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Genetic Associations With Hypoxemia and Pulmonary Arterial Pressure in COPD*

Peter J. Castaldi, MD, Craig P. Hersh, MD, MPH, John J. Reilly, MD, FCCP, and Edwin K. Silverman, MD, PhD

Institute for Clinical Research and Health Policy Studies (Dr. Castaldi), Tufts Medical Center, Boston, MA; Channing Laboratory (Drs. Hersh and Silverman) and Pulmonary and Critical Care Division (Dr. Reilly), Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Abstract

Background—Hypoxemia, hypercarbia, and pulmonary arterial hypertension are known complications of advanced COPD. We sought to identify genetic polymorphisms associated with these traits in a population of patients with severe COPD from the National Emphysema Treatment Trial (NETT).

Methods—In 389 participants from the NETT Genetics Ancillary Study, single-nucleotide polymorphisms (SNPs) were genotyped in five candidate genes previously associated with COPD susceptibility (EPHX1, SERPINE2, SFTPB, TGFB1, and GSTP1). Linear regression models were used to test for associations among these SNPs and three quantitative COPD-related traits (Pa_{O_2} , $Paco_2$, and pulmonary artery systolic pressure). Genes associated with hypoxemia were tested for replication in probands from the Boston Early-Onset COPD Study.

Results—In the NETT Genetics Ancillary Study population, SNPs in microsomal epoxide hydrolase (EPHX1) [$p = 0.01$ to 0.04] and serpin peptidase inhibitor, clade E, member 2 (SERPINE2) [$p = 0.04$ to 0.008] were associated with hypoxemia. One SNP within surfactant protein B (SFTPB) was associated with pulmonary artery systolic pressure ($p = 0.01$). In probands from the Boston Early-Onset COPD Study, SNPs in EPHX1 and in SERPINE2 were associated with the requirement for supplemental oxygen.

Conclusions—In participants with severe COPD, SNPs in EPHX1 and SERPINE2 were associated with hypoxemia in two separate study populations, and SNPs from SFTPB were associated with pulmonary artery pressure in the NETT participants.

Keywords

case-control studies; COPD; genetics; phenotype; single-nucleotide polymorphism

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Correspondence to: Craig P. Hersh, MD, MPH, Channing Laboratory, Brigham and Women's Hospital, 181 Longwood Ave, Boston, MA 02115; craig.hersh@channing.harvard.edu.

COPD is the fourth leading cause of mortality in the United States.¹ Cigarette smoking is the major environmental risk factor for COPD, but there are important genetic determinants of disease susceptibility that have yet to be elucidated.²

Studying COPD as a dichotomous phenotype can dilute valid genetic associations when the case group consists of genetically distinct subgroups. Some genetic studies³ have examined COPD-associated traits such as exercise capacity or shortness of breath, identifying significant associations that were confirmed in separate populations. Genetic associations with COPD-related traits can help to define distinct subtypes of COPD.

Based on previous published associations with COPD susceptibility and known biological function, we selected five candidate genes (EPHX1, SERPINE2, SFTPB, GSTP1, and TGFB1) to test for association with COPD-related traits.^{4–10} Microsomal epoxide hydrolase (EPHX1) is involved in xenobiotic metabolism of the polycyclic aromatic hydrocarbons found in cigarette smoke. It is expressed in lung tissue, and two known variants of the gene alter its rate of enzymatic activity.¹¹ Serpin peptidase inhibitor, clade E, member 2 (SERPINE2) is located on chromosome 2q, a genomic region that has demonstrated genetic linkage to FEV₁/FVC ratio in multiple human populations.^{12,13} Gene expression studies in mice have demonstrated that SERPINE2 is a highly expressed gene during murine alveogenesis, with significant down-regulation later in lung development. Similar analyses in human lung tissue from patients with emphysema demonstrated correlations between SERPINE2 gene expression and FEV₁, diffusing capacity of the lung for carbon monoxide, and total lung capacity.⁹ Surfactant protein B (SFTPB) is an essential component of surfactant that is expressed in type 2 alveolar cells and bronchiolar epithelial cells.¹⁴ Severe deficiency of SFTPB is associated with neonatal respiratory distress syndrome, whereas partial deficiency can result in chronic lung conditions that present later in life.^{15–17} Glutathione S-transferase P1 (GSTP1) is a member of the glutathione transferase family of enzymes and is involved in xenobiotic metabolism by binding electrophilic compounds to reduced glutathione. It is highly expressed in normal human lung tissue.¹⁸ Transforming growth factor- β 1 (TGFB1) has diverse effects including immune regulation, tissue repair, and cell growth and differentiation. There is significant evidence in animal models of a role for TGFB1 in COPD, as mouse strains with abnormalities in TGFB1 activation or in TGFB1-binding proteins both develop emphysema.^{19,20}

