ORIGINAL RESEARCH

Common Genetic Variants Associated with Resting Oxygenation in Chronic Obstructive Pulmonary Disease

Merry-Lynn N. McDonald[1], Michael H. Cho[1,2], Inga-Cecilie Sørheim[3], Sharon M. Lutz[4], Peter J. Castaldi[1], David A. Lomas[5], Harvey O. Coxson[6], Lisa D. Edwards[7], William MacNee[8], Jørgen Vestbo[9], Julie C. Yates[7], Alvar Agusti[10], Peter M. A. Calverley[11], Bartolome Celli[3], Courtney Crim[7], Stephen I. Rennard[12], Emiel F. M. Wouters[13], Per Bakke[3], Ruth Tal-Singer[14], Bruce E. Miller[14], Amund Gulsvik[3], Richard Casaburi[15], J. Michael Wells[16], Elizabeth A. Regan[17], Barry J. Make[17], John E. Hokanson[18], Christoph Lange[19], James D. Crapo[17], Terri H. Beaty[20], Edwin K. Silverman[1,2], and Craig P. Hersh[1,2], on behalf of the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints and COPDGene Investigators

[1]Channing Division of Network Medicine, and [2]Division of Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts; [3]Department of Clinical Science, University of Bergen, Bergen, Norway; [4]Colorado School of Public Health, Denver, Colorado; [5]Wolfson Institute for Biomedical Research, University College London, London, United Kingdom; [6]University of British Columbia Department of Radiology and Vancouver General Hospital, Vancouver, British Columbia, Canada; [7]GlaxoSmithKline, Research Triangle Park, North Carolina; [8]Department of Respiratory and Environmental Medicine, University of Edinburgh, Edinburgh, Scotland; [9]Department of Respiratory Medicine, Manchester Academic Health Sciences Centre, University Hospital of South Manchester, Manchester, United Kingdom; [10]Thoracic Institute, Hospital Clinic, Institut D'Investigacions Biomediques August Pi i Synyer (IDIBAPS), University of Barcelona and Centro investigación biomedica en red de enfermedades respiratorias (CIBERES), Barcelona, Spain; [11]Department of Pulmonary and Rehabilitation Medicine, University of Liverpool, Liverpool, United Kingdom; [12]Department of Medicine, University of Nebraska Medical Center, Omaha, Nebraska; [13]Department of Respiratory Medicine, Maastricht University Medical Center, Maastricht, The Netherlands; [14]GlaxoSmithKline, King of Prussia, Pennsylvania; [15]Rehabilitation Clinical Trials Center, Los Angeles Biomedical Research Institute at Harbor–University of California Los Angeles Medical Center, Torrance, California; [16]Divison of Pulmonary, Allergy and Critical Care Medicine, University of Alabama at Birmingham, Birmingham, Alabama; [17]Division of Pulmonary Sciences and Critical Care Medicine, National Jewish Health, Denver, Colorado; [18]Department of Epidemiology, Colorado School of Public Health, University of Colorado Denver, Denver, Colorado; [19]Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts; and [20]Johns Hopkins School of Public Health, Baltimore, Maryland

Abstract

Hypoxemia is a major complication of chronic obstructive pulmonary disease (COPD) that correlates with disease prognosis. Identifying genetic variants associated with oxygenation may provide clues for deciphering the heterogeneity in prognosis among patients with COPD. However, previous genetic studies have been restricted to investigating COPD candidate genes for association with hypoxemia. To report results from the first genome-wide association study (GWAS) of resting oxygen saturation (as measured by pulse oximetry [Sp_{O_2}]) in subjects with COPD, we performed a GWAS of Sp_{O_2} in two large, well characterized COPD populations: COPDGene, including both the non-Hispanic white (NHW) and African American (AA) groups, and Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE). We identified several suggestive loci ($P < 1 \times 10^{-5}$) associated with Sp_{O_2} in COPDGene in the NHW ($n = 2810$) and ECLIPSE ($n = 1758$) groups, and two loci on chromosomes 14 and 15 in the AA group ($n = 820$) from COPDGene achieving a level of genome-wide significance ($P < 5 \times 10^{-8}$). The chromosome 14 single-nucleotide polymorphism, rs6576132, located in an intergenic region, was nominally replicated ($P < 0.05$) in the NHW group from COPDGene. The chromosome 15 single-nucleotide polymorphisms were rare in subjects of European ancestry, so the results could not be replicated. The chromosome 15 region contains several genes, including *TICRR* and *KIF7*, and is proximal to *RHCG* (Rh family C glyocoprotein gene). We have identified two loci associated with resting oxygen saturation in AA subjects with COPD, and several suggestive regions in subjects of European descent with COPD. Our study highlights the importance of investigating the genetics of complex traits in different racial groups.

Keywords: chronic obstructive pulmonary disease; hypoxemia; pulse oximetry; genome-wide association study; oxygen saturation

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Correspondence and requests for reprints should be addressed to Merry-Lynn McDonald, Ph.D., Channing Division of Network Medicine, Brigham and Women's Hospital, Boston, MA 02115. E-mail: remrm@channing.harvard.edu

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Clinical Relevance

Hypoxemia is a major complication of chronic obstructive pulmonary disease (COPD) that is correlated with increased risk of mortality. From our analysis of the first genome-wide association study of resting oxygen saturation in COPD, we have highlighted genes and pathways that may provide clues for deciphering heterogeneity in prognosis among patients with COPD.

Chronic obstructive pulmonary disease (COPD) is a major cause of disability, and the third leading cause of death in the United States (1). Chronic hypoxemia is a strong predictor of mortality in COPD (2, 3). However, its relationship with the severity of airflow limitation is weak. We hypothesized that identifying genetic variants associated with the severity of hypoxemia could help decipher the heterogeneity in COPD.

Blood oxygen saturation has been termed the "fifth vital sign," and can be easily measured by pulse oximetry (Sp_{O_2}) in clinical settings (4). Sp_{O_2} is an indirect measure of arterial oxygen saturation (5). Low Sp_{O_2} values are generally caused by abnormalities of pulmonary gas exchange and, in rare cases, by mutations in hemoglobin proteins (6, 7). Furthermore, genetic variants in several genes have been associated with oxygen saturation in individuals living at high altitudes (8–10). Thus, similar to many other genetic traits, there is evidence of both Mendelian and complex determinants of resting oxygen saturation. This motivated us to investigate the genetic etiology of resting oxygen saturation in subjects with COPD through a genome-wide association of resting oxygen saturation in two large cohorts of well characterized patients with COPD (COPDGene and Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints [ECLIPSE]).

