Genome-Wide Association Analysis of Single-Breath DL_{CO}

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Abstract

DL_{CO} is a widely used pulmonary function test in clinical practice and a particularly useful measure for assessing patients with chronic obstructive pulmonary disease (COPD). We hypothesized that elucidating genetic determinants of DL_{CO} could lead to better understanding of the genetic architecture of COPD. We estimated the heritability of DL_{CO} using common genetic variants and performed genome-wide association analyses in four cohorts enriched for subjects with COPD (COPDGene [Genetic Epidemiology of COPD], NETT [National Emphysema Treatment Trial], GenKOLS [Genetics of Chronic Obstructive Lung Disease study], and TESRA [Treatment of Emphysema With a Gamma-Selective Retinoid Agonist study]) using a combined European ancestry white dataset and a COPDGene African American dataset. We assessed our genome-wide significant and suggestive associations for DL_{CO} in previously reported genome-wide association studies of COPD and related traits. We also characterized associations of known COPD-associated variants and DL_{CO}. We estimated the SNP-based heritability of DL_{CO} in the

European ancestry white population to be 22% ($P = 0.0004$). We identified three genome-wide significant associations with DL_{CO}: variants near *TGFB2*, *CHRNA3*, and *PDE11A* loci ($P < 5 \times 10^{-8}$). In addition, 12 loci were suggestively associated with DL_{CO} in European ancestry white ($P < 1 \times 10^{-5}$ in the combined analysis and $P < 0.05$ in both COPDGene and GenKOLS), including variants near *NEGR1*, *CADM2*, *PCDH7*, *RETREG1*, *DACT2*, *NRG1*, *ANKRD18A*, *KRT86*, *NTN4*, *ARHGAP28*, *INSR*, and *PCBP3*. Some DL_{CO}-associated variants were also associated with COPD, emphysema, and/or spirometric values. Among 25 previously reported COPD loci, *TGFB2*, *CHRNA3/CHRNA5*, *FAM13A*, *DSP*, and *CYP2A6* were associated with DL_{CO} ($P < 0.001$). We identified several genetic loci that were significantly associated with DL_{CO} and characterized effects of known COPD-associated loci on DL_{CO}. These results could lead to better understanding of the heterogeneous nature of COPD.

Keywords: chronic obstructive pulmonary disease; DL_{CO}; genome-wide association study

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DL_{CO} is a widely used pulmonary function test in clinical practice (1). It measures the capacity of pulmonary alveolar and capillary surfaces for gas exchange (2). DL_{CO} has been used in diagnosing and determining the severity of multiple lung diseases (1), as well as in determining their prognosis (3) and predicting their response to treatment (4, 5). DL_{CO} is frequently abnormal in chronic obstructive pulmonary disease (COPD) and is associated with emphysema measured from computed tomography (6) as well as with pulmonary vascular disease. DL_{CO} testing can also identify emphysema in smokers with normal spirometry (7), indicating that classifying COPD by the degree of airflow obstruction by spirometry is limited (8, 9). Genetic association studies can provide useful insights into the biological mechanisms of complex diseases (10). This approach has been applied to many respiratory diseases, including COPD (11), and quantitative traits such as emphysema (12) and spirometry (13). Recently, researchers in a genome-wide association study (GWAS) of DL_{CO} reported a significant heritability estimate of DL_{CO} in population-based cohorts, although no genome-wide significant associations for DL_{CO} have been identified (14). We hypothesized that elucidating genetic determinants of DL_{CO} in cohorts including larger proportions of COPD cases could lead to better understanding of the genetic architecture of COPD. To this end, we estimated the heritability of DL_{CO} using common genetic variants and performed genome-wide association analyses in four cohorts enriched for subjects with COPD.

Methods

Study Populations and DL_{CO} Measurements

We included individuals from a study population of current smokers or ex-smokers enriched for COPD cases (COPDGene [Genetic Epidemiology of COPD]), a COPD case-control cohort (GenKOLS [Genetics of Chronic Obstructive Lung Disease study]), and two clinical trials of subjects with COPD (NETT [National Emphysema Treatment Trial] and TESRA [Treatment of Emphysema With a Gamma-Selective Retinoid Agonist study]) in genetic association analysis.

