ORIGINAL RESEARCH

IREB2 and *GALC* Are Associated with Pulmonary Artery Enlargement in Chronic Obstructive Pulmonary Disease

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Abstract

Pulmonary hypertension is associated with advanced chronic obstructive pulmonary disease (COPD), although pulmonary vascular changes occur early in the course of the disease. Pulmonary artery (PA) enlargement (PAE) measured by computed tomography correlates with pulmonary hypertension and COPD exacerbation frequency. Genome-wide association studies of PAE in subjects with COPD have not been reported. To investigate whether genetic variants are associated with PAE within subjects with COPD, we investigated data from current and former smokers from the COPDGene Study and the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints study. The ratio of the diameter of the PA to the diameter of the aorta (A) was measured using computed tomography. PAE was defined as PA/A greater than 1. A genome-wide association study for COPD with PAE was performed using subjects with COPD without PAE (PA/A $\leq$ 1) as a control group. A secondary analysis used smokers with normal spirometry as a control group. Genotyping was performed on Illumina platforms. The results were summarized using fixed-effect meta-analysis. Both meta-analyses revealed a genome-wide significant locus on chromosome 15q25.1 in *IREB2* (COPD with versus without PAE, rs7181486; odds ratio [OR] = 1.32; $P = 2.10 \times 10^{-8}$; versus smoking control subjects, rs2009746; OR = 1.42; $P = 1.32 \times 10^{-9}$). PAE was also associated with a region on 14q31.3 near the *GALC* gene (rs7140285; OR = 1.55; $P = 3.75 \times 10^{-8}$). Genetic variants near *IREB2* and *GALC* likely contribute to genetic susceptibility to PAE associated with COPD. This study provides evidence for genetic heterogeneity associated with a clinically important COPD vascular subtype.

Keywords: chronic obstructive pulmonary disease; genome-wide association; pulmonary hypertension; subtyping

Chronic obstructive pulmonary disease (COPD) is characterized by the progressive development of airflow limitation that is not fully reversible and marked phenotypic heterogeneity. COPD was the third leading cause of mortality in the United States in 2010 (1). Although COPD susceptibility is mainly attributable to cigarette smoking, not all heavy smokers develop COPD for

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Clinical Relevance

Our study is the first genome-wide association study of pulmonary artery enlargement (PAE) associated with chronic obstructive pulmonary disease (COPD). PAE is usually observed in advanced COPD, and it is also a significant predictor of COPD exacerbations. We have found that genetic variants near *IREB2* and *GALC* are associated with susceptibility to PAE within subjects with COPD. This study suggests that a clinically important vascular subtype of COPD is closely linked to genetic heterogeneity.

reasons that are still unclear, but likely involve differences in genetic backgrounds (2–5).

Pulmonary hypertension (PH), a well established complication of COPD, is one of the most common forms of secondary PH (6). Typically, PH is associated with severe airflow limitation and chronic hypoxemia. However, there is a poor correlation between lung function parameters and pulmonary artery (PA) pressures, suggesting that factors other than airway obstruction may play a role in its etiology. Recent studies have reported that a proportion of patients with only moderate airflow limitation have severe PH (7, 8), which has been termed "disproportionate" PH. This subgroup has been suggested as a distinct phenotype, which may benefit from a different therapeutic approach, such as selective vasodilators (6–8). Furthermore, pulmonary vascular changes occur early in the course of COPD (9), and patients with COPD without resting PH frequently have exercise-induced PH (10). PH in COPD is associated with decreased survival (11–14). The ratio of the diameter of the PA to the diameter of the aorta (A) can be measured by computed tomography (CT), which correlates with PA pressure gauged by right heart catheterization (15–18). Recently, Wells and colleagues (19) demonstrated that PA/A greater than 1 was associated with COPD exacerbation frequency.

Although variants in several genes have been identified in association with Mendelian or near-Mendelian forms of PA hypertension, genetic risk factors for PH in COPD have not been well studied. A genome-wide association study (GWAS) of PA enlargement (PAE) within subjects with COPD has not been reported. We hypothesized that genetic susceptibility to PAE would differ among subjects with COPD. We addressed this hypothesis by comparing subjects with COPD with PA/A greater than 1 measured by CT to subjects with COPD with PA/A of 1 or less as a control group, as well as to smoking control subjects.

Materials and Methods

Study Cohorts

Subjects were current and former smokers from two studies: the COPDGene (Genetic Epidemiology of COPD) Study (NCT00608764, www.copdgene.org), and ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; NCT00292552; www.eclipse-copd.com). Study design and details of each study have been previously published (20, 21). Subjects in COPDGene were either self-identified non-Hispanic white (NHW) or African American (AA), and those included from ECLIPSE were of white European ancestry.

Measurement of the Diameters of the PA and Aorta

An investigator who was unaware of the subjects' clinical or genetic characteristics measured vascular diameters from axial images of baseline chest CT scans in the COPDGene and ECLIPSE cohorts by using inspiratory acquisitions with Digital Imaging and Communications in Medicine software (OsiriX DICOM Viewer, version 4.0, 32-bit; www.osirix-viewer.com), as previously described (19). The interpreter measured the diameter of the main PA at the level of its bifurcation and the diameter of the ascending aorta (A) by averaging two measurements taken 90° apart in its maximum dimension using the same images, and then a ratio of PA to A (PA/A) was calculated. The interobserver κ was 0.75 (95% confidence interval = 0.67–0.82) and intraobserver κ was 0.92 (95% confidence interval = 0.83–1.0) (19).

Variable Definitions

PAE was defined as PA/A measured by CT greater than 1. COPD cases with PAE were defined as having both PA/A greater than 1 and COPD severity of at least spirometry grade 2 (post-bronchodilator forced expiratory volume at 1 s [FEV_1]/forced vital capacity < 0.7 and $FEV_1 < 80\%$ predicted), defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) (22). For COPD cases with PAE, primary analysis was performed using subjects with COPD (GOLD 2–4) but no PAE (PA/A $\leq$ 1) as control subjects to explore genetic heterogeneity within subjects with COPD.