Materials and Methods

Study Participants

Details of the COPDGene and ECLIPSE studies have been previously described (11–13). Briefly, the COPDGene Study (NCT00608764, www.copdgene.org) enrolled non-Hispanic white (NHW) and African American (AA) subjects with COPD and control subjects, aged 45–80 years, with at least 10 pack-years of lifetime smoking history in 21 U.S. centers (14). The ECLIPSE study (SCO104960, NCT00292552; www.eclipse-copd.com) recruited patients with COPD and control subjects aged 45–75 years with a smoking history of at least 10 pack-years from 46 centers across 12 countries (11). Subjects with other significant lung diseases were excluded from both studies, although COPDGene did not exclude enrollment of subjects with a history of asthma. Both COPDGene and ECLIPSE had a clinical center in Denver, Colorado at an altitude of 1.6 km above sea level. Analyses were limited to patients with COPD, defined by post-bronchodilator FEV_1/forced vital capacity less than 0.7 and an FEV_1 less than 80% predicted (Global Initiative for Chronic Obstructive Lung Disease grade 2 or greater) (15).

Oxygen Saturation

Sp_{O_2} was measured on a finger without nail polish after the subject had remained seated for at least 5 minutes in COPDGene, and at least 10 minutes in ECLIPSE, and recorded only if a strong pulse was apparent. The median value was recorded after observing the pulse oximetry over a 1-minute period. Subjects treated with supplemental oxygen discontinued it while being monitored with the oximeter. In the analysis for both studies, if the pulse oximeter reported a reading of less than 82%, an Sp_{O_2} value of 82% was recorded (14).

Genetic Markers

Standard quality control steps were performed on DNA samples and single-nucleotide polymorphism (SNP) data as previously described (12–14, 16). Genotyping for the COPDGene population was performed using the Illumina Human Omni 1-Quad (Illumina, San Diego, CA), and for the ECLIPSE population the Illumina HumanHap 550v3 chips were used. The COPDGene data have been deposited into dbGaP (accession number phs000179.v1.p1). For both studies, additional genotypes were imputed using the 1,000 Genomes Reference panel (17). Only SNPs with an imputation quality score of 0.8 or greater were included in the analysis. A total of 4,749,595 imputed variants in the NHW subjects with COPD from COPDGene, 4,753,821 imputed variants in ECLIPSE subjects with COPD, and 6,160,662 imputed variants in the AA subjects with COPD from COPDGene passed quality control.

Statistical Analysis

Unless otherwise specified, statistical analyses were performed in R or PLINK (18). Sp_{O_2} values were log transformed to normalize their frequency distribution. Each SNP with minor allele frequency (MAF) of 5% or greater was tested for its correlation with resting oxygenation, as measured by the $\log_{10}$-transformed Sp_{O_2}, using an additive model adjusted for age, sex, pack-years of smoking, Denver as a study site, and principal components to summarize genetic background. Sensitivity analyses were performed by adjusting for FEV_1 and/or current smoking, and also by excluding participants from Denver. Analyses were performed in the COPDGene NHW and AA groups and the ECLIPSE study separately. The initial analysis was performed in NHW cases from COPDGene, the largest group, with replication in ECLIPSE. We then investigated the COPDGene AA cases while using the other two populations as replication samples. For the loci reaching genome-wide significance (GWS) in the AA population from COPDGene, we used HaploReg2 (19), with an r^2 value of 0.8 based on linkage disequilibrium information of 1,000 Genomes African Ancestry population, to assess evidence that SNPs were located in regulatory regions. Meta-analyses were performed between NHW cases from COPDGene and ECLIPSE, in addition to all three populations, including the AA subjects from COPDGene. Gene-based analyses were performed using VEGAS (20), software that generates a gene-based result based on single SNP *P* values from the genome-wide association study (GWAS) and linkage disequilibrium patterns (20). VEGAS assigns SNPs to genes based on physical position ($\pm$ 50 kb) of known genes in the University of California, Santa Cruz (Santa Cruz, CA) genome browser. Regional association plots were generated using LocusZoom (21).

Results

Characterization of Participants

Table 1 presents the main clinical characteristics of the participants

Table 1. Characteristics of Subjects with Chronic Obstructive Pulmonary Disease Included in the Analysis

Characteristic	COPDGene		ECLIPSE
	Non-Hispanic White	African American	
n	2,810	820	1,758
Males, %	55.7	55.1	67.2
Age, yr	64.7 (8.2)	59 (8.2)	63.6 (7.1)
BMI, kg/m^2	28.1 (6.1)	28 (6.8)	26.7 (5.6)
Smoking exposure, pack-years	56.3 (28)	42.4 (23)	50.3 (27)
Enrollment in Denver, %	22.7	4.3	1.2
FEV_1 % predicted	49.6 (18)	52.2 (17.8)	43.5 (14.9)
FEV_1/FVC, %	0.49 (0.13)	0.53 (0.12)	0.44 (0.11)
Sp_{O_2}, %*	95 (17)	97 (17)	95 (18)
Severe hypoxemia, %†	7	4	4

Definition of abbreviations: BMI, body mass index; ECLIPSE, Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints study; FVC, forced vital capacity; Sp_{O_2}, oxygen saturation measured using pulse oximetry.
Values presented are mean and SD unless otherwise noted.
*Median (range).
†$Sp_{O_2} \leq 88\%$.

represented in this investigation. In ECLIPSE, subjects with COPD consisted of a higher proportion of males (67.2%) than COPDGene NHW and AA cases (55.7 and 55.1%, respectively). The AA case population was, on average, slightly younger and also had a lower mean exposure to smoking, as measured by pack-years, than the NHW and ECLIPSE cases. The ECLIPSE subjects were, on average, slightly less overweight (mean body mass index = 26.7 kg/m^2), but had lower lung function measurements than subjects with COPD from the COPDGene study. ECLIPSE also had fewer participants recruited from Denver than either COPDGene case populations. The percentage of subjects with COPD with severe hypoxemia, defined as Sp_{O_2} of 88% or less, was higher in the NHW (7%) than both the AA and ECLIPSE cases (4%). However, the median and range of Sp_{O_2} in each population were comparable with the medians for all three study populations, centered at approximately 95%. We also examined the subset of subjects within each study recruited from Denver, the site with the highest altitude (*see* Table E1 in the online supplement). Subjects recruited from Denver were slightly less overweight and had lower lung function than the respective total case populations. Furthermore, the median percent Sp_{O_2} was lower and there were more cases with severe hypoxemia recruited from Denver compared with the total study populations.