Detailed descriptions of these cohorts and their genotyping procedures have been published previously (12, 15–17). We imputed genotypes using the Haplotype Reference Consortium reference panel version 1.1 via the University of Michigan imputation server (18) for COPDGene, NETT, and TESRA, as well as the Sanger Imputation Server (19) for GenKOLS. We analyzed variants with minor allele frequency of 1% or higher, minimum imputation quality of 0.7, and Hardy-Weinberg equilibrium ($P > 10^{-5}$).

In COPDGene, DL_{CO} was measured at the 5-year follow-up visit using the EasyOne Pro system (nidd Medical Technologies, Inc.). In GenKOLS, DL_{CO} was measured at the baseline visit using SensorMedics V_{max}22D (VIASYS Healthcare/Cardinal Health). In NETT and TESRA, DL_{CO} was measured before randomization using available equipment at each clinical site. Predicted values for DL_{CO} were based on equations published by Miller and colleagues (20). All participating cohorts conformed to European Respiratory Society/American Thoracic Society standards for measurement of single-breath carbon monoxide uptake in the lung (21–23). As previously described (24), we adjusted raw measurements of DL_{CO} for barometric pressure (i.e., altitude at a performing site) “altitude adjusted” before subsequent analyses.

Estimation of SNP-based Heritability

We estimated the heritability of DL_{CO} using a SNP-based method for unrelated subjects (the GREML-LDMS module in GCTA software) (25, 26). SNP-based heritability is the proportion of phenotypic variance explained by any set of SNPs (27). We estimated the heritability separately in European ancestry white (EA) and African American (AA) subjects (see the data supplement). We adjusted for age, sex, height, peripheral blood hemoglobin concentration, and pack-years of smoking. We also stratified the analysis by COPD affection status and sex (see the data supplement).

Genome-Wide Association Analyses

We performed genome-wide association analyses in EA and AA subjects separately (i.e., pooling individuals from COPDGene non-Hispanic white [NHW], GenKOLS, NETT, and TESRA for the former, and only COPDGene AA for the latter). To limit the

impact of statistical outliers, we transformed altitude-adjusted DL_{CO} measurements using inverse normalized transformation. We used linear mixed models with DL_{CO} as the outcome, adjusting for the same covariates as the heritability analysis. We corrected for population substructure and cryptic relatedness using efficient mixed-model association (EMMA) implemented in Efficient and Parallelizable Association Container Toolbox (EPACTS)/EMMA eXpedited (EMMAX) software (28, 29). We examined a quantile-quantile plot for evidence of population stratification. A genome-wide significance threshold at $P < 5 \times 10^{-8}$ was used. To ensure that the signals were supported by both of our largest cohorts, we also determined significance using $P < 0.05$ in both COPDGene NHW and GenKOLS in the combined EA dataset. We also performed sensitivity analysis by adjusting for various covariates (see the data supplement). Details on candidate causal gene and pathway analyses and phenotypic associations are described in the data supplement.

Results

We first assessed the contribution of genetics to DL_{CO} measurements by estimating the heritability. We then performed genome-wide association analyses of DL_{CO} in EA and AA subjects separately. We also examined associations of previously reported genome-wide significant loci for COPD and COPD-related phenotypes.

Characteristics of Study Subjects

In general, subjects in NETT had more severe COPD than other cohorts (lower DL_{CO} and FEV₁ percent predicted) (Table 1). DL_{CO} values were comparable among subjects in COPDGene NHW and GenKOLS, whereas individuals in COPDGene AA, TESRA, and NETT had generally lower values. To maximize the power to discover genetic associations and heritability estimation, we performed the analyses by pooling individuals with the same ancestry, resulting in a combined dataset with EA individuals (COPDGene NHW, GenKOLS, NETT, and TESRA) and a COPDGene AA dataset. In total, we included 5,586 EA and 1,349 AA individuals in the analysis.