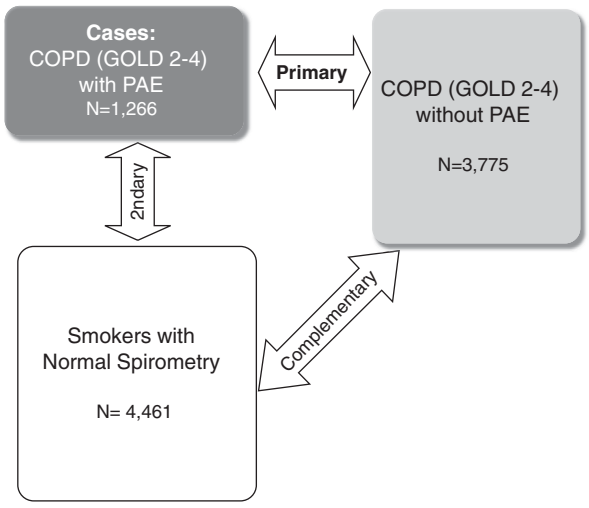

Figure 1. Genome-wide association study design for subjects with chronic obstructive pulmonary disease (COPD) with a ratio of pulmonary artery (PA) diameter to aorta (A) diameter greater than 1. GOLD (Global Initiative for Chronic Obstructive Lung Disease) 2–4 was defined as having a post-bronchodilator forced expiratory volume at 1 second (FEV_1)/forced vital capacity (FVC) less than 0.7 and FEV_1 less than 80% predicted. Normal spirometry was defined as a post-bronchodilator FEV_1/FVC of 0.7 or greater and an FEV_1 of 80% predicted or greater. PAE, PA enlargement.

The secondary analyses used current or former smokers with normal spirometry (post-bronchodilator FEV_1/forced vital capacity ≥ 0.7 and $FEV_1 \geq 80\%$ predicted) as a control group. In addition, we performed GWAS of subjects with COPD without PAE relative to smoking control subjects for comparison to our results in subjects with COPD with PAE (Figure 1).

Genotyping Quality Control and Imputation

Illumina platforms (HumanOmniExpress for the COPDGene cohort and HumanHap 550V3 for the ECLIPSE cohort; Illumina, Inc., San Diego, CA) were used for genotyping. Details of genotyping and imputation quality control have been previously published (23) and are described in the online supplement. Briefly, we performed imputation on the COPDGene cohorts using MaCH (24) and minimac (25) using 1,000 Genomes (26) Phase I v3 European and cosmopolitan reference panels for the NHWs and AAs, respectively (23). Previous studies have described details on genotyping quality control and imputation for the ECLIPSE cohort (27–29). Variants passing genotyping or imputation quality control in all cohorts were included for analysis.

Statistical Analysis

We performed logistic regression analysis of single-nucleotide polymorphisms (SNPs) under an additive model of inheritance with affection status in each cohort with adjustment for age, sex, pack-years of cigarette smoking, and genetic ancestry–based principal components using PLINK 1.07 (30), as previously described (27, 29). Complementary analysis included post-bronchodilator FEV_1 % predicted and/or exacerbation frequency as adjustment variables. Imputed genotypes were analyzed in a similar manner using SNP dosage data in PLINK 1.07 (30). Separate analyses were performed in COPDGene NHWs, COPDGene AAs, and ECLIPSE European ancestry subjects. Fixed-effects meta-analysis (31) was undertaken using METAL (version 2011-3-25) (32) and R 2.15.1 (www.r-project.org) with the meta-package. We evaluated heterogeneity by calculating both I^2 (33) and P values for Cochran's Q. I^2 describes the percentage of total variation across studies that is due to heterogeneity rather than chance. $I^2 = 100\% \times (Q - \mathrm{df})/Q$, where Q is Cochran's heterogeneity statistic and df the degrees of freedom. Genomic inflation factors (34) were calculated using GenABEL (35). Regional association plots were generated using LocusZoom (36), with linkage disequilibrium (LD) calculated using the 1,000 Genomes European reference data.

To evaluate differences of odds ratios (ORs) for previously known genome-wide significant SNPs between two different meta-analyses, permutation testing was performed. For each cohort, we randomly reassigned the phenotypes (COPD with or without PAE) of each individual to another individual in the dataset. Each random reassignment of the data represented one possible sampling of individuals under the null hypothesis, and this process was repeated a predefined number of times (N) to generate an empirical distribution with resolution N. Logistic regression was performed, and the results were combined using meta-analysis. We repeated this procedure 10,000 times to obtain the null distribution of differences of effect sizes. Our baseline difference of effect size for each SNP between two meta-analyses was compared with the permutation results, which was described by the null distribution to obtain a P value.

To search other SNPs independently associated with COPD with PAE, we also

Table 1. Baseline Characteristics of Subjects with Chronic Obstructive Pulmonary Disease, Stratified by Pulmonary Artery: Ascending Aorta Ratio Values, and Smokers with Normal Spirometry

	COPDGene NHWs			COPDGene AAs			ECLIPSE		
	COPD			COPD			COPD		
Characteristics	PA/A > 1	PA/A ⩽ 1	Control Subjects	PA/A > 1	PA/A ⩽ 1	Control Subjects	PA/A > 1	PA/A ⩽ 1	Control Subjects
n	535	2,128	2,534	260	494	1,749	471	1,153	178
Age, yr	64.2 (8.4)	64.8 (8.1)	59.5 (8.7)	59.3 (8.1)	58.7 (8.1)	52.8 (6.0)	63.7 (7.2)	63.7 (7.0)	57.5 (9.4)
Sex, % male	42.1	59.1	49.3	40.0	62.6	58.1	62.2	67.1	57.9
Pack-years	54.6 (25.1)	56.8 (28.6)	37.8 (20.3)	41.0 (22.3)	43.3 (23.0)	36.4 (20.1)	47.8 (24.4)	51.9 (28.6)	32.1 (24.8)
Current smoker, %	27.3	36.7	39.6	52.3	65.8	87.4	31.4	36.4	40.1
FEV_1 % predicted	45.5 (17.4)	50.8 (17.9)	96.8 (11.0)	47.1 (18.0)	55.1 (16.7)	98.4 (12.2)	43.2 (14.8)	49.5 (15.6)	107.9 (13.7)
Spirometry grade, %*									
GOLD 2	41.3	53.5		46.2	62.8		29.8	46.8	
GOLD 3	37.2	30.7		33.1	28.1		50.0	41.3	
GOLD 4	21.5	15.8		20.8	9.1		20.2	11.9	
PA, cm	3.3 (0.4)	2.7 (0.4)	—	3.3 (0.4)	2.8 (0.4)	—	3.5 (0.5)	2.9 (0.4)	—
A, cm	3.1 (0.3)	3.3 (0.4)	—	3.1 (0.4)	3.2 (0.4)	—	3.2 (0.4)	3.4 (0.4)	—
PA/A	1.07 (0.08)	0.84 (0.10)	—	1.09 (0.10)	0.88 (0.09)	—	1.10 (0.10)	0.85 (0.10)	—
Exacerbation frequency per year	1.32 (1.52)	0.61 (1.10)		1.06 (1.41)	0.47 (1.04)		1.14 (1.34)	0.73 (1.16)	