We focused our association analyses on three COPD-related traits, hypoxemia, hypercarbia, and pulmonary arterial hypertension, which result in increased morbidity and mortality.^{21,22} We hypothesized that within a population of subjects with severe COPD from the National Emphysema Treatment Trial (NETT), certain genetic polymorphisms would be associated with pulmonary artery systolic pressure (PASP), PaO₂, and PaCO₂, independent of the severity of airflow obstruction. To test this hypothesis, we examined single-nucleotide polymorphisms (SNPs) in the five selected genes previously associated with COPD susceptibility for their association with these three phenotypes. For two genes with SNPs associated with PaO₂ in NETT participants, we performed a replication analysis in a separate population of subjects from the Boston Early-Onset COPD Study.

Materials and Methods

Study Participants

The current analysis was performed in severe COPD subjects in two populations, the NETT Genetics Ancillary Study and the Boston Early-Onset COPD Study. Subject enrollment and data collection in the NETT and the Boston Early-Onset COPD Study have been described elsewhere.^{23–25} The study populations consisted of 389 participants from the NETT Genetics Ancillary Study and 139 probands from the Boston Early-Onset COPD Study.

After providing written informed consent, participants from both studies provided blood samples for genetic analysis. Both studies were approved by institutional review boards at participating centers.

The 389 participants in the NETT Genetics Ancillary Study are a subset of the overall NETT cohort. Study enrollment and phenotype characteristics in the NETT have been previously reported.^{23,26} All participants were screened for α_1 -antitrypsin deficiency. Participants with severe deficiency were excluded from further analysis. NETT participants had marked air-flow obstruction ($FEV_1 \leq 45\%$), hyperinflation (total lung capacity $\geq 100\%$ predicted) and chest CT scan evidence of bilateral emphysema. Exclusion criteria included mean PASP ≥ 35 mm Hg (≥ 38 mm Hg in Denver), peak PASP ≥ 45 mm Hg (≥ 50 mm Hg in Denver), $P_{aCO_2} > 60$ mm Hg (> 55 mm Hg in Denver), and $P_{aO_2} < 45$ mm Hg (< 30 mm Hg in Denver). Arterial blood gas measurements made while the patient was breathing room air were obtained for all patients prior to randomization. Pulmonary artery pressures were obtained by echocardiography and/or right heart catheterization. A total of 325 participants in the NETT Genetics Ancillary Study had peak PASP values recorded. The echocardiographic and right heart catheterization techniques used in the NETT have been described previously.^{27,28}

The Boston Early-Onset COPD Study is a family-based study in which probands with a physician's diagnosis of COPD were ascertained according to the following criteria: age < 53 years; $FEV_1 < 40\%$ predicted; and the absence of severe α_1 -antitrypsin deficiency. This analysis was performed on the probands from the study cohort. Study enrollment and phenotyping in the Boston Early-Onset COPD Study has been previously described.¹² For this analysis, oxygen use was quantified by responses to a follow-up questionnaire and medical record review.²⁵

Genotyping

A set of SNPs from each gene was selected based on linkage disequilibrium (LD) patterns and previously reported associations with COPD. All analyzed SNPs had a minor allele frequency $\geq 10\%$. (Table 1 in the online supplemental material lists the genes and the SNPs tested within each gene.) The following three platforms were used for genotyping: TaqMan 5' to 3' exonuclease assay (Applied Biosystems; Foster City, CA); allele specific hybridization (Illumina Golden Gate Assay; San Diego, CA); or unlabeled minisequencing reactions with mass spectrometry (Sequenom; San Diego, CA).

Statistical Analysis

SNPs in the five candidate genes were tested for association with P_{aO_2} , P_{aCO_2} , and PASP in the 389 NETT participants using linear regression models adjusting for pertinent covariates under an additive genetic model. Genotypes were coded 0, 1, or 2 based on the number of copies of the minor allele. Two regression models were constructed. Model 1 adjusts for traditional demographic variables as well as for FEV_1 percent predicted. Model 2 includes all of the model 1 covariates as well as a term for the percentage of CT scan-quantified emphysema using a threshold of -950 Hounsfield units. (See Table 3 for specific covariates for each model.)