Table 1. Baseline Characteristics of Study Subjects, by Cohort

	COPDGene Non-Hispanic White Subjects		COPDGene African American Subjects		Norway GenKOLS		NETT	TESRA
	GOLD 2–4	GOLD 0, 1, PRISm	GOLD 2–4	GOLD 0, 1, PRISm	GOLD 2–4	GOLD 0	GOLD 3–4	GOLD 2–4
<i>n</i>	1,253	2,210	343	1,006	596	792	354	381
Female sex, <i>n</i> (%)	552 (44.05)	1,167 (52.81)	167 (48.69)	503 (50)	201 (33.72)	395 (49.87)	123 (34.75)	116 (30.45)
Current smokers, <i>n</i> (%)	355 (28.33)	594 (26.88)	185 (53.94)	686 (68.19)	306 (51.34)	326 (41.16)	0	0
Pack-years	53.42 (24.63)	40.81 (22.89)	44.8 (22.52)	37.31 (20.56)	32.2 (18.99)	19.71 (13.62)	66.31 (30.97)	45.97 (23.68)
DL _{CO} , % predicted	61.81 (21.45)	84.98 (17.59)	53.89 (18.87)	73.39 (16.6)	64.22 (18.02)	86.82 (14.77)	35.46 (11.62)	51.85 (13.24)
FEV ₁ , % predicted	53.2 (16.91)	92.41 (14.63)	54.7 (15.1)	91.15 (15.93)	57.32 (13.57)	94.93 (9.26)	28.09 (6.89)	49.5 (9.63)
FEV ₁ /FVC	0.51 (0.12)	0.75 (0.07)	0.55 (0.11)	0.78 (0.07)	0.56 (0.1)	0.79 (0.04)	0.32 (0.06)	0.43 (0.09)

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; COPDGene = Genetic Epidemiology of COPD study; FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; GenKOLS = Genetics of Chronic Obstructive Lung Disease study; GOLD = Global Initiative for Chronic Obstructive Lung Disease; GOLD 0 = smokers with normal spirometry; GOLD 1 = FEV₁ greater than or equal to 80% predicted and FEV₁/FVC less than 0.7; GOLD 2 = FEV₁ less than 80% predicted and FEV₁/FVC less than 0.7; GOLD 3 = FEV₁ less than 50% predicted and FEV₁/FVC less than 0.7; GOLD 4 = FEV₁ less than 30% predicted and FEV₁/FVC less than 0.7; NETT = National Emphysema Treatment Trial; PRISm = preserved ratio with impaired spirometry with FEV₁ less than 80% predicted and FEV₁/FVC greater than or equal to 0.7; TESRA = Treatment of Emphysema with a Gamma-Selective Retinoid Agonist study.

Heritability Estimation Using SNPs

To estimate the importance of genetic determinants of DL_{CO}, we calculated the proportion of the variance after inverse normal transformation of altitude-adjusted DL_{CO} measurements that could be explained by all common autosomal SNPs (minor allele frequency, $\geq 1\%$) as the SNP-based heritability. We estimated the SNP-based heritability of DL_{CO} in EA subjects ($n = 4,932$) to be 22% (SE, 7%), which was significantly greater than zero ($P = 0.0004$). We also adjusted for current smoking status and identified the same heritability estimate (22%; SE, 7%; $P = 0.0004$). The heritability estimate in AA subjects ($n = 1,262$) was not significantly different from zero (12% with SE 28%; $P = 0.32$). The heritability estimates in EA subjects were similar when we stratified the analysis to individuals with COPD (20%; SE, 14%; $P = 0.06$; $n = 2,331$) and without COPD (20%; SE, 13%; $P = 0.06$; $n = 2,601$). The heritability estimates in female subjects (15%; SE, 14%; $P = 0.11$; $n = 2,276$) and male subjects (18%; SE, 12%; $P = 0.06$; $n = 2,656$) were similar.

Genome-Wide Association Analysis

Using a combined dataset of EA subjects, we identified two genome-wide significant loci for DL_{CO} near *TGFB2* and *CHRNA3* ($P < 5 \times 10^{-8}$ in the combined analysis and $P < 0.05$ in COPDGene and GenKOLS) (Table 2 and Figures 1 and 2).