Definition of abbreviations: AA, African American; COPD, chronic obstructive pulmonary disease; COPDGene, Genetic Epidemiology of COPD Study; ECLIPSE, Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; FEV_1, forced expiratory volume at 1 second; GOLD, Global Initiative for Chronic Obstructive Lung Disease; NHW, non-Hispanic white; PA/A, ratio of the diameter of the main pulmonary artery (PA) at the level of its bifurcation and the diameter of the ascending aorta (A) in its maximum dimension measured by computed tomography.
Data are presented as mean (SD) or percentage, as appropriate.
*For subjects with post-bronchodilator FEV_1/forced vital capacity < 0.7; GOLD 2 = $FEV_1 \geq 50\%$ predicted and $<80\%$ predicted; GOLD 3 = $FEV_1 \geq 30\%$ predicted and $<50\%$ predicted; GOLD 4 = $FEV_1 < 30\%$ predicted.

performed region-based conditional analyses using logistic regression, adjusting for the most significant (lead) SNP in each genome-wide significant region using genotyped or dosage data, as appropriate. All SNPs within a 250-kb window on either side of the lead SNP were tested for association with COPD with PAE. For region-based analyses conditional on the top SNP, a threshold of P less than 5×10^{-4} was considered significant to reflect an approximate adjustment for a 500-kb interval (23, 29).

Results

A total of 1,266 subjects with COPD with PAE, 3,775 subjects with COPD without PAE, and 4,461 smokers with normal spirometry from the combined COPDGene and ECLIPSE cohorts passed quality control and were not outliers by genetic ancestry. Table 1 shows baseline characteristics for these subjects.

GWAS of Subjects with COPD with PAE Relative to Those without PAE

Our primary association analysis on the presence or absence of PAE (defined by CT measurement of PA/A $>$ 1 versus $\leq$1) included 1,266 subjects with COPD with PAE and 3,775 subjects with COPD without PAE. The quantile–quantile plot showed no evidence of significant population stratification (Figure 2A; λ = 1.00). Figure 3A shows genome-wide significant associations on chromosomes 15q25.1 and 14q31.3. The most significantly associated SNP was rs7181486 on 15q25.1 with a meta-analysis P value of 2.10×10^{-8} and OR of 1.32, located within the iron-responsive element–binding protein 2 gene (*IREB2*). (Table 2 and Figure 4). All of the genome-wide significant SNPs on 15q25.1 were in strong LD ($r^2 > 0.6$) with the previously described lead COPD risk SNP, rs11858836, near *IREB2* (29).

We identified one novel additional locus on 14q31.3 near the *GALC* gene encoding galactosylceramidase; the top SNP at this locus was rs7140285, with a P value of 3.75×10^{-8}. It was in strong LD (r^2 = 0.68) with rs1805078, which is a missense SNP of *GALC* ($P = 5.49 \times 10^{-7}$).

Because of a significant difference of mean FEV_1 % predicted between subjects with COPD with PAE and those without PAE (Table 1), we performed an additional meta-analysis including FEV_1 % predicted as an adjustment variable (*see* Figure E1 in the online supplement). The results of the top SNPs ($P < 1 \times 10^{-6}$) are shown in Table E1. The significance of rs7181486 was reduced to 5.58×10^{-7} and the significance of rs7140285 was reduced to 1.50×10^{-7}. Thus, adjustment for COPD severity, as captured by FEV_1, attenuated but did not remove the *IREB2* and *GALC* associations with PAE.

Because subjects with COPD with PAE and those without PAE showed different exacerbation frequency (Table 1), an additional meta-analysis including exacerbation frequency per year as an adjustment variable was performed (Figure E2). Although the significance of rs7181486 was increased to 6.68×10^{-9}, rs7140285 was reduced to 1.87×10^{-7} (Table E2). An additional meta-analysis including adjustment for both FEV_1 and exacerbation frequency showed slightly reduced significance of these two SNPs, rs718146 ($P = 9.20 \times 10^{-8}$) and rs7140285 ($P = 5.33 \times 10^{-7}$) (Figure E3 and Table E3).

GWAS of Subjects with COPD with PAE Relative to Smokers with Normal Spirometry

A GWAS of PAE associated with COPD (GOLD 2–4) included the same number of cases and 4,461 smokers with normal spirometry as a control group (Table 1).

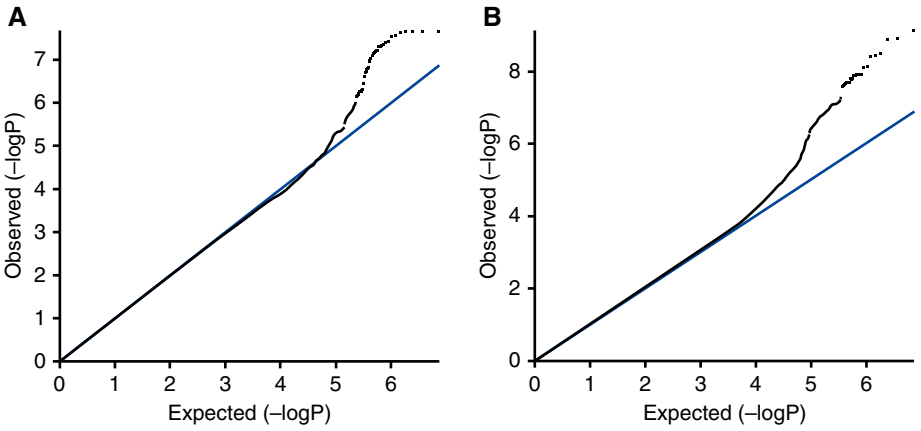

Figure 2. The quantile–quantile plots (*black*) for the meta-analysis including 1,000 Genomes project imputed data of (*A*) subjects with COPD with PAE (PA/A $>$ 1) versus those without PAE (PA/A $\leq$ 1), and (*B*) subjects with COPD with PAE versus smokers with normal spirometry, after adjustment for age, sex, pack-years of cigarette smoking, and genetic ancestry using principal components. The reference lines (*blue*) show the values where the observed (−logP) is equal to the expected (−logP).