Distribution of Sp_{O_2} in Subjects with COPD

The distribution of Sp_{O_2} in subjects with COPD in COPDGene and ECLIPSE was not normally distributed (*see* Figure E1). As a result, a log transformation was used to normalize the trait. Figure E1 displays the distribution of residuals of the log-transformed trait after adjusting for sex, lifetime smoking intensity, age, Denver site, and population substructure for each COPD case population.

GWAS of Sp_{O_2} in Subjects with COPD with European Ancestry

In the GWAS of the NHW population from COPDGene, no SNP reached GWS in tests of association with $\log(Sp_{O_2})$, nor did any SNP reach GWS in the meta-analysis between the COPDGene and ECLIPSE (data not shown). In Table 2, the top 10 SNPs associated with $\log(Sp_{O_2})$ sorted by chromosome and physical position are listed for the COPDGene NHW and ECLIPSE analyses. Several SNPs on chromosome 1, approximately 71 kb 5′ of the gene, *RUNX3* (runt-related transcription factor 3), were associated ($P = 1.2 \times 10^{-6}$) with $\log(Sp_{O_2})$ in the NHW group in COPDGene. Several SNPs near *NRXN1* (neurexin-1-α) on chromosome 2 were associated with $\log(Sp_{O_2})$ ($P < 7.1 \times 10^{-7}$). SNP rs6893408 on chromosome 5 located within the *FGF1* (fibroblast growth factor 1) gene was associated with $\log(Sp_{O_2})$ in COPDGene (Beta = −0.0026 $\log[Sp_{O_2}]$ units; $P = 4.8 \times 10^{-7}$), which corresponded to a mean difference in Sp_{O_2} of −1.0% Sp_{O_2} for each minor allele. Three SNPs, with *TRIQK* (triple QxxK/R motif containing) as the closest gene, were moderately associated with resting oxygenation in the NHW population. Several SNPs in the *CBR4* (carnbonyl reductase family member 4) gene region on chromosome 4 and single SNPs in the genes, *OPCML* (Opioid Binding Protein/Cell Adhesion) and *BRSK2* (BR serine/threonine kinase 2), were associated with $\log(Sp_{O_2})$ in the ECLIPSE cases; however, these results did not reach GWS. The top results in COPDGene NHW and ECLIPSE subjects were examined in the other populations (Tables E2 and E3). As a secondary analysis, we also tested variants in the two largest populations, COPDGene NHW and ECLIPSE, with MAF greater than 1%. No variant reached a level of GWS in the largest population: the NHW from COPDGene. However, one variant with a MAF of 2.3%, rs7868621 on chromosome 9, reached GWS (Beta = −0.0080; SE = 0.0014; $P = 4.3 \times 10^{-8}$) in ECLIPSE. This variant did not replicate in either COPDGene population ($P_{NHW} = 0.80$ and $P_{AA} = 0.21$, respectively).

We also examined the top results adjusting for FEV_1, current smoking, and both together (Table E4). The top results did not change substantially when FEV_1 and/or current smoking were considered. Furthermore, we found that the top associations in ECLIPSE were robust to excluding participants from Denver (Table 2). However, after excluding cases from Denver (22.7% of the total NHW case population), the top results in the NHW population became less significant.

GWAS of Sp_{O_2} in AA Subjects with COPD

There were two SNPs with a MAF greater than 5% that were significantly associated with $\log(Sp_{O_2})$ among the COPDGene AA cases (Table 3). Further examination of variants in these regions with MAF less than 5% identified three more variants on chromosome 15 significantly associated with Sp_{O_2} (MAF, ∼4%). The chromosome 14 SNP, rs6576132 (Beta = 0.0039; $P = 5.0 \times 10^{-8}$), replicated nominally among the COPDGene NHW case population (Beta = 0.0009; $P = 0.011$). This Beta

Table 2. Top 10 Single-Nucleotide Polymorphisms Associated with $\log_{10}(Sp_{O_2})$ in Non-Hispanic White Subjects with Chronic Obstructive Pulmonary Disease from the COPDGene and ECLIPSE Studies