We identified 12 additional suggestive loci, including *NEGR1*, *CADM2*, *PCDH7*, *RETREG1*, *DACT2*, *NRG1* (neuregulin 1), *ANKRD18A*, *KRT86*, *NTN4*, *ARHGAP28*, *INSR*, and *PCBP3* loci ($P < 1 \times 10^{-5}$ in the combined analysis and $P < 0.05$ in COPDGene and GenKOLS) (Table 2 and Table E1 in the data supplement). In the analysis of AA subjects, we identified one genome-wide significant locus near *PDE11A* ($P = 2.4 \times 10^{-9}$) (Table E2 and Figure 3). This variant was quite rare in Europeans (minor allele frequency 0.02% in Europeans compared with 2.4% in African populations, both from the 1000 Genomes Project [30]). We identified 41 additional suggestive loci ($P < 1 \times 10^{-5}$) in AA subjects (Table E2). All genome-wide significant and suggestive loci were nominally significant in either COPD (Global Initiative for Chronic Obstructive Lung Disease stages 2–4) and/or non-COPD (Global Initiative for Chronic Obstructive Lung Disease stages 0, 1, and Preserved Ratio Impaired Spirometry) (see Table 1) in the stratified analysis (Tables E3 and E4). Of interest, the *PDE11A* variant was only significant in the AA subjects without COPD. Our results were robust to adjustment for cohorts (Table E5) and after adding or excluding current smoking status as a covariate (Figures E1–E4). DL_{CO} associations for genome-wide significant and most suggestive variants persisted after

adjusting for emphysema and lung function ($P < 0.05$) (Tables E6 and E7).

Identification of Functional Variants and Candidate Genes

To identify potential functional genetic variants at DL_{CO}-associated loci, we queried regulatory annotations of top associated variants and their variants in linkage disequilibrium ($r^2 > 0.8$) at three genome-wide significant loci (*TGFB2*, *CHRNA3*, and *PDE11A*) in EA or AA subjects and 12 suggestive loci in EA subjects. We observed overlap between various regulatory elements, including active transcription start site and enhancer in lung-related cells, and variants near *CHRNA3* and *TGFB2* (Table E8). Variants near *PDE11A* overlapped enhancer histone marks in bone marrow-derived cultured mesenchymal stem cells and muscle satellite cultured cells. We also searched for protein-altering variants (missense and nonsense) at these genome-wide significant and suggestive loci. We found one missense variant in *CHRNA3* that was in linkage disequilibrium with the lead variant ($r^2 = 0.98$). To identify candidate causal genes, especially for loci harboring multiple genes, we used multitissue gene expression data (see the data supplement). At the *CHRNA3* locus, the genetic component of gene expression of *PSMA4* was strongly associated with

Table 2. Genome-Wide Significant and Suggestive Loci in European Ancestry Subjects

Marker Name (dbSNP)	Locus	Closest Gene	Effect Allele	Effect Allele Frequency (%)	Effect Size (95% CI)	P Value	Candidate Causal Genes from S-PrediXcan Analysis (Only Top Three Shown)
rs17091046	1p31.1	<i>NEGR1</i>	A	8	0.16 (0.09 to 0.24)	7.16E-06	—
rs3009947	1q41	<i>TGFB2</i>	T	48	-0.11 (-0.15 to -0.07)	8.85E-09	<i>TGFB2</i> (1.3e-05), <i>LYPLAL1</i> (4.4e-03)
rs6768455	3p12.1	<i>CADM2</i>	T	43	0.1 (0.06 to 0.14)	1.92E-06	<i>CADM2</i> (7.3e-04)
rs7664851	4p15.1	<i>PCDH7</i>	C	14	0.13 (0.08 to 0.19)	3.03E-06	—
rs71609316	5p15.1	<i>RETREG1</i>	C	5	0.2 (0.11 to 0.29)	8.72E-06	—
rs2127333	6q27	<i>DACT2</i>	A	44	-0.09 (-0.13 to -0.05)	4.62E-06	RP11-503C24.3 (2.8e-02), RP11-503C24.2 (3.6e-02)
rs138138889	8p12	<i>NRG1</i>	C	2	-0.39 (-0.54 to -0.23)	6.16E-07	—
rs10974058	9p13.1	<i>ANKRD18A</i>	C	15	-0.13 (-0.19 to 0.08)	2.97E-06	<i>SPATA31A1</i> (1.5e-02)
rs150823320	12q13.13	<i>KRT86</i>	A	2	-0.32 (-0.46 to -0.18)	6.66E-06	<i>KRT18</i> (1.8e-03), <i>KRT86</i> (4.0e-02)
rs7303901	12q22	<i>NTN4</i>	C	68	-0.1 (-0.14 to -0.06)	1.54E-06	<i>LTA4H</i> (2.7e-04), <i>NTN4</i> (2.5e-03), <i>CCDC38</i> (3.0e-03)
rs112878080	15q25.1	<i>CHRNA3</i>	G	37	-0.13 (-0.17 to -0.09)	2.90E-10	<i>PSMA4</i> (3.3e-08), <i>IREB2</i> (4.7e-07), <i>ACO27228.1</i> (1.8e-06)
rs79439691	18p11.31	<i>ARHGAP28</i>	T	2	0.37 (0.21 to 0.53)	3.70E-06	—
rs72998171	19p13.2	<i>INSR</i>	C	3	-0.27 (-0.39 to -0.15)	6.25E-06	<i>EMR4P</i> (8.1e-03), <i>ACER1</i> (1.3e-02), CTD-3193O13.10 (1.9e-02)
rs116946461	21q22.3	<i>PCBP3</i>	A	2	-0.32 (-0.45 to -0.18)	2.29E-06	—