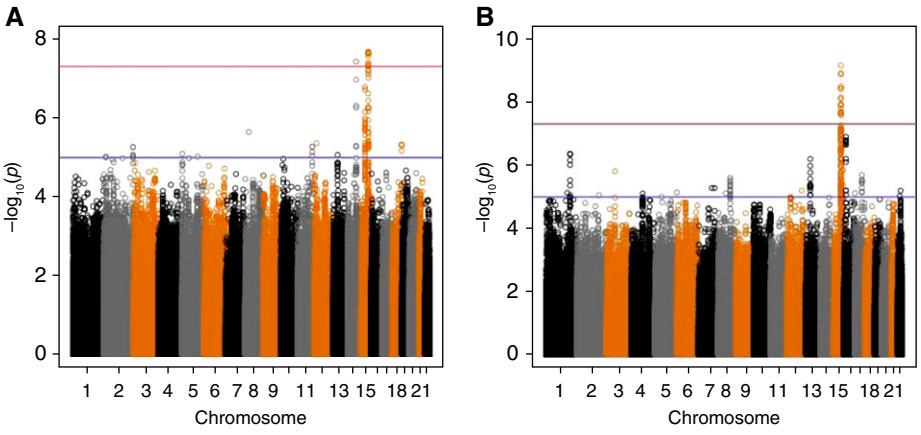

Figure 3. Manhattan plots of $-\log_{10} P$ for the meta-analysis including 1,000 Genomes project imputed data of (*A*) subjects with COPD with PAE (PA/A $>$ 1) versus those without PAE (PA/A $\leq$ 1), and (*B*) subjects with COPD with PAE versus smokers with normal spirometry, after adjustment for age, sex, pack-years of cigarette smoking, and genetic ancestry using principal components.

Table 2. Top Results of the Genome-Wide Association Study for Subjects with Chronic Obstructive Pulmonary Disease with a Pulmonary Artery:Ascending Aorta Ratio Greater Than 1 versus Those with a Pulmonary Artery:Ascending Aorta Ratio of 1 or Less in the Cohorts of COPDGene Non-Hispanic Whites, COPDGene African Americans, and ECLIPSE Studies*

Locus	Nearest Gene	SNP	Risk Allele	FRQ		COPDGene NHW		COPDGene AA		ECLIPSE		Overall			
				EUR†	AA	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	I^2	*Q*
15q25.1	*IREB2*	rs7181486	C	0.39	0.24	1.22‡ (1.06–1.40)	5.62×10^{-3}	1.58‡ (1.23–2.04)	3.53×10^{-4}	1.36‡ (1.16–1.60)	1.40×10^{-4}	1.32 (1.20–1.45)	2.10×10^{-8}	43.6	0.17
15q25.1	*IREB2*	rs56219465	G	0.39	0.24	1.22‡ (1.06–1.40)	5.64×10^{-3}	1.59‡ (1.23–2.04)	3.55×10^{-4}	1.36‡ (1.16–1.60)	1.40×10^{-4}	1.32 (1.20–1.45)	2.12×10^{-8}	43.6	0.17
15q25.1	*IREB2*	rs17483929	A	0.38	0.23	1.21 (1.06–1.39)	5.85×10^{-3}	1.59 (1.23–2.04)	3.51×10^{-4}	1.36‡ (1.16–1.60)	1.40×10^{-4}	1.32 (1.20–1.45)	2.24×10^{-8}	44.2	0.17
15q25.1	*IREB2*	rs2009746	G	0.39	0.22	1.21‡ (1.06–1.39)	6.15×10^{-3}	1.62‡ (1.25–2.10)	2.58×10^{-4}	1.36‡ (1.16–1.60)	1.49×10^{-4}	1.32 (1.20–1.45)	2.25×10^{-8}	49.9	0.14
15q25.1	*IREB2*	rs72738718	C	0.39	0.24	1.22‡ (1.06–1.40)	5.90×10^{-3}	1.57‡ (1.22–2.02)	4.37×10^{-4}	1.37‡ (1.16–1.60)	1.33×10^{-4}	1.32 (1.20–1.45)	2.37×10^{-8}	41.1	0.18
15q25.1	*IREB2*	rs72736802	T	0.39	0.48	1.25‡ (1.08–1.45)	3.52×10^{-3}	1.38‡ (1.10–1.72)	5.67×10^{-3}	1.44‡ (1.20–1.71)	5.77×10^{-5}	1.34 (1.21–1.48)	2.72×10^{-8}	0	0.48
14q31.3	*GALC*	rs7140285	T	0.08	0.12	1.51‡ (1.20–1.89)	4.20×10^{-4}	1.68‡ (1.19–2.37)	3.12×10^{-3}	1.53‡ (1.17–2.01)	2.21×10^{-3}	1.55 (1.33–1.81)	3.75×10^{-8}	0	0.87
15q25.1	*AGPHD1*	rs8034191	C	0.39	0.17	1.23 (1.08–1.41)	2.70×10^{-3}	1.66 (1.25–2.20)	4.60×10^{-4}	1.33 (1.13–1.56)	4.49×10^{-4}	1.31 (1.19–1.45)	4.06×10^{-8}	42.2	0.18
15q25.1	*IREB2*	rs55983731	T	0.39	0.24	1.21‡ (1.06–1.39)	6.47×10^{-3}	1.54‡ (1.19–1.98)	9.01×10^{-4}	1.36‡ (1.16–1.60)	1.38×10^{-4}	1.31 (1.19–1.44)	4.39×10^{-8}	33.1	0.22
15q25.1	*IREB2*	rs17483686	T	0.39	0.24	1.21‡ (1.05–1.39)	6.72×10^{-3}	1.53‡ (1.19–1.98)	9.50×10^{-4}	1.36‡ (1.16–1.60)	1.38×10^{-4}	1.31 (1.19–1.44)	4.74×10^{-8}	33.0	0.22
15q25.1	*IREB2*	rs17483721	C	0.38	0.23	1.21 (1.05–1.39)	6.74×10^{-3}	1.53 (1.19–1.97)	9.81×10^{-4}	1.36‡ (1.16–1.60)	1.38×10^{-4}	1.31 (1.19–1.44)	4.90×10^{-8}	32.7	0.23

Definition of abbreviations: AA, African American; CI, confidence interval; COPDGene, Genetic Epidemiology of COPD Study; ECLIPSE, Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; EUR, European white; FRQ, risk allele frequency; I^2, percentage of the variability in effect estimates due to heterogeneity; NHW, non-Hispanic white; OR, odds ratio; *Q*, *P* values for Cochran's *Q* test; SNP, single-nucleotide polymorphism.

*Adjusted for age, sex, pack-years of cigarette smoking, and genetic ancestry, as summarized in the principal components.

†EUR frequency data are from the meta-analysis of COPDGene NHW and ECLIPSE.

‡Imputed genotypes.

Figure 2B shows a corresponding quantile–quantile plot (lambda = 1.02). A genome-wide significant locus was identified on chromosome 15q25.1 (Figure 3B), and this locus includes five genes, which were cholinergic receptor, nicotinic, α 5 (neuronal) (*CHRNA5*), proteasome (prosome, macropain) subunit, α type, 4 (*PSMA4*), *IREB2*, aminoglycoside phosphotransferase domain containing 1 (*AGPHD1*), and cholinergic receptor, nicotinic, α 3 (neuronal) (*CHRNA3*) (Table 3 and Figure 5). The top SNP was rs17486278 (OR = 1.42; $P = 6.93 \times 10^{-10}$) and located within *CHRNA5*. These genome-wide significant SNPs were either identical to, or in strong LD ($r^2 \geqslant 0.80$) with, SNPs previously discovered in GWASs of COPD susceptibility (23, 37, 38).

