male heavy smokers: association with lung function decline. *Thorax* 2011;66:782–787.
10. Nishimura M, Makita H, Nagai K, Konno S, Nasuhara Y, Hasegawa M, Shimizu K, Betsuyaku T, Ito YM, Fuke S, *et al*.; Hokkaido COPD Cohort Study Investigators. 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;104:1890–1895.
12. Patel BD, Coxson HO, Pillai SG, Agustí AGN, Calverley PMA, Donner CF, Make BJ, Müller NL, Rennard SI, Vestbo J, *et al*.; International COPD Genetics Network. Airway wall thickening and emphysema show independent familial aggregation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2008;178:500–505.
13. Zhou JJ, Cho MH, Castaldi PJ, Hersh CP, Silverman EK, Laird NM. Heritability of chronic obstructive pulmonary disease and related phenotypes in smokers. *Am J Respir Crit Care Med* 2013;188: 941–947.
14. Urban Z, Gao J, Pope FM, Davis EC. Autosomal dominant cutis laxa with severe lung disease: synthesis and matrix deposition of mutant tropoelastin. *J Invest Dermatol* 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, *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;189:408–418.
16. Castaldi PJ, Cho MH, San José Estépar R, McDonald M-LN, Laird N, Beaty TH, Washko G, Crapo JD, Silverman EK; COPDGene Investigators. Genome-wide association identifies regulatory Loci associated with distinct local histogram emphysema patterns. *Am J Respir Crit Care Med* 2014;190:399–409.
17. Kong X, Cho MH, Anderson W, Coxson HO, Muller N, Washko G, Hoffman EA, Bakke P, Gulsvik A, Lomas DA, *et al*.; ECLIPSE Study NETT Investigators. Genome-wide association study identifies BICD1 as a susceptibility gene for emphysema. *Am J Respir Crit Care Med* 2011;183:43–49.
18. Dijkstra AE, Postma DS, van Ginneken B, Wielpütz MO, Schmidt M, Becker N, Owsijewitsch M, Kauczor H-U, de Koning HJ, Lammers JW, *et al*. Novel genes for airway wall thickness identified with combined genome-wide association and expression analyses. *Am J Respir Crit Care Med* 2015;191:547–556.
19. Cho MH, Castaldi PJ, Hersh CP, Hobbs BD, Barr RG, Tal-Singer R, Bakke P, Gulsvik A, San José Estépar R, Van Beek EJ, *et al*.; NETT Genetics, ECLIPSE, and COPDGene Investigators. A genome-wide association study of emphysema and airway quantitative imaging phenotypes [abstract]. *Am J Respir Crit Care Med* 2015;191: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;7:32–43.
21. Vestbo J, Anderson W, Coxson HO, Crim C, Dawber F, Edwards L, Hagan G, Knobil K, Lomas DA, MacNee W, *et al*.; ECLIPSE investigators. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). *Eur Respir J* 2008;31: 869–873.
22. Fishman A, Martinez F, Naunheim K, Piantadosi S, Wise R, Ries A, Weinmann G, Wood DE; National Emphysema Treatment Trial Research Group. A randomized trial comparing lung-volume-reduction

surgery with medical therapy for severe emphysema. *N Engl J Med* 2003;348:2059–2073.
23. Pillai SG, Ge D, Zhu G, Kong X, Shianna KV, Need AC, Feng S, Hersh CP, Bakke P, Gulsvik A, *et al*.; ICGN Investigators. A genome-wide association study in chronic obstructive pulmonary disease (COPD): identification of two major susceptibility loci. *PLoS Genet* 2009;5: e1000421.
24. Cho MH, Boutaoui N, Klanderman BJ, Sylvia JS, Ziniti JP, Hersh CP, DeMeo DL, Hunninghake GM, Litonjua AA, Sparrow D, *et al*. Variants in FAM13A are associated with chronic obstructive pulmonary disease. *Nat Genet* 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, *et al*.; ICGN Investigators; ECLIPSE Investigators; COPDGene Investigators. A genome-wide association study of COPD identifies a susceptibility locus on chromosome 19q13. *Hum Mol Genet* 2012;21:947–957.
26. Cho MH, McDonald M-LN, Zhou X, Mattheisen M, Castaldi PJ, Hersh CP, Demeo DL, Sylvia JS, Ziniti J, Laird NM, *et al*.; NETT Genetics, ICGN, ECLIPSE and COPDGene Investigators. Risk loci for chronic obstructive pulmonary disease: a genome-wide association study and meta-analysis. *Lancet Respir Med* 2014;2:214–225.
27. DeMeo DL, Hersh CP, Hoffman EA, Litonjua AA, Lazarus R, Sparrow D, Benditt JO, Criner G, Make B, Martinez FJ, *et al*. 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 Jr, 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;44:955–959.
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;88:586–598.
31. de Bakker PI, Ferreira MA, 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–R128.
32. Lin DY, Zeng D. Proper analysis of secondary phenotype data in case-control association studies. *Genet Epidemiol* 2009;33:256–265.
33. Hao K, Bossé Y, Nickle DC, Paré PD, Postma DS, Laviolette M, Sandford A, Hackett TL, Daley D, Hogg JC, *et al*. 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–D934.
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;38: W90–W95.
37. Lee PH, O'Dushlaine C, Thomas B, Purcell SM. INRICH: interval-based enrichment analysis for genome-wide association studies. *Bioinformatics* 2012;28:1797–1799.
38. Wilk JB, Shrine NRG, Loehr LR, Zhao JH, Manichaikul A, Lopez LM, Smith AV, Heckbert SR, Smolonska J, Tang W, *et al*. Genome-wide association studies identify CHRNA5/3 and HTR4 in the development of airflow obstruction. *Am J Respir Crit Care Med* 2012; 186:622–632.