Definition of abbreviations: CI = confidence interval; dbSNP = Single Nucleotide Polymorphism Database.

Data shown are for genome-wide significant and suggestive loci ($P < 1 \times 10^{-5}$) in a combined European ancestry white dataset that were also nominally significant in both COPDGene (Genetic Epidemiology of Chronic Obstructive Pulmonary Disease study) non-Hispanic white individuals and in GenKOLS (Genetics of Chronic Obstructive Lung Disease study) ($P < 0.05$).

DL_{CO} ($P = 3.3 \times 10^{-8}$), together with *IREB2*, *CHRNA3*, *ACSBG1*, *CHRNA4*, and *ADAMTS7* ($P < 1.0 \times 10^{-3}$) (Table E1). We identified significant associations of *TGFB2* ($P = 1.3 \times 10^{-5}$) and *LYPLAL1*

($P = 4.4 \times 10^{-3}$) at the *TGFB2* locus (Table E1). Among 12 suggestive loci, 6 loci contained significant gene associations, including *LTA4H* (leukotriene A4 hydrolase) at the *NTN4* locus (Table E1).

Pathway Enrichment Analysis

We identified nominal enrichment of gene pathways including a set of genes in the protein-protein interaction subnetwork of *RHOJ*, a gene specific to endothelial cell-mediating angiogenesis (31) ($P = 1.3 \times 10^{-6}$), and genes with 3',5'-cyclic nucleotide PDE activity ($P = 0.008$) (Table E9) using DEPICT (32). The latter pathway includes *PCBP3*, *CHRNA4*, *INSR*, *CADM2*, *ARHGAP28*, *DACT2*, and *ANKRD18A* genes.

Genetic Variants Associated with DL_{CO} and COPD-related Traits

We assessed the associations of DL_{CO} -associated variants with COPD, spirometry, and emphysema. As reported previously, variants at *CHRNA3* and *TGFB2* loci were strongly associated with COPD (17, 33), emphysema (12), and spirometric values (34, 35). Nine of 12 suggestive DL_{CO} loci were associated with COPD, emphysema, or spirometry ($P < 0.05$) (Table E10). DL_{CO} variants associated with COPD (11) or severe COPD (33) were near *PCDH7*, *NRG1*, *ARHGAP28*, and *PCBP3* ($P < 0.05$) (Table E10). Four DL_{CO} -associated loci were associated with emphysema, including variants near *NRG1*, *KRT86*, *NTN4*, and *PCBP3* ($P \leq 0.04$) (Table E10).

Figure 1. Regional association plot for rs3009947 (*TGFB2* locus at 1q41) using a combined European ancestry white dataset.

Figure 2. Regional association plot for rs112878080 (*CHRNA3* locus at 15q25.1) using a combined European ancestry white dataset.

We then sought to investigate the association of previously reported COPD-associated variants (11, 12, 33) with DL_{CO} . Of 25 COPD-associated variants, 13 were associated with DL_{CO} at nominal significance ($P < 0.05$) (Table 3), including variants previously described in

quantitative emphysema (*AGER*, *DLC1*, and *SERPINA10*). In addition to two genome-wide significant loci (*TGFB2* and *CHRNA3/CHRNA5*), three loci at *FAM13A*, *DSP*, and *CYP2A6* were significant after Bonferroni correction ($P < 2 \times 10^{-3}$) (Table 3). Effect sizes of all 25 COPD-

associated were not significantly different between DL_{CO} and emphysema (quantitative emphysema, percentage of low-attenuation area below -950 Hounsfield units) (Figure E5).