Complementary Analyses

Because the meta-analysis of subjects with COPD with PAE relative to smoking control subjects showed 15q25.1 as the only genome-wide significant locus, we performed additional analyses to ascertain whether previously implicated genome-wide significant COPD risk loci had different effects between COPD with PAE and COPD without PAE. A meta-analysis of GWASs for subjects with COPD without PAE relative to smoking control subjects identified *FAM13A* on 4q22.1 as the top gene, which was followed by *HHIP-AS1* on 4q31.21 and *CHRNA3* (Table E4 and Figure E4). ORs and *P* values of previously known COPD risk SNPs among our results from meta-analyses for COPD with PAE or without PAE are summarized in Table E3. Permutation testing demonstrated that the effect estimates in the meta-analysis of subjects with COPD with PAE were stronger compared with those without PAE at three SNPs on 15q25 ($P < 0.0001$ for rs12914385, rs8034191, and rs11858836; Table 4).

To determine whether there is likely to be more than one functional variant located within the genome-wide significant regions, we performed analyses conditioning on the top (lead) SNP in these meta-analysis. All SNPs present in 250-kb flanks around the top signal were examined. Although no evidence suggestive of secondary associations ($P < 5 \times 10^{-4}$) existed in the meta-analyses of COPD with PAE relative to COPD without PAE on 15q25 (conditioning on rs7181486) and 14q31.3 (conditioning

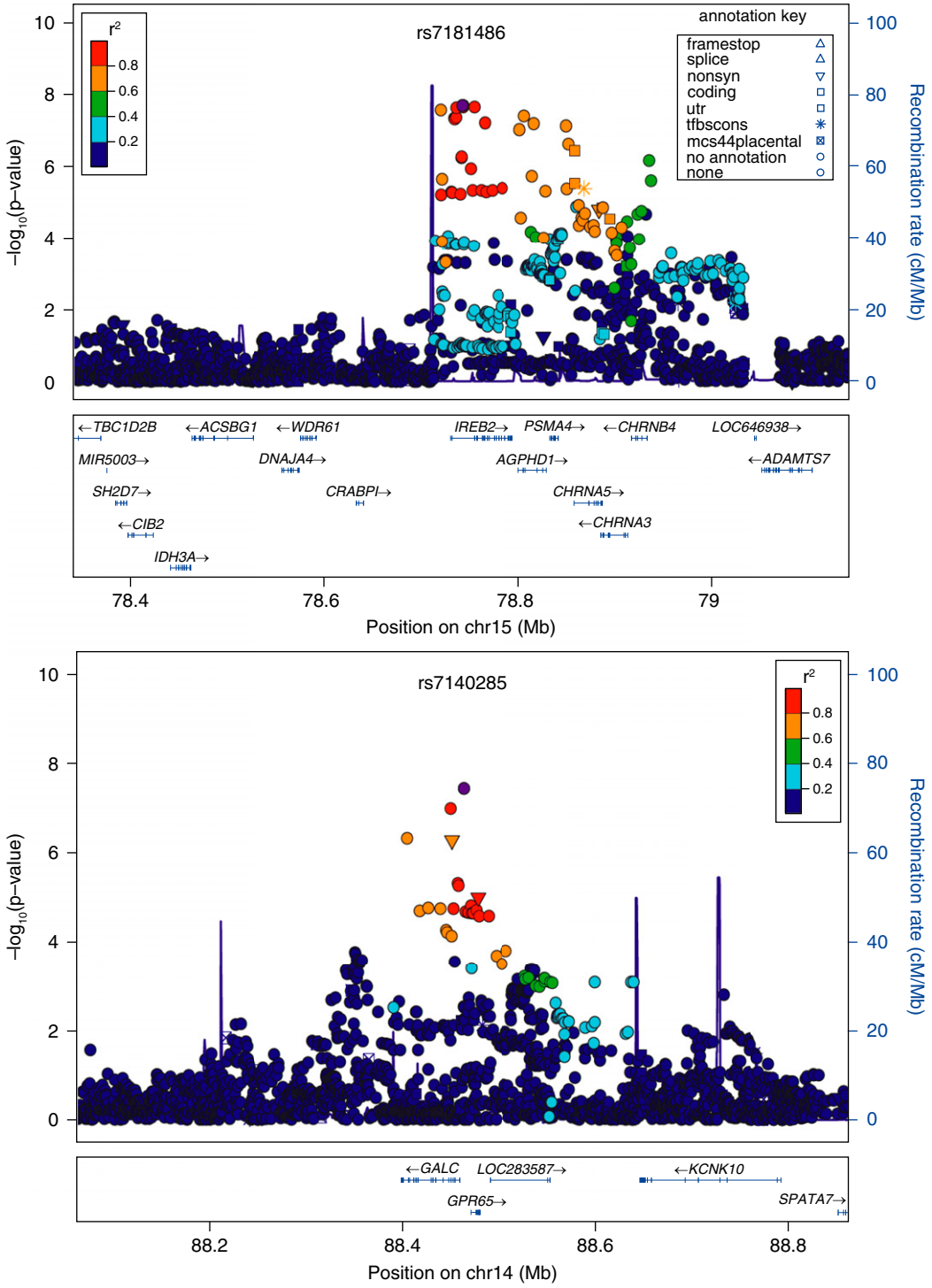

Figure 4. Local association plots for genome-wide significant loci in the meta-analysis of subjects with COPD with PA/A greater than 1 versus subjects with COPD with PA/A of 1 or lower in COPDGene (Genetic Epidemiology of COPD) non-Hispanic whites (NHWs), African Americans (AAs), and ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints). The *x axis* is chromosomal position, and the *y axis* shows the $-\log_{10}$ *P* value. The most significant single-nucleotide polymorphism (SNP) at each locus is labeled in *purple*, with other SNPs colored by degree of linkage disequilibrium (LD; r^2). Plots were created using LocusZoom.

on rs7140285), we found evidence suggestive of secondary associations in the meta-analysis of COPD with PAE relative to smoking control subjects on 15q25 (conditioning on rs17486278) in two SNPs (rs9920506, $P = 2.17 \times 10^{-4}$; rs3813567, $P = 4.43 \times 10^{-4}$) located within *CHRNB4*, coding neuronal acetylcholine receptor subunit β-4.