							Excluding Participants from Denver				
CHR	SNP	Position	MAF (%)	BETA	95% CI	*P* Value	MAF (%)	BETA	95% CI	*P* Value	Closest Gene
COPDGene non-Hispanic white subjects with COPD (*n* = 2,810)											
1	rs10903128	25,363,338	27.7	−0.0020	−0.0028 to −0.0012	1.2E−06	26.9	−0.0010	−0.0018 to −0.0002	0.013	*RUNX3* (~72 kB)
1	rs452044	25,363,656	27.9	−0.0020	−0.0028 to −0.0012	1.2E−06	27.8	−0.0010	−0.0017 to −0.0002	0.015	*RUNX3* (~72 kB)
1	rs453532	25,363,707	27.7	−0.0020	−0.0028 to −0.0012	1.2E−06	27.0	−0.0010	−0.0018 to −0.0002	0.013	*RUNX3* (~72 kB)
2	rs10190452	52,230,237	45.8	0.0019	0.0011 to 0.0027	4.3E−07	45.7	0.0013	0.0007 to 0.0019	1.2E−04	*NRXN1* (~97 kB)
2	rs6736403	52,233,463	45.6	0.0019	0.0011 to 0.0026	4.1E−07	45.7	0.0013	0.0006 to 0.0020	1.5E−04	*NRXN1* (~97 kB)
2	rs7566343	52,240,977	44.9	0.0018	0.0010 to 0.0026	7.1E−07	44.8	0.0014	0.0008 to 0.0020	8.8E−05	*NRXN1* (~97 kB)
5	rs6893408	142,022,777	14.3	−0.0026	−0.0036 to −0.0016	4.8E−07	14.1	−0.0012	−0.0022 to −0.0003	0.014	*FGF1*
8	rs536161	93,513,027	27.8	−0.0019	−0.0027 to −0.0011	2.5E−06	28.4	−0.0010	−0.0018 to −0.0002	0.013	*TRIQK* (~380 kB)
8	rs511954	93,514,434	27.9	−0.0019	−0.0027 to −0.0011	2.4E−06	28.4	−0.0010	−0.0018 to −0.0002	0.013	*TRIQK* (~380 kB)
8	rs482619	93,514,906	28.2	−0.0019	−0.0028 to −0.0011	2.4E−06	28.1	−0.0010	−0.0017 to −0.0002	0.013	*TRIQK* (~380 kB)
ECLIPSE subjects with COPD (*n* = 1,758)											
4	rs115325116	169,933,895	6.4	−0.0047	−0.0065 to −0.0029	2.3E−07	6.4	−0.0047	−0.006 to −0.003	2.6E−07	*CBR4* (~2 kB)
4	rs180977786	169,933,903	6.6	−0.0047	−0.0065 to −0.0029	2.4E−07	6.6	−0.0047	−0.006 to −0.003	2.7E−07	*CBR4* (~2 kB)
4	rs17615397	169,937,521	5.7	−0.0050	−0.0070 to −0.0030	3.8E−07	5.7	−0.0050	−0.007 to −0.003	4.2E−07	*CBR4* (~2 kB)
4	rs74581741	169,940,336	5.7	−0.0050	−0.0070 to −0.0030	3.8E−07	5.7	−0.0050	−0.007 to −0.003	4.2E−07	*CBR4* (~2 kB)
4	rs17615362	169,934,087	6.4	−0.0046	−0.0064 to −0.0028	4.0E−07	6.3	−0.0046	−0.006 to −0.003	4.4E−07	*CBR4* (~2 kB)
4	rs17543620	169,934,725	6.4	−0.0046	−0.0064 to −0.0028	4.0E−07	6.3	−0.0046	−0.006 to −0.003	4.5E−07	*CBR4* (~2 kB)
4	rs148456540	169,937,248	5.4	−0.0052	−0.0072 to −0.0032	4.4E−07	5.4	−0.0051	−0.007 to −0.003	5.3E−07	*CBR4* (~2 kB)
4	rs77404015	169,934,424	6.5	−0.0044	−0.0062 to −0.0026	1.0E−06	6.5	−0.0044	−0.006 to −0.003	1.1E−06	*CBR4* (~2 kB)
11	rs4379857	133,183,604	5.5	−0.0045	−0.0063 to −0.0026	2.2E−06	5.5	−0.0045	−0.006 to −0.003	2.0E−06	*OPCML*
11	rs138091420	1,399,692	8.3	−0.0038	−0.0054 to −0.0022	2.3E−06	8.3	−0.0036	−0.005 to −0.002	6.0E−06	*BRSK2* (11 kB)

Definition of abbreviations: CHR, chromosome; CI, confidence interval; COPD, chronic obstructive pulmonary disease; ECLIPSE, Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints study; MAF, minor allele frequency; SNP, single-nucleotide polymorphism; Sp_{O_2}, oxygen saturation measured using pulse oximetry.
The term "Position" indicates the Human Genome Build 19 physical position in base pairs, and "Closest gene" is the closest gene to the SNP, where SNP distance to gene is denoted in parentheses when SNP is not within gene.

Table 3. SNPs Associated at a Level of Genome-Wide Significance* with $\log_{10}(Sp_{O_2})$ in the African American Population from COPDGene

			COPDGene African American Subjects with COPD (n = 820)				Excluding Participants from Denver				
CHR	SNP	Position	MAF (%)	BETA	95% CI	*P* Value	MAF (%)	BETA	95% CI	*P* Value	Closest Gene
14	rs6576132	28,728,111	43.4	0.004	0.003 to 0.005	4.9E−08	43.6	0.003	0.002 to 0.005	1.3E−06	*FOXG1* (~ 508 kB)
15	rs8038108	90,089,276	4.8	−0.009	−0.013 to −0.006	4.8E−09	4.6	−0.009	−0.012 to −0.006	1.7E−08	*LINC00928* (~ 22 kB)
15	rs116033091	90,076,553	4.6	−0.009	−0.012 to −0.006	2.2E−08	4.4	−0.008	−0.011 to −0.005	2.1E−07	*LINC00928* (~ 9 kB)
15	rs8025537	90,093,403	4.2	−0.009	−0.012 to −0.006	2.5E−08	4.5	−0.008	−0.011 to −0.005	6.0E−08	*TICRR* (~ 25 kB)
15	rs147566087	90,096,263	5.5	−0.008	−0.011 to −0.005	4.6E−08	5.3	−0.008	−0.011 to −0.005	7.8E−08	*TICRR* (~ 25 kB)

Definition of abbreviations: CHR, chromosome; CI, confidence interval; COPD, chronic obstructive pulmonary disease; ECLIPSE, Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints study; MAF, minor allele frequency; SNP, single-nucleotide polymorphism; Sp_{O_2}, oxygen saturation measured using pulse oximetry.
The term "Position" indicates the Human Genome Build 19 physical position in base pairs, and "Closest gene" is the closest gene to the SNP, where SNP distance to gene is denoted in parentheses when SNP is not within gene.
*$P \leq 5.0 \times 10^{-8}$.

corresponded to a mean difference of 1.0% Sp_{O_2} for each minor allele. Figure E3A illustrates Sp_{O_2} stratified by rs6576132 genotype. The minor allele for this SNP is common, with an MAF of 43.4%. Approximately 145 AA subjects with COPD carried the CC genotype, with a median Sp_{O_2} of 97.7%. This is in comparison to roughly 250 AA subjects with COPD who carried the AA genotype, with a median Sp_{O_2} of 95.5%. However, there were no genes annotated to this region as is depicted in the regional association plot (Figure 1A). The five SNPs associated with $\log(Sp_{O_2})$ on chromosome 15 had MAFs that ranged from 4.2 to 5.5% in the AA population, but these markers were rare in European populations (MAF = 0.02% in COPDGene NHW cases). For this reason, we were unable to replicate this region in the NHW or ECLIPSE populations (Table E5). We assessed whether the top SNPs in Table 3 were located in regulatory regions using HaploReg2. Several of these SNPs, chr14: rs6576132, chr15: rs116033091, and chr15: rs8038108, are predicted to alter regulatory motifs. No additional loci reached GWS in the meta-analysis of all three populations (data not shown).