Discussion

In the present study, we investigated the underlying genetic determinants of DL_{CO} by estimating SNP-based heritability and performing genome-wide association analysis. We identified significant heritability for DL_{CO} in EA subjects but not in AA subjects, potentially related to the smaller sample size of AA subjects. We identified three genome-wide significant loci for DL_{CO} : two from a combined EA dataset (COPDGene NHW, GenKOLS, NETT, and TESRA) and one from COPDGene AA. We also investigated the effects of known COPD-associated loci on DL_{CO} , and we found that a subset of previously reported COPD-associated loci were at least nominally associated with DL_{CO} .

Among European populations, COPDGene and GenKOLS had comparable values of DL_{CO} (percent predicted), whereas two other cohorts (NETT and TESRA) were lower. This difference likely resulted from the study designs of NETT and TESRA, which focused on patients with more severe COPD, who often have lower DL_{CO} .

We confirmed the previously estimated heritability of DL_{CO} in unrelated individuals. Our heritability estimate of DL_{CO} at 22% (SE, 7%) was consistent with the estimate in unrelated individuals from the Rotterdam study at 23% (SE, 10%) (14) and a twin study at 15% (in males; SE not reported) (36). These estimates may imply that there is a similar extent of genetic control to DL_{CO} in our COPD-enriched populations and general population samples. However, these estimates from common SNPs in unrelated individuals were lower than estimated in related individuals from the Framingham Heart Study (49%; SE, 3%) or a twin study of females (39%; SE not reported) (36), as previously reported (14). Though the twin study previously showed sexual dimorphism of DL_{CO} heritability (36), our heritability estimates in male and female subjects were similar. However, comparing the heritability estimates among studies

Figure 3. Regional association plot for rs116825096 (*PDE11A* locus at 2q31.2) using a COPDGene (Genetic Epidemiology of Chronic Obstructive Pulmonary Disease study) African American dataset.

Table 3. DL_{CO} Associations of 25 Previously Reported Chronic Obstructive Pulmonary Disease and Emphysema-associated Variants in a Combined European Ancestry White Dataset

rsID	Closest Gene	Cytoband	Risk/ Alternative Allele	β (95% CI)	P Value
rs10429950	TGFB2	1q41	T/C	-0.09 (-0.13 to -0.04)	1.24E-04
rs16825267	PID1	2q36.3	C/G	-0.04 (-0.12 to 0.03)	2.61E-01
rs1529672	RARB	3p24.2	C/A	-0.07 (-0.12 to -0.02)	5.49E-03
rs2955083	EEFSEC	3q21.3	A/T	-0.03 (-0.09 to 0.03)	2.78E-01
rs6837671	FAM13A	4q22.1	G/A	-0.07 (-0.11 to -0.03)	5.59E-04
rs2047409	TET2	4q24	A/G	-0.02 (-0.06 to 0.02)	3.37E-01
rs11727735	GSTCD	4q24	A/G	-0.00 (-0.08 to 0.07)	9.31E-01
rs13141641	HHIP	4q31.21	T/C	-0.04 (-0.08 to 0)	4.58E-02
rs7733088	HTR4	5q32	G/A	0.01 (-0.03 to 0.05)	6.97E-01
rs113897301*	ADAM19	5q33.3	AT/A	-0.03 (-0.07 to 0.01)	1.90E-01
rs2076295	DSP	6p24.3	T/G	-0.08 (-0.11 to -0.04)	1.03E-04
rs2070600	AGER	6p21.32	C/T	-0.11 (-0.2 to -0.01)	2.60E-02
rs2806356	ARMC2	6q21	C/T	-0.02 (-0.07 to 0.03)	4.45E-01
rs9399401	ADGRG6	6q24.1	T/C	-0.02 (-0.06 to 0.03)	4.51E-01
rs75200691	DLG1	8p22	T/G	-0.07 (-0.13 to -0.01)	1.84E-02
rs721917	SFTPD	10q22.3	G/A	-0.03 (-0.07 to 0.01)	8.86E-02
rs626750	MMP12	11q22.2	G/A	-0.06 (-0.11 to -0.01)	1.10E-02
rs754388	RIN3	14q32.12	C/G	-0.04 (-0.09 to 0.01)	9.73E-02
rs45505795	SERPINA10	14q32.13	C/G	-0.12 (-0.24 to 0)	4.88E-02
rs1441358	THSD4	15q23	G/T	-0.05 (-0.09 to -0.01)	1.52E-02
rs17486278	CHRNA5	15q25.1	C/A	-0.12 (-0.16 to -0.08)	8.95E-10
rs17707300	CCDC101	16p11.2	C/T	-0.05 (-0.09 to -0.01)	1.50E-02
rs7186831*	CFDP1	16q23.1	A/G	0.01 (-0.03 to 0.05)	6.63E-01
rs647097	MTCL1	18p11.22	C/T	-0.01 (-0.05 to 0.03)	5.52E-01
rs12459249	CYP2A6	19q13.2	C/T	-0.07 (-0.11 to -0.03)	1.06E-03