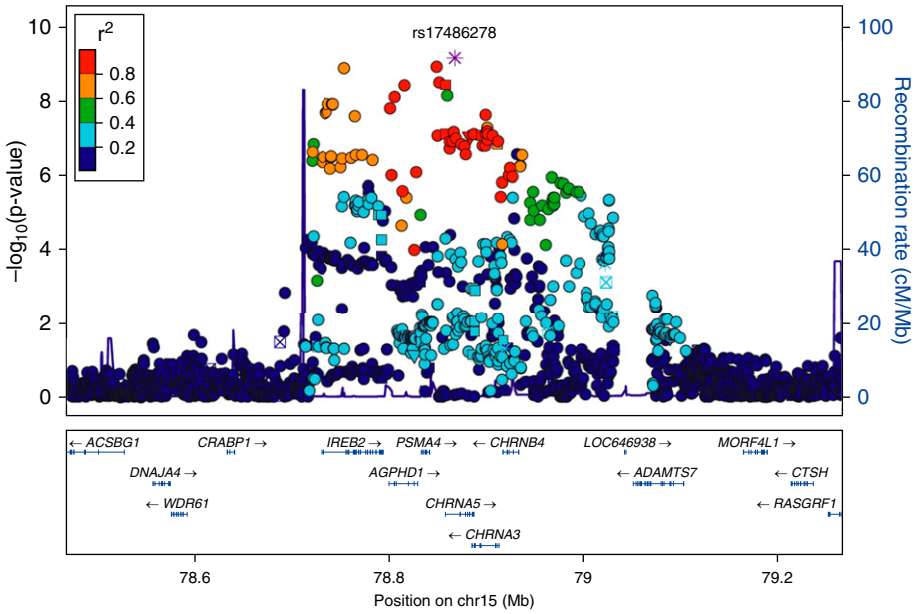

Figure 5. Local association plots for significant loci in the meta-analysis of subjects with COPD with PA/A greater than 1 versus smoking control subjects in COPDGene NHWs, AAs, and ECLIPSE. The *x axis* is chromosomal position, and the *y axis* shows the $-\log_{10} P$ value. The most significant SNP at each locus is labeled in *purple* (denoted by *asterisk*, as it is located in a transcription factor binding site conserved in a multiple-species alignment), with other SNPs colored by degree of LD (r^2). Plots were created using LocusZoom.

Discussion

This study is the first GWAS of PAE in COPD, and the first reported genetic association analysis of an important vascular subtype using PA/A. Our most significant finding was at the *IREB2* locus with the presence of PAE, which has been previously implicated as a predictor for severe exacerbations and total exacerbation frequency of COPD using the same case subjects as our current study (19).

A genomic region on chromosome 15q25.1 including *CHRNA5/3*, *AGPHD1*, and *IREB2* is a well established locus for COPD susceptibility (37). Although this region clearly contains genetic determinants for nicotine addiction, mediation analysis suggested that there may be two COPD genetic determinants in that region—one related to smoking, and one independent of smoking (39). A study of sputum gene expression also demonstrated that expression quantitative trait loci for *IREB2* and *CHRNA5* are not in LD, suggesting two susceptibility genes in this region (40). Our meta-analysis of three GWASs for subjects with COPD with PAE versus smoking control subjects showed the same locus with SNPs in multiple genes reaching the genome-wide significant threshold, whereas the meta-analysis for subjects with COPD with PAE versus those without PAE revealed only *IREB2* and *AGPHD1* as genes that contained genome-wide significant SNPs. A recent meta-analysis for severe COPD including our subjects identified 15q25 as the most genome-wide significant region (23). Considering the relatively low mean FEV_1 % predicted in our subjects with COPD with PAE, the most strongly associated genomic region in our current analysis of subjects with COPD with PAE relative to smoking control subjects seemed to be consistent with the previous locus for severe COPD. On the other hand, PA/A data in smoking control subjects were not available, and it is possible that some subjects could have PAE in this group, which may affect our results. The significance of the top SNP, rs17486278, located within *CHRNA5*, in the analysis of COPD with PAE relative to smoking control subjects was reduced to 4.08×10^{-6} in the analysis of PAE versus no PAE within subjects with COPD. Although the association of the *CHRNA3* locus with COPD has been reported to be significantly mediated by smoking-related phenotypes, *IREB2* appears to affect COPD independently of smoking (39, 41). *IREB2* may have a different role for COPD pathogenesis from the other genes on 15q25.1, and our study provides additional evidence for this hypothesis.

The protein product of *IREB2*, also known as iron-regulatory protein 2 (IRP2), is an RNA-binding protein that, together with IREB1, participates in maintaining human cellular iron metabolism. Even though iron is a vital element, it is toxic at high concentrations. Therefore, iron acquisition and storage are strictly controlled. Translation and/or stability of mRNAs encoding proteins required for iron storage, acquisition, and utilization are regulated through the binding of IRPs. According to body iron amount, IRPs modulate the expression of those proteins relevant to iron uptake, export, and sequestration (42). When systemic iron is too low, IRPs decrease iron storage and increase iron uptake (43). Hypoxemia is commonly associated with COPD progression; post-translational regulation of *IREB2* is dependent on oxygen as well as iron (44). Two recent papers (45, 46) have identified a mechanism for iron and oxygen sensing for IRP2-mediated post-transcriptional regulation of iron metabolism; IRP2 does not directly sense iron, but rather the iron/oxygen sensor is the enzyme that effects IRP2 degradation.

Chronic alveolar hypoxia may play an important mechanistic role; however, pulmonary vascular remodeling has been observed in lung specimens from patients with mild-to-moderate COPD without chronic hypoxemia (9). Even those without resting hypoxemia and/or PH could have hypoxemia or PH during exertion. Genetic variants of *IREB2* may determine individual variability of pulmonary vascular response to exertional or resting hypoxemia, influencing PA pressures independently of the severity of airflow limitation.

One of the genome-wide significant SNPs for PAE in subjects with COPD was rs7140285 on 14q31.3 near *GALC*, which encodes galactosylceramidase, which was the most significant SNP in the analysis of COPD with PAE relative to those without PAE with adjustment for FEV_1 % predicted. Mutations in *GALC* have been associated with Krabbe disease, also known as globoid cell leukodystrophy or galactosylceramide lipidosis. It is a rare, often fatal degenerative disorder that affects the myelin sheath of the nervous system, and involves dysfunctional metabolism of sphingolipids (47). This disorder is inherited in an autosomal recessive pattern. Recently, a study demonstrated that galactosylceramidase deficiency inhibits angiogenesis in murine

Table 3. Top Results of the Genome-Wide Association Study for Subjects with Chronic Obstructive Pulmonary Disease with a Pulmonary Artery:Ascending Aorta Ratio Greater Than 1 versus Smokers with Normal Spirometry in the Cohorts of COPDGene Non-Hispanic Whites, COPDGene African Americans, and ECLIPSE Studies*