Figures E3A and E3B show Sp_{O_2} stratified by genotype for the single chromosome 14 SNP and two chromosome 15 SNPs exhibiting GWS. The *KIF7* (kinesin family member 7) gene is annotated to the chromosome 15 region (Figure 1B). This region is also in close proximity to the *RHCG* (Rh family, C glycoprotein) gene; however, the genetic region associated with $\log(Sp_{O_2})$ in AA subjects was separated from *RHCG* by a recombination hot spot. Similar to the analysis in the two populations of European descent, adjusting for the effect of FEV_1 and/or current smoking in the AA cases had no major effect on the top results (Table E6). However, when subjects recruited from Denver (4.3% of the total AA case population) were excluded from the analysis, the association on chromosome 14 no longer reached GWS (Beta = 0.003; $P = 1.3 \times 10^{-6}$; Table 3). However, the association with rs8038108 on chromosome 15 remained of GWS (Beta = −0.009; $P = 1.7 \times 10^{-8}$).

Gene-Based Associations with Sp_{O_2} in COPD

We next performed a gene-based analysis for 17,640 genes, as opposed to a single

SNP–based analysis, in each population using VEGAS. None of the gene-based results withstood Bonferonni correction ($P < 2.8 \times 10^{-6}$). The top results from each population are listed in Table E7. For comparison, we also examined the results excluding participants from Denver. In the COPDGene NHW population, the top gene was *BIN1* on chromosome 2 ($P_{top20\%} = 9.0 \times 10^{-6}$). The next highest-ranking gene was *TGFBR3*, which our group has previously shown to be associated with emphysema (22). The top gene associated with log(Sp_{O_2}) in ECLIPSE was *CLEC12A* ($P_{top20\%} = 3.0 \times 10^{-5}$). The top gene in the AA population was *HSD17B3* ($P_{top20\%} = 1.1 \times 10^{-5}$). We also looked specifically for associations with hemoglobin genes in the hemoglobin α and β gene regions on chromosomes 11 and 16 in each population (Table E8). Interestingly, none of these genes reached nominal significance ($P < 0.05$) in the NHW case population. However, *HBA2* ($P_{top20\%} = 0.039$) and *HBM* ($P_{top20\%} = 0.044$) were nominally significant in the AA COPD case population.

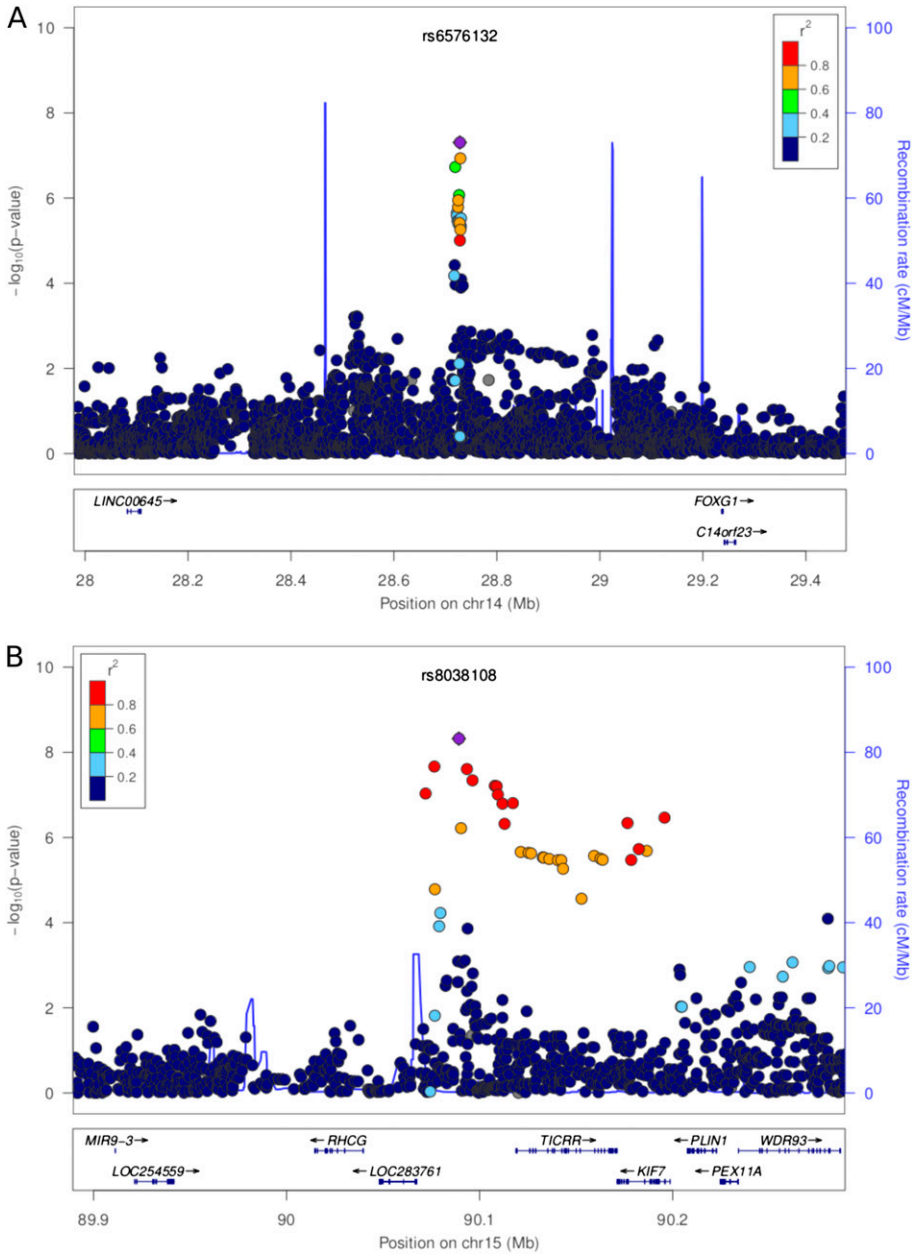

Figure 1. Regional association plots around single-nucleotide polymorphisms reaching genome-wide significance for association with log(Sp_{O_2}) in African American subjects from COPDGene. (*A*) Chromosome 14; (*B*) chromosome 15. Sp_{O_2}, oxygen saturation measured using pulse oximetry.