Definition of abbreviations: CI = confidence interval; LD = linkage disequilibrium.

*These two variants were replaced by their variants in LD: rs113897301 with rs11134779 (r^2 in 1000 GENOMES: phase_3:CHS = 1), and rs7186831 with rs8051407 (r^2 in 1000 GENOMES: phase_3:CEU = 1).

with different designs and populations is challenging (37). Further study on larger populations to test the sex-specific genetic determinants of DL_{CO} is needed to investigate these issues. We performed the first GWAS of DL_{CO} in COPD-enriched populations. Most previously reported genetic association studies of DL_{CO} investigated limited regions in the genome and/or specific disease conditions such as cystic fibrosis (38, 39). An early study found significant association between a missense variant (p.Glu27Gln) in *ADRB2* (β_2 -adrenergic receptor) in individuals with cystic fibrosis but not in healthy individuals (39). Another study also found a significant association between a missense variant (p.Thr663Ala) in *SCNN1A* (sodium channel epithelial 1 α -subunit) and DL_{CO} in response to exercise (38). Recently, a GWAS of DL_{CO} and the carbon monoxide transfer coefficient (K_{CO}) in a general population sample identified association of the *ADGRG6* locus with K_{CO} but not for DL_{CO} (14). The previously reported

K_{CO} -associated variant in *ADGRG6* (rs17280293) is nominally significant in our study of DL_{CO} ($P = 0.03$).

To interpret our GWAS findings, we assessed the overlap of DL_{CO}-associated variants with regulatory regions, their protein-altering effects, and predicted gene expression in various cell types. Most of the genome-wide significant and suggestive variants overlap with various regulatory regions in lung and blood cell types. Only a missense variant in *CHRNA5* was likely to affect protein structure. We integrated gene expression from multiple tissues and prioritized various effector genes for follow-up studies.

The genome-wide significant association for DL_{CO} near *TGFB2* was previously associated with COPD (17, 33), emphysema (12), and lung function (34). The association of this variant and DL_{CO} persisted after adjusting for emphysema and lung function. *TGFB2* encodes a ligand that activates transforming growth factor- β signaling, a pathway critical to development of lung and blood vessels.

Tgfb2-null mice show dilated conducting airways and collapsed respiratory airways (40). *TGFB2* also affects lung branching in a rat model (41), as well as mucin production (42). Its gene expression levels and protein concentrations in the lung were significantly altered, together with airway changes, after cigarette smoke exposure in a murine model (43). The various isoforms of transforming growth factor- β may have complementary roles in COPD pathogenesis, which is suggested by distinct expression patterns of those isoforms in the lower airways of patients with COPD (44) and in rats (43).

At the *TGFB2* locus, gene expression data also identified *LYPLAL1* (lysophospholipase-like 1) as a potential causative gene. The predicted amino acid sequence of this gene was similar to those of members of the phospholipase/carboxylesterase family, particularly for lysophospholipases (45). Phospholipases in the A2 superfamily have previously been implicated in COPD (46). This gene product interacts with *SCLT1*, which was suggestively associated with COPD in a prior analysis (33). *SCLT1* encodes a component of centrioles that is critical for ciliogenesis (47), which was also implicated in genetic studies of lung function levels (34, 48). A long noncoding RNA in this locus, *TGB2-OT1* (*TGFB2* overlapping transcript 1), regulates autophagy in vascular endothelial cells (49), whereas its contribution to DL_{CO} has yet to be determined.