Locus	Nearest Gene	SNP	Risk Allele	FRQ		COPDGene NHW		COPDGene AA		ECLIPSE		Overall			
				EUR†	AA	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	I^2	*Q*
15q25.1	*CHRNA5*	rs17486278	C	0.37	0.29	1.43‡ (1.23–1.65)	2.05×10^{-6}	1.54‡ (1.24–1.91)	9.26×10^{-5}	1.22‡ (0.92–1.62)	1.69×10^{-1}	1.42 (1.27–1.59)	6.93×10^{-10}	0	0.43
15q25.1	*PSMA4*	rs58365910	C	0.38	0.26	1.43‡ (1.24–1.66)	1.82×10^{-6}	1.51‡ (1.20–1.88)	3.55×10^{-4}	1.25‡ (0.94–1.67)	1.21×10^{-1}	1.42 (1.27–1.59)	1.21×10^{-9}	0	0.61
15q25.1	*IREB2*	rs2009746	G	0.37	0.21	1.38‡ (1.20–1.60)	1.30×10^{-5}	1.59‡ (1.26–2.00)	8.33×10^{-5}	1.32‡ (0.99–1.75)	5.82×10^{-2}	1.42 (1.27–1.59)	1.32×10^{-9}	0	0.52
15q25.1	*CHRNA5*	rs2036527	A	0.38	0.23	1.44 (1.24–1.67)	1.05×10^{-6}	1.43 (1.14–1.79)	1.93×10^{-3}	1.25‡ (0.94–1.67)	1.23×10^{-1}	1.41 (1.26–1.57)	3.15×10^{-9}	0	0.69
15q25.1	*CHRNA5*	rs55781567	G	0.37	0.28	1.44‡ (1.24–1.67)	1.22×10^{-6}	1.42‡ (1.14–1.78)	1.94×10^{-3}	1.25‡ (0.94–1.66)	1.25×10^{-1}	1.40 (1.25–1.57)	3.68×10^{-9}	0	0.69
15q25.1	*AGPHD1*	rs8031948	T	0.38	0.17	1.43‡ (1.23–1.65)	1.89×10^{-6}	1.51‡ (1.17–1.94)	1.36×10^{-3}	1.27‡ (0.95–1.68)	1.03×10^{-1}	1.42 (1.26–1.59)	3.74×10^{-9}	0	0.65
15q25.1	*CHRNA5*	rs190065944	A	0.29	0.23	1.67‡ (1.35–2.07)	2.05×10^{-6}	1.57‡ (1.18–2.09)	1.96×10^{-3}	1.36‡ (0.90–2.06)	1.41×10^{-1}	1.59 (1.36–1.86)	7.04×10^{-9}	0	0.69
15q25.1	*AGPHD1*	rs8034191	C	0.38	0.17	1.43 (1.24–1.66)	1.75×10^{-6}	1.44 (1.13–1.84)	3.49×10^{-3}	1.26 (0.95–1.67)	1.09×10^{-1}	1.40 (1.25–1.57)	7.80×10^{-9}	0	0.72
15q25.1	*IREB2*	rs56219465	G	0.37	0.23	1.39‡ (1.20–1.60)	1.19×10^{-5}	1.43‡ (1.15–1.79)	1.59×10^{-3}	1.31‡ (0.99–1.75)	5.89×10^{-2}	1.39 (1.24–1.55)	1.20×10^{-8}	0	0.90
15q25.1	*IREB2*	rs72738718	C	0.37	0.23	1.39‡ (1.20–1.61)	1.16×10^{-5}	1.43‡ (1.14–1.79)	1.65×10^{-3}	1.31‡ (0.99–1.75)	6.01×10^{-2}	1.39 (1.24–1.55)	1.21×10^{-8}	0	0.90
15q25.1	*IREB2*	rs7181486	C	0.37	0.23	1.39‡ (1.20–1.60)	1.18×10^{-5}	1.43‡ (1.15–1.79)	1.61×10^{-3}	1.31‡ (0.99–1.75)	5.88×10^{-2}	1.39 (1.24–1.55)	1.21×10^{-8}	0	0.90
15q25.1	*IREB2*	rs17483929	A	0.38	0.23	1.39 (1.20–1.60)	1.19×10^{-5}	1.43 (1.14–1.79)	1.68×10^{-3}	1.31‡ (0.99–1.75)	5.89×10^{-2}	1.39 (1.24–1.55)	1.25×10^{-8}	0	0.90
15q25.1	*AGPHD1*	rs11852372	C	0.35	0.17	1.45‡ (1.25–1.70)	2.03×10^{-6}	1.42‡ (1.10–1.82)	6.25×10^{-3}	1.27‡ (0.94–1.72)	1.14×10^{-1}	1.41 (1.25–1.59)	1.57×10^{-8}	0	0.75
15q25.1	*IREB2*	rs55983731	T	0.37	0.23	1.39‡ (1.20–1.60)	1.18×10^{-5}	1.41‡ (1.12–1.76)	2.97×10^{-3}	1.31‡ (0.99–1.75)	5.87×10^{-2}	1.38 (1.23–1.54)	2.01×10^{-8}	0	0.93
15q25.1	*IREB2*	rs17483686	T	0.37	0.23	1.39‡ (1.20–1.60)	1.19×10^{-5}	1.41‡ (1.12–1.76)	2.98×10^{-3}	1.31‡ (0.99–1.75)	5.87×10^{-2}	1.38 (1.23–1.54)	2.06×10^{-8}	0	0.93
15q25.1	*IREB2*	rs17483721	C	0.61	0.77	1.39 (1.20–1.60)	1.21×10^{-5}	1.40 (1.12–1.76)	3.01×10^{-3}	1.31‡ (0.99–1.75)	5.87×10^{-2}	1.38 (1.23–1.54)	2.16×10^{-8}	0	0.93
15q25.1	*CHRNA3*	rs56077333	A	0.35	0.17	1.46‡ (1.25–1.70)	1.63×10^{-6}	1.41‡ (1.09–1.83)	7.98×10^{-3}	1.24‡ (0.92–1.68)	1.58×10^{-1}	1.41 (1.25–1.59)	2.38×10^{-8}	0	0.65
15q25.1	*IREB2*	rs72738736	T	0.37	0.23	1.38‡ (1.19–1.59)	1.79×10^{-5}	1.42‡ (1.13–1.77)	2.50×10^{-3}	1.32‡ (0.99–1.75)	5.72×10^{-2}	1.38 (1.23–1.54)	2.54×10^{-8}	0	0.93

Definition of abbreviations: AA, African American; CI, confidence interval; COPDGene, Genetic Epidemiology of COPD Study; ECLIPSE, Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; EUR, European white; FRQ, risk allele frequency; I^2, percentage of the variability in effect estimates due to heterogeneity; NHW, non-Hispanic white; OR, odds ratio; *Q*, *P* values for Cochran's *Q* test; SNP, single-nucleotide polymorphism.
*Adjusted for age, sex, pack-years of cigarette smoking and genetic ancestry, as summarized in the principal components.
†EUR frequency data is from the meta-analysis of COPDGene NHW and ECLIPSE.
‡Imputed genotypes.

aortic endothelial cells (48). It was also shown that small interfering RNA *GALC* knockdown inhibits human umbilical vein endothelial cell proliferation and migration, suggesting a pivotal role of *GALC* in endothelial neovascular responses. In addition, ceramides have been implicated in both COPD pathogenesis and vascular endothelial cell death through alterations in cellular apoptosis (49, 50). Even though a case report suggested lung involvement of Krabbe disease (51), it needs to be determined whether there is any role of GALC in the development of PH associated with COPD.