39. Soler Artigas M, Loth DW, Wain LV, Gharib SA, Obeidat M, Tang W, Zhai G, Zhao JH, Smith AV, Huffman JE, *et al*.; International Lung Cancer Consortium; GIANT consortium. Genome-wide association and large-scale follow up identifies 16 new loci influencing lung function. *Nat Genet* 2011;43:1082–1090.
40. Hancock DB, Artigas MS, Gharib SA, Henry A, Manichaikul A, Ramasamy A, Loth DW, Imboden M, Koch B, McArdle WL, *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 LV, Burton PR, Johnson T, Obeidat M, Zhao JH, Ramasamy A, Zhai G, Vitart V, *et al*.; Wellcome Trust Case Control Consortium; NSHD Respiratory Study Team. Genome-wide association study identifies five loci associated with lung function. *Nat Genet* 2010;42:36–44.
42. Cheng DT, Kim DK, Cockayne DA, Belousov A, Bitter H, Cho MH, Duvoix A, Edwards LD, Lomas DA, Miller BE, *et al*.; TESRA and ECLIPSE Investigators. 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;188:948–957.
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; 189:419–427.
44. Sørheim I-C, Bakke P, Gulsvik A, Pillai SG, Johannessen A, Gaarder PI, Campbell EJ, Agustí A, Calverley PMA, Donner CF, *et al*. α_1-Antitrypsin protease inhibitor MZ heterozygosity is associated with airflow obstruction in two large cohorts. *Chest* 2010;138: 1125–1132.
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;40:843–847.
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;23:1308–1313.
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;47:326–337.
48. Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, Zwahlen M, Kampf C, Wester K, Hober S, *et al*. Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol* 2010;28:1248–1250.
49. Lonsdale J, Thomas J, Salvatore M, Phillips R, Lo E, Shad S, Hasz R, Walters G, Garcia F, Young N, *et al*.; GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013;45: 580–585.
50. Campbell JD, McDonough JE, Zeskind JE, Hackett TL, Pechkovsky DV, Brandsma C-A, Suzuki M, Gosselink JV, Liu G, Alekseyev YO, *et al*. 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. *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:421–429.
54. Savale L, Chaouat A, Bastuji-Garin S, Marcos E, Boyer L, Maitre B, Sarni M, Housset B, Weitzenblum E, Matrat M, *et al*. Shortened telomeres in circulating leukocytes of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2009; 179:566–571.
55. Tsuji T, Aoshiba K, Nagai A. Alveolar cell senescence in patients with pulmonary emphysema. *Am J Respir Crit Care Med* 2006;174:886–893.
56. Alder JK, Guo N, Kembou F, Parry EM, Anderson CJ, Gorgy AI, Walsh MF, Sussan T, Biswal S, Mitzner W, *et al*. Telomere length is a determinant of emphysema susceptibility. *Am J Respir Crit Care Med* 2011;184:904–912.

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;189:753–754.
58. Barnes PJ. Emerging pharmacotherapies for COPD. *Chest* 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;143:1025–1035.
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. *J Clin Invest* 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: 734–742.
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;283:29447–29460.
63. Thomashow MA, Shimbo D, Parikh MA, Hoffman EA, Vogel-Claussen J, Hueper K, Fu J, Liu C-Y, Bluemke DA, Ventetuolo CE, *et al*. 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;188:60–68.
64. Stratelis G, Fransson SG, Schmekel B, Jakobsson P, Mölstad S. High prevalence of emphysema and its association with BMI: a study of smokers with normal spirometry. *Scand J Prim Health Care* 2008;26: 241–247.
65. Hoffman EA, Ahmed FS, Baumhauer H, Budoff M, Carr JJ, Kronmal R, Reddy S, Barr RG. Variation in the percent of emphysema-like lung in a healthy, nonsmoking multiethnic sample. The MESA lung study. *Ann Am Thorac Soc* 2014;11:898–907.
66. Wan ES, Cho MH, Boutaoui N, Klanderman BJ, Sylvia JS, Ziniti JP, Won S, Lange C, Pillai SG, Anderson WH, *et al*.; Evaluation of Chronic Obstructive Pulmonary Disease Longitudinally to Identify Predictive Surrogate End-Points (ECLIPSE); Norway-Bergen cohort; National Emphysema Treatment Trial; COPD Gene investigators. Genome-wide association analysis of body mass in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 2011;45:304–310.
67. Diaz AA, Come CE, Ross JC, San José Estépar R, Han MK, Loring SH, Silverman EK, Washko GR; COPDGene Investigators. Association between airway caliber changes with lung inflation and emphysema assessed by volumetric CT scan in subjects with COPD. *Chest* 2012; 141:736–744.
68. Smith BM, Hoffman EA, Rabinowitz D, Bleecker E, Christenson S, Couper D, Donohue KM, Han MK, Hansel NN, Kanner RE, *et al*.; The Multi-Ethnic Study of Atherosclerosis (MESA) COPD Study and the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS). Comparison of spatially matched airways reveals thinner airway walls in COPD. *Thorax* 2014;69: 987–996.
69. Wilk JB, Chen TH, Gottlieb DJ, Walter RE, Nagle MW, Brandler BJ, Myers RH, Borecki IB, Silverman EK, Weiss ST, *et al*. A genome-wide association study of pulmonary function measures in the Framingham Heart Study. *PLoS Genet* 2009;5:e1000429.
70. Hancock DB, Eijgelsheim M, Wilk JB, Gharib SA, Loehr LR, Marciante KD, Franceschini N, van Durme YMTA, Chen T-HH, Barr RG, *et al*. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nat Genet* 2010;42:45–52.