PDE11A encodes a member of the PDE family, which comprise enzymes that degrade cAMP and cGMP (50). Variants at the *PDE11A* locus were associated with asthma (51). Although the role of *PDE11A* in COPD is unknown, *PDE4* has been used as a COPD therapeutic target for roflumilast (52, 53). Using pathway enrichment analysis, we implicated the 3',5'-cyclic nucleotide PDE activity, which is the function of PDE enzymes. PDEs participate in signal transduction by degrading the phosphodiester bond in second messengers (cAMP and cGMP). PDE inhibitors have been suggested as treatments for inflammatory and pulmonary-related diseases, especially PDE-4 inhibitors for COPD (54) and PDE-5 inhibitors for pulmonary arterial hypertension (55). Although the specific role of these genes affecting DL_{CO} is unknown, this pathway may suggest a link

between COPD and pulmonary vascular diseases.

Among suggestively associated loci with DL_{CO} in our study, we identified candidate genes that may be of biological relevance to COPD. *LTA4H* encodes an enzyme governing neutrophilic inflammation with a proinflammatory action through leukotriene B4 and degradation of the neutrophil chemoattractant Pro-Gly-Pro (PGP) as an antiinflammatory activity (56, 57). Sputum concentrations of leukotriene B4 and PGP were associated with stable COPD and acute exacerbations of COPD (58, 59). Cigarette smoke-induced emphysematous mice showed suppression of *LTA4H*'s aminopeptidase activity and accumulated PGP (60, 61). *NRG1* encodes a membrane glycoprotein that plays a role in cell signaling and organ development (62). Various isoforms of *NRG1* exert different effects on lung epithelium, including regulating the expression of airway goblet cell mucins (63), lung development (64, 65), and airway remodeling (66).

We also characterized effects of DL_{CO} -associated variants in previously reported GWAS (COPD, emphysema, and spirometry), as well as associations of 25 previously reported COPD- and emphysema-associated variants, on DL_{CO} . We identified nominal associations of suggestive DL_{CO} -associated variants and COPD-related phenotypes, although these published results included many of the

same study populations in association analysis. Including COPD cases that generally had lower DL_{CO} values may drive the genetic association of DL_{CO} in our study. However, we identified some DL_{CO} -associated variants that were not associated with COPD or spirometry, suggesting that they are DL_{CO} -specific genetic effects. Thirteen COPD-associated variants were nominally associated with DL_{CO} ($P < 0.05$). Because DL_{CO} measures both parenchymal and vascular abnormalities in the lung, these varying effects of COPD-associated genetic variants on this physiological parameter may suggest opportunities for better characterizing COPD heterogeneity using this additional information.

Our study has some limitations. Although our population was enriched for COPD, our results were robust after stratification by COPD status. We did not replicate significant variants due to either an ancestry-specific variant near *PDE11A* in AA subjects or lack of other European cohorts for replication. However, further replication of our findings is needed to validate our results. Differences in the study populations between GWAS analyses could contribute to incomplete replication, but confidence in our reported associations would be increased by replication. Although this study has a large population combining four cohorts, the power to detect genetic effects of modest effect was limited. Increasing the sample size could lead to further discovery of genetic loci associated

with DL_{CO} . Incorporating related phenotypes such as K_{CO} could also provide useful information in addition to DL_{CO} , although the nonuniformity of the K_{CO} in different sites and underestimation of lung volume in COPD can be problematic (1). Because we investigated genetic determinants of DL_{CO} in cohorts enriched for subjects with COPD, genetic architecture of DL_{CO} in individuals with normal spirometry may be different. However, prior investigations on other measures of lung function (FEV₁ and FEV₁/FVC) in subjects with COPD recapitulated genetic findings from the general population (35). Percent predicted values for DL_{CO} were lower in AA subjects than in EA subjects. This is likely due to prediction equations that do not account for differences between ethnicities (67). This racial difference in predicted values did not affect our analysis, because we used raw altitude-adjusted DL_{CO} measurements and analyzed each ethnicity separately.

In conclusion, we have estimated the heritability and performed genome-wide association analysis of DL_{CO} . We identified several genetic loci that were significantly associated with DL_{CO} and characterized effects of COPD-associated loci on DL_{CO} . This work could lead to better understanding of the heterogeneous nature of COPD. ■

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