Discussion

This study has identified two genetic regions that are significantly associated with resting oxygen saturation among AA subjects with COPD. We were able to replicate nominally ($P < 0.05$) the association on chromosome 14 in the NHW subjects from COPDGene. The SNPs we found associated on chromosome 15 with Sp_{O_2} in AA subjects with COPD were rare in populations of European descent. However, this region encompassed several candidate genes for further investigation, including *TICRR*, *KIF7*, and *RHCG*. Although we identified several suggestive associations using the two populations of European descent, NHW subjects from COPDGene and ECLIPSE, our investigation underscores the importance of investigating the genetics of complex traits in additional racial groups, such as AA subjects.

Previous Studies

Previous genetic studies of oxygen saturation in COPD focused on genes known to influence COPD susceptibility rather than oxygen saturation *per se* (23, 24). On the other hand, there have been few investigations into the genetics of oxygen saturation in other respiratory diseases, and these have mainly been restricted to candidate gene studies. Schroder and colleagues (25) found evidence for association of serotonin transporter polymorphism with decreased arterial oxygen saturation, and also with increasing apnea–hypopnea index in older adults. de Lima Marson and colleagues (26) searched for cystic fibrosis modifier genes by assessing genetic interactions with cystic fibrosis transmembrane conductance regulator gene mutations, but did not find a significant association with oxygen saturation itself. The role of environmental factors, such as altitude, oxygen use, exercise capacity, and quality of life, has been investigated in relation to hypoxemia in patients with COPD (27). Here, we report results from the first GWAS of resting oxygen saturation in patients with COPD.

Interpretation of Findings

The median and range of Sp_{O_2} in the three COPD case populations were similar at

roughly 95%. The NHW population had a higher percentage of subjects with COPD with severe hypoxemia at 7% in comparison with 4% in the AA and ECLIPSE groups. This difference is slightly lower than the prevalence Kim and colleagues (27) reported for the prevalence of severe hypoxemia of 7.7% (n = 1,061) in the first 2,500 subjects with COPD enrolled in COPDGene. In the current report, the NHW subjects with COPD from COPDGene had the highest percentage of subjects recruited in Denver. Furthermore, the NHW cases also had more pack-years of smoking on average than the other two case populations. Smoking history is known to influence Sp_{O_2} levels even in the general population (28). For this reason, we included these variables as covariates in the GWAS, and also assessed the effects of current smoking on the results.

Our analysis of Sp_{O_2} in two COPD case populations of European descent, the NHW subjects with COPD from COPDGene and ECLIPSE, revealed suggestive associations with variants near several genes, including *BRSK2*, *CBR4*, *RUNX3*, and *FGF1*. BRSK2 is a brain-specific kinase (29). *CBR4*, a carbonyl reductase gene, is thought to play a role in biosynthesis of fatty acids in the mitochondria (30). *RUNX3* is a transcription factor essential for CD8 T cell development (31). A related gene, *RUNX1*, which also contains a Runt domain, is essential for hematopoiesis, and chromosomal rearrangements in this gene are commonly found in patients with leukemia (32, 33). In our study, we observed several variants with suggestive association near *RUNX3* in the NHW COPD case population in COPDGene. The variants are approximately 71 kB from *RUNX3*, and there is evidence of transcriptional regulation in lymphoblastoid cells (19). Furthermore, a SNP in *FGF1* demonstrated suggestive association with Sp_{O_2} in our study. FGF1 is a member of the fibroblast growth factor family, which is involved in a broad range of processes. FGF1, in particular, influences endothelial cell migration and proliferation, in addition to playing a role in angiogeneisis and wound healing (34).

Most interestingly, our analysis revealed two regions associated with resting oxygen saturation in AA subjects with COPD. The region on chromosome 14 also replicated in the NHW population from COPDGene. This region is intergenic and bounded by genes, *FOXG1* (forkhead box G1) and *LINC00645* (noncoding RNA645), by recombination peaks (Figure 1A). The second region on chromosome 15 tags the genes, *TICRR* and *KIF7*, and is also proximal to *RHCG*, but is separated by a recombination peak (Figure 1B). *RHCG* is an intriguing candidate gene for blood oxygen saturation, as it codes for an Rh protein, which is important for maintaining the structure of the red blood cell membrane (35). *TICRR* codes for the treslin protein involved with the initiation of DNA replication (36). *KIF7* is an essential component of the sonic hedgehog signaling pathway, and also for the development of the diaphragm (37). Furthermore, *RUNX3*, for which we showed suggestive associations with resting blood oxygen in the NHW subjects with COPD, is involved with Indian Hedgehog signaling in epithelial cells in the stomach and intestine (38). The *HHIP* (hedgehog interacting protein) gene is associated with lung function and COPD (13, 16, 39–41); however, its contribution to the pathogenesis of COPD remains to be elucidated (38, 41). The robustness of our top findings after adjusting for FEV_1 suggests that we have identified true genetic associations with resting oxygen saturation as opposed to associations with COPD severity.

We also performed a gene-based analysis in each COPD case population testing for association with resting oxygen saturation. Although no gene withstood correction for multiple testing, the gene, *TGFBR3*, which has been previously associated with emphysema, was among the top genes associated in the NHW subjects with COPD from COPDGene (22). We saw no evidence of association with common variants in hemoglobin genes on chromosomes 11 and 16.