Table 4. Genome-Wide Association Study Meta-Analysis Results of Subjects with Chronic Obstructive Pulmonary Disease with a Pulmonary Artery:Ascending Aorta Ratio Greater Than 1 versus Smoking Control Subjects Compared with Those of Subjects with Chronic Obstructive Pulmonary Disease with a Pulmonary Artery:Ascending Aorta Ratio of 1 or Less versus Smoking Control Subjects for Previously Identified COPD Risk Alleles

Locus	Gene	SNP	Risk Allele	COPD with PA/A > 1 versus Smoking Control Subjects		COPD with PA/A ⩽ 1 versus Smoking Control Subjects		Permutation Testing to Assess Difference of OR between Two Meta-Analyses, 10,000 Times
				OR	*P* Value	OR	*P* Value	*P* Value
4q22	*FAM13A*	rs2869967*	C	1.25	9.14×10^{-5}	1.28	8.73×10^{-10}	NS
4q22	*FAM13A*	rs4416442†	C	1.27	1.66×10^{-5}	1.28	1.74×10^{-10}	NS
4q22	*FAM13A*	rs7671167*,‡	T	1.25	7.39×10^{-5}	1.22	2.32×10^{-7}	NS
4q22	*FAM13A*	rs1964516‡	T	1.17	6.32×10^{-3}	1.23	2.38×10^{-7}	0.015
4q31	*HHIP*	rs13141641†,‡	T	1.28	5.68×10^{-5}	1.23	7.94×10^{-7}	0.013
4q31	*HHIP*	rs13118928‡	A	1.22	9.25×10^{-4}	1.21	4.25×10^{-6}	NS
15q25	*CHRNA3*	rs12914385†	T	1.36	1.61×10^{-7}	1.23	5.98×10^{-7}	<0.0001
15q25	*AGPHD1*	rs8034191§	C	1.40	7.80×10^{-9}	1.12	7.97×10^{-3}	<0.0001
15q25	*IREB2*	rs11858836‡	A	1.38	3.90×10^{-7}	1.16	1.32×10^{-3}	<0.0001
19q13	*RAB4B*	rs2604894‡	G	1.16	1.01×10^{-2}	1.10	1.53×10^{-2}	0.010
14q32	*RIN3*	rs754388†	C	1.38	3.49×10^{-5}	1.29	1.19×10^{-6}	0.007
14q32	*RIN3*	rs17184313†	C	1.27	2.15×10^{-3}	1.27	8.53×10^{-6}	NS

Definition of abbreviations: COPD, chronic obstructive pulmonary disease; NS, not significant; OR, odds ratio; PA/A, ratio of the diameter of the main pulmonary artery (PA) at the level of its bifurcation and the diameter of the ascending aorta (A) in its maximum dimension measured by computed tomography; SNP, single-nucleotide polymorphism.
*Cho and colleagues (Ref. 27).
†Cho and colleagues (Ref. 23).
‡Cho and colleagues (Ref. 29).
§Pillai and colleagues (Ref. 37).

Our study has several limitations. First, although our strongest genetic associations with COPD with PAE were found with SNPs near *IREB2*, there are multiple other genes in the chromosome 15q25.1 region that could be responsible for the PAE associations. We have not determined which genetic variants in or near *IREB2* are functional variants for the development of PH in COPD. Although *IREB2* protein and mRNA were increased in lung tissue samples from subjects with COPD in comparison to control subjects (52), the specific role of IREB2 in the pathogenesis of PH associated with COPD and functional differences according to *IREB2* variants will need to be evaluated. Second, a replication analysis has not been explored, even though this was a meta-analysis of three GWASs using the largest COPD cohorts to date. Third, PA/A of our smoking control subjects was not included, although the prevalence of PAE in control subjects was lower than that in subjects with GOLD 2–4 (J.M.W., unpublished data). Because age-associated increases in PA systolic pressure in the general population have been reported (53), a portion of our smoking control subjects might have PA/A greater than 1, considering their mean age. Fourth, PA pressures were not measured, although the ratio of PA/A has been shown to correlate with hemodynamically gauged PA pressure (15–17) even better than echocardiography in severe COPD (18), and PA/A greater than 1 has been proven to be a predictor of severe exacerbation of COPD (19). It is also possible that PAE, at least in some subjects, could be caused by COPD-related factors unrelated to hypoxemia. PAE could be caused by not only resting PH, but also peripheral vascular pruning with centralization of blood flow, undiagnosed cardiovascular disease, or a combination of these mechanisms (10, 54–56). Despite these limitations, we emphasize that our phenotype was recently validated in a well designed study including a large number of subjects with COPD, longitudinal follow up, and replication (19), and that we examined our hypothesis in the same set of well phenotyped subjects. Finally, our study did not address whether *IREB2* is responsible for "disproportionate" PH associated with relatively mild airway limitation. Although some subjects with COPD with PAE had moderate airflow limitation (GOLD 2), mean FEV_1 % predicted was lower than among those without PAE. However, our complementary analysis including adjustment for FEV_1 % predicted revealed that rs7181486 in *IREB2* was one of the most significant SNPs, but was not significant genome wide. Moreover, the results from the meta-analysis for COPD with PAE relative to smoking control subjects were somewhat different from a recent meta-analysis of subjects with severe COPD relative to smoking control subjects, including our subjects, which demonstrated *MMP3/12*, *RIN3*, and *TGFB2*, as well as four previously described genome-wide significant loci, as the most significant associations (23). These results suggest that our genome-wide significant region, particularly *IREB2*, is likely to be from a vascular subtype, not purely an indicator of the severity of airflow limitation.

We have reported the first GWAS of subjects with COPD with PAE relative to those without PAE as well as to smoking control subjects, and identified *IREB2* and *GALC* as potential susceptibility genes associated with PH in COPD. This study strongly supports that phenotypic heterogeneity of COPD is closely linked to genetic heterogeneity. Additional GWASs for specific COPD subtypes are likely to provide further insight into different roles of genetic variants contributing to COPD heterogeneity. ■

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