Genes that were associated with P_{aO_2} were then analyzed in the Boston Early-Onset COPD Study probands to confirm the associations from the NETT cohort. In the Boston Early-Onset COPD analysis, hypoxemic cases were defined as individuals who received oxygen supplementation 24 h per day; nonhypoxemic control subjects did not receive any oxygen supplementation. Thirty-five participants were not classified due to intermittent oxygen use or missing data. Genotype-phenotype associations were tested in an unadjusted analysis

using the Armitage trend test, and in adjusted analysis using logistic regression adjusting for FEV₁.

Statistical power calculations for the linear regression models in the NETT participants were performed with a statistical software package (Quanto, version 1.2.3; University of Southern California; Los Angeles, CA)²⁹ for an additive genetic model with a two-sided α of 0.05, a sample size of 380, and a minor allele frequency of 10%. For Pa_{o2}, our study had 85% power to detect an allelic effect size of 4 mm Hg for Pa_{o2}. For PASP, our study had 93% power to detect an allelic effect size of 2.5 mm Hg.

LD was calculated and displayed for the SNPs within each gene (Haploview, version 3.32; Broad Institute; Cambridge, MA).³⁰ Haplotype analysis was performed using the expectation-maximization algorithm and score tests (Haplo.stats, version 1.3.1; Mayo Clinic; Rochester, MN).³¹

Results

Study Participants

Baseline characteristics of the NETT and Boston Early-Onset COPD Study participants are described in Tables 1 and 2, respectively. Both populations are characterized by severe COPD and a history of heavy smoking. Compared to the NETT population, the Boston Early-Onset COPD Study probands are younger and composed of a larger percentage of women.

For the case-control analysis of hypoxemia in the Boston Early-Onset COPD Study probands, comparison of baseline variables between groups is included in Table 2. FEV₁ was slightly lower in hypoxemic case subjects than non-hypoxemic control subjects. There were no other significant differences between the case and control groups in other baseline demographic variables, including age, sex, and number of pack-years of smoking.

NETT Candidate Gene Association Analysis

The SNPs tested in each gene and the tests for Hardy-Weinberg equilibrium are included in Table 1 in the online supplemental material. Using a threshold of $p > 0.001$, all analyzed SNPs were in Hardy-Weinberg equilibrium in NETT subjects.

The results of the association analysis among SNPs in the five candidate genes and three phenotypes are shown in Table 3. SNPs in TGFB1 and GSTP1 were not associated with any of the three phenotypes, and no SNPs in any of the genes were significantly associated with Paco₂. Six SNPs from two genes were significantly associated with Pa_{o2}, and two SNPs from one gene were associated with PASP.

In model 1, a regression model adjusted for age, sex, number of pack-years of cigarette smoking, FEV₁, and the Denver study site (to adjust for elevation above sea level at this site), the two genes with SNPs associated with Pa_{o2} were EPHX1 and SERPINE2. Three SNPs in both EPHX1 ($p = 0.01$ to 0.04) and SERPINE2 ($p = 0.04$ to 0.008) were associated with Pa_{o2}. The most strongly associated SNP in EPHX1 was rs1051741 (Fig 1). Two of the three significantly associated EPHX1 SNPs, rs1051741 and rs2292558, are in tight LD in our study population ($r^2 = 0.92$). The three significantly associated SNPs in SERPINE2 are located within a 3-kb segment near the 3' end of the gene and are in LD in our study population and in Caucasian HapMap samples (HapMap CEU; International HapMap Project; <http://www.hapmap.org/thehapmap.html.en>).³² In model 2, which included a CT scan emphysema term in addition to the covariates from model 1, adjustment for CT scan emphysema did not change the magnitude of association for any of these associated SNPs.

One SNP in SFTPB was associated with PASP ($p = 0.01$ in model 1; $p = 0.002$ in model 2) in both regression models. A second SNP within 500 base pairs of this SNP was associated with PASP ($p = 0.09$ in model 1; $p = 0.01$ in model 2) in the regression model adjusting for CT scan emphysema. These SFTPB SNPs were in LD in our study population ($r^2 = 0.63$). Two nonsynonymous coding variants in EPHX1 that have been previously associated with COPD, rs1051740 (Tyr113His) and rs2234922 (His139Arg), were not associated with any of the three study phenotypes.

Haplotype analysis results using two SNP sliding windows are displayed in Table 4. Analysis with two and three SNP sliding windows identified significant associations between haplotypes in EPHX1 and SERPINE2 with Pa_{O_2} , and in SFTPB with PASP. However, the SNPs driving the associations with these haplotypes were the same SNPs identified in single SNP analysis, and the magnitude of the haplotype associations was similar to that found in single SNP analysis.