Potential Limitations

Our study has several limitations. Our study lacks an AA replication population for the findings achieving GWS in the AA subjects from COPDGene. It is well established that the pattern of linkage disequilibrium between individuals of European and African descent differs (42, 43). In our report, the variants that we have identified as associated with Sp_{O_2} in the AA population on chromosome 15 were extremely rare in European populations. However, the variants in the chromosome 14 region are common in both the AA and NHW populations. For these more common variants, we were able to nominally replicate ($P < 0.05$) statistical associations in the larger NHW population. With complex traits, it is possible that different variants are associated in populations with different racial/ethnic populations.

Furthermore, the top result on chromosome 14 in the COPDGene AA cases became less significant when participants from Denver were excluded, but the top results on chromosome 15 were robust to excluding subjects from Denver. This indicates that the chromosome 14 finding is confounded by living at high altitude, whereas the chromosome 15 finding is not. Although genetic variants and genes have been associated adaptation to hypoxia in high-altitude populations (44–49), our results did not show overlap with these findings, which is not surprising given the differences in phenotype. Additional studies of AA subjects with COPD living at high altitude would be required to further explore the relationship between the chromosome 14 variant and resting oxygen levels.

Conclusions

This study identifies two novel regions associated with resting oxygen saturation on chromosomes 14 and 15 among AA subjects with COPD. Several genes involved in the hedgehog and transforming growth factor β pathways support the relevance of these pathways in COPD. Additional studies will be required to determine the functional variants and mechanisms by which the chromosome 14 and 15 loci could influence resting oxygen saturation in COPD. Our report underscores the importance of investigating multiple racial populations in genetic studies of complex disease. ■

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COPDGene Investigators—Core Units

Administrative Core: James Crapo, M.D. (principal investigator [PI]), Edwin Silverman, M.D., Ph.D. (PI), Barry Make, M.D., Elizabeth Regan, M.D., Rochelle Lantz, Lori Stepp, Sandra Melanson.

Genetic Analysis Core: Terri Beaty, Ph.D., Nan Laird, Ph.D., Christoph Lange, Ph.D., Michael Cho, M.D., Stephanie Santorico, Ph.D., John Hokanson, M.P.H., Ph.D., Dawn DeMeo, M.D., M.P.H., Nadia Hansel, M.D., M.P.H., Craig Hersh, M.D., M.P.H., Peter Castaldi, M.D., M.Sc., Merry-Lynn McDonald, Ph.D., Jin Zhou, Ph.D., Manuel Mattheisen, M.D., Emily Wan, M.D., Megan Hardin, M.D., Jacqueline Hetmanski, M.S., Margaret Parker, M.S., Tanda Murray, M.S., Marilyn Foreman, M.D., M.S., Sharon M. Lutz, M.P.H., Ph.D., Elizabeth Regan, M.D., Ph.D., Edwin K. Silverman, M.D., Ph.D.

Imaging Core: David Lynch, MB, Joyce Schroeder, M.D., John Newell, Jr., M.D., John Reilly, M.D., Harvey Coxson, Ph.D., Philip Judy, Ph.D., Eric Hoffman, Ph.D., George Washko, M.D., Raul San Jose Estepar, Ph.D., James Ross, M.Sc., Mustafa Al Qaisi, M.D., Jordan Zach, Alex Kluiber, Jered Sieren, Tanya Mann, Deanna Richert, Alexander McKenzie, Jaleh Akhavan, Douglas Stinson, Christian W. Cox, M.D., Deanna Cusick, Jennifer G. Dy, Ph.D., Shoshana Ginsburg, M.S., Arkadiusz Sitek, Ph.D., Edwin van Beek, M.D., Ph.D., M.Ed.

Pulmonary Function Testing Quality Assurance Core, *LDS Hospital, Salt Lake City, UT*: Robert Jensen, Ph.D.

Biological Repository, *Johns Hopkins University, Baltimore, MD*: Homayoon Farzadegan, Ph.D., Stacey Meyerer, Shivam Chandan, Samantha Bragan

Data Coordinating Center and Biostatistics, *National Jewish Health, Denver, CO*: Douglas Everett, Ph.D., Andre Williams, Ph.D., Carla Wilson, M.S., Anna Forssen, M.S., Amber Powell, Joe Piccoli

Epidemiology Core, *University of Colorado School of Public Health, Denver, CO*: John Hokanson, M.P.H., Ph.D., Marci Sontag, Ph.D., Jennifer Black-Shinn, M.P.H., Gregory Kinney, M.P.H., Ph.D.c, Sharon Lutz, M.P.H., Ph.D.

COPDGene Investigators: Clinical Centers

Ann Arbor Veterans Affairs, Ann Arbor, MI: Jeffrey Curtis, M.D., Ella Kazerooni, M.D.

Baylor College of Medicine, Houston, TX: Nicola Hanania, M.D., M.S., Philip Alapat, M.D., Venkata Bandi, M.D., Kalpalatha Guntupalli, M.D., Elizabeth Guy, M.D., Antara Mallampalli, M.D., Charles Trinh, M.D., Mustafa Atik, M.D., Hasan Al-Azzawi, M.D., Marc Willis, D.O., Susan Pinero, M.D., Linda Fahr, M.D., Arun Nachiappan, M.D., Collin Bray, M.D., L. Alexander Frigini, M.D., Carlos Farinas, M.D., David Katz, M.D., Jose Freytes, M.D., Anne Marie Marciel, M.D.

Brigham and Women's Hospital, Boston, MA: Dawn DeMeo, M.D., M.P.H., Craig Hersh, M.D., M.P.H., George Washko, M.D., Francine Jacobson, M.D., M.P.H., Hiroto Hatabu, M.D., Ph.D., Peter Clarke, M.D., Ritu Gill, M.D., Andetta Hunsaker, M.D., Beatrice Trotman-Dickenson, M.B.B.S., Rachna Madan, M.D.

Columbia University, New York, NY: R. Graham Barr, M.D., Dr.PH., Byron Thomashow, M.D., John Austin, M.D., Belinda D'Souza, M.D.

Duke University Medical Center, Durham, NC: Neil MacIntyre, Jr., M.D., Lacey Washington, M.D., H. Page McAdams, M.D.

Reliant Medical Group, Worcester, MA: Richard Rosiello, M.D., Timothy Bresnahan, M.D., Joseph Bradley, M.D., Sharon Kuong, M.D., Steven Meller, M.D., Suzanne Roland, M.D.

Health Partners Research Foundation, Minneapolis, MN: Charlene McEvoy, M.D., M.P.H., Joseph Tashjian, M.D.

Johns Hopkins University, Baltimore, MD: Robert Wise, M.D., Nadia Hansel, M.D., M.P.H., Robert Brown, M.D., Gregory Diette, M.D., Karen Horton, M.D.

Los Angeles Biomedical Research Institute at Harbor–University of California Los Angeles Medical Center, Torrance, CA: Richard Casaburi, M.D., Ph.D., Janos Porszasz, M.D., Ph.D., Hans Fischer, M.D., Matt Budoff, M.D.

Michael E. DeBakey Veterans Affairs Medical Center, Houston, TX: Amir Sharafkhaneh, M.D., Charles Trinh, M.D., Hirani Kamal, M.D., Roham Darvishi, M.D., Marc Willis, D.O., Susan Pinero, M.D., Linda Fahr, M.D., Arun Nachiappan, M.D., Collin Bray, M.D., L. Alexander Frigini, M.D., Carlos Farinas, M.D., David Katz, M.D., Jose Freytes, M.D., Anne Marie Marciel, M.D.

Minneapolis Veterans Affairs, Minneapolis, MN: Dennis Niewoehner, M.D., Quentin Anderson, M.D., Kathryn Rice, M.D., Audrey Caine, M.D.

Morehouse School of Medicine, Atlanta, GA: Marilyn Foreman, M.D., M.S., Gloria Westney, M.D., M.S., Eugene Berkowitz, M.D., Ph.D.

National Jewish Health, Denver, CO: Russell Bowler, M.D., Ph.D., David Lynch, M.B., Joyce Schroeder, M.D., Valerie Hale, M.D., John Armstrong, II, M.D., Debra Dyer, M.D., Jonathan Chung, M.D., Christian Cox, M.D.

Temple University, Philadelphia, PA: Gerard Criner, M.D., Victor Kim, M.D., Nathaniel Marchetti, D.O., Aditi Satti, M.D., A. James Mamary, M.D., Robert Steiner, M.D., Chandra Dass, M.D., Libby Cone, M.D.

University of Alabama, Birmingham, AL: William Bailey, M.D., Mark Dransfield, M.D., Michael Wells, M.D., Surya Bhatt, M.D., Hrudaya Nath, M.D., Satinder Singh, M.D.

University of California, San Diego, CA: Joe Ramsdell, M.D., Paul Friedman, M.D.

University of Iowa, Iowa City, IA: Alejandro Cornellas, M.D., John Newell, Jr., M.D., Edwin J. R. van Beek, M.D., Ph.D.

University of Michigan, Ann Arbor, MI: Fernando Martinez, M.D., Mei Lan Han, M.D., Ella Kazerooni, M.D.

University of Minnesota, Minneapolis, MN: Christine Wendt, M.D., Tadashi Allen, M.D.

University of Pittsburgh, Pittsburgh, PA: Frank Sciurba, M.D., Joel Weissfeld, M.D., M.P.H., Carl Fuhrman, M.D., Jessica Bon, M.D., Danielle Hooper, M.D.

University of Texas Health Science Center at San Antonio, San Antonio, TX: Antonio Anzueto, M.D., Sandra Adams, M.D., Carlos Orozco, M.D., Mario Ruiz, M.D., Amy Mumbower, M.D., Ariel Kruger, M.D., Carlos Restrepo, M.D., Michael Lane, M.D.

Principal Investigators and Centers Participating in the ECLIPSE Study Include:

Bulgaria: Y. Ivanov (Pleven); K. Kostov (Sofia). *Canada*: J. Bourbeau (Montreal, PQ); M. Fitzgerald (Vancouver, BC); P. Hernandez (Halifax, NS); K. Killian (Hamilton, ON); R. Levy (Vancouver, BC); F. Maltais (Montreal, PQ); D. O'Donnell (Kingston, ON). *Czech Republic*: J. Krepelka (Prague). *Denmark*: J. Vestbo (Hvidovre). *The Netherlands*: E. Wouters (Horn–Maastricht). *New Zealand*: D. Quinn (Wellington). *Norway*: P. Bakke (Bergen). *Slovenia*: M. Kosnik (Golnik). *Spain*: A. Agusti, J. Sauleda (P. de Mallorca). *Ukraine*: Y. Feschenko, V. Gavrisyuk, L. Yashina (Kiev); N. Monogarova (Donetsk). *United Kingdom*: P. Calverley (Liverpool); D. Lomas (Cambridge); W. MacNee (Edinburgh); D. Singh (Manchester); J. Wedzicha (London). *United States*: A. Anzueto (San Antonio, TX); S. Braman (Providence, RI); R. Casaburi (Torrance, CA); B. Celli (Boston, MA); G. Giessel (Richmond, VA); M. Gotfried (Phoenix, AZ); G. Greenwald (Rancho Mirage, CA); N. Hanania (Houston, TX); D. Mahler (Lebanon, NH); B. Make (Denver, CO); S. Rennard (Omaha, NE); C. Rochester (New Haven, CT); P. Scanlon (Rochester, MN); D. Schuller (Omaha, NE); F. Sciurba (Pittsburgh, PA); A. Sharafkhaneh (Houston, TX); T. Siler (St. Charles, MO); E. Silverman (Boston, MA); A. Wanner (Miami, FL); R. Wise (Baltimore, MD); R. ZuWallack (Hartford, CT).

Steering Committee:

H. Coxson (Canada), C. Crim (GlaxoSmithKline, USA), L. Edwards (GlaxoSmithKline, USA), D. Lomas (UK), W. MacNee (UK), E. Silverman (USA), R. Tal Singer (Co-chair, GlaxoSmithKline, USA), J. Vestbo (Co-chair, Denmark), J. Yates (GlaxoSmithKline, USA).

Scientific Committee:

A. Agusti (Spain), P. Calverley (UK), B. Celli (USA), C. Crim (GlaxoSmithKline, USA), B. Miller (GlaxoSmithKline, USA), W. MacNee (Chair, UK), S. Rennard (USA), R. Tal-Singer (GlaxoSmithKline, USA), E. Wouters (The Netherlands), J. Yates (GlaxoSmithKline, USA).

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