Boston Early-Onset COPD Study Case-Control Analysis

The two genes with SNPs associated with Pa_{O_2} in the NETT population, EPHX1 and SERPINE2, were examined in the Boston Early-Onset COPD Study probands in order to confirm the associations. The identical SNP set was analyzed. SNPs were evaluated in an unadjusted analysis for oxygen dependence as well as in a logistic regression model adjusting for FEV_1 . All analyzed SNPs were in Hardy-Weinberg equilibrium in the Boston Early-Onset COPD Study probands.

The results of association tests for SNPs in EPHX1 and SERPINE2 with hypoxemia are displayed in Table 5. In EPHX1, three SNPs ($p = 0.02$ to 0.05) were associated with supplemental oxygen use in the unadjusted analysis. In the logistic regression model adjusting for FEV_1 , the same three SNPs were associated ($p = 0.01$ to 0.004). An additional EPHX1 SNP (rs1877724) within 1 kb of one SNP discussed earlier (rs2854450) was also significantly associated in the logistic model and marginally associated in the unadjusted analysis.

In SERPINE2, one SNP (rs1025734) was significantly associated in both the unadjusted analysis ($p = 0.006$) and the logistic regression model ($p = 0.01$). This SNP is located approximately 4.5 kb downstream from the gene. While both genes contain regions significantly associated with the outcomes of interest in the NETT and Boston Early-Onset COPD Study populations, no individual SNP was significantly associated in both cohorts.

Discussion

In this study, we tested five candidate genes for association with three COPD-related phenotypes. We found associations in EPHX1 and SERPINE2 for Pa_{O_2} , and in SFTPB for PASP. To confirm the associations with Pa_{O_2} , we tested the same SNPs from EPHX1 and SERPINE2 in a different population and demonstrated gene-level, but not SNP-level, association with a closely related phenotype.

This is the first study of the relationship between genetic polymorphisms and hypoxemia in subjects with COPD. While there have been no prior studies directly addressing genetic determinants of hypoxemia in COPD, there have been previous candidate gene studies of pulmonary hypertension in COPD patients. In a cohort of 103 individuals with COPD, Eddahibi et al³³ reported an association between pulmonary hypertension and a variant in the Serotonin transporter (SLC6A4) gene. Yildiz et al³⁴ reported an association between endothelial nitric oxide synthase (NOS3) variants and pulmonary hypertension in a sample of 40 cases; there have been similarly sized studies of the relationship between the

angiotensin-converting enzyme insertion-deletion polymorphism and pulmonary hypertension in COPD patients, but these studies have had conflicting results.^{34,35,36}

Regarding potential biological explanations for our observed genetic associations with hypoxemia, EPHX1 and SERPINE2 are both excellent candidate genes for COPD susceptibility based on the known detoxifying function of EPHX1 and the gene expression and human linkage data available for SERPINE2. Although we continued to observe significant associations with hypoxemia after adjustment for both FEV₁ level and a quantitative measure of overall emphysema severity, it is possible that the associations of SNPs in EPHX1 and SERPINE2 with hypoxemia may be due to the effects of these SNPs on emphysema susceptibility or COPD severity. It is possible that our statistical adjustment for emphysema does not capture important features of emphysema extent and distribution that could impact levels of hypoxemia, or that small airway disease is influencing the development of hypoxemia. It is also possible that the observed associations with hypoxemia may represent a pathophysiologic process separate from the development of COPD, although this is less likely based on our method of candidate gene selection.

At the SNP level, while no individual SNP was significantly associated with hypoxemia, the associated SNPs did come from similar genomic regions based on the LD patterns present in our samples. In EPHX1, a genomic region encompassing the 5' promoter region and exon 1 was significantly associated with hypoxemia in our analyses of both NETT and Boston Early-Onset COPD Study participants. This is an area of alternative splicing of the RNA transcript.³⁷ One exon 1 splice variant is expressed predominantly in the liver, whereas another variant is present in other body tissues, including the lung. Each splice variant has its own 5' promoter region, suggesting that this region may play a tissue-specific role in regulating EPHX1 messenger RNA levels.

The EPHX1 SNP most strongly associated with hypoxemia in NETT participants, rs1051741, is a synonymous coding SNP in exon 8 that has previously been associated³⁸ with emphysema distribution in the NETT population. While this would not change the amino acid structure of the resulting protein, synonymous amino acid substitutions can affect messenger RNA levels through differences in transfer RNA availability.³⁹ Alternatively, this associated SNP may be in LD with a nearby functional variant.

In SERPINE2, the most highly associated SNPs from the NETT and Boston Early-Onset COPD Study populations fall within a 15-kb region spanning the 3' end of SERPINE2. There is no known function for these intronic SNPs; however, in both study populations, this region was in moderate LD, suggesting that these signals could be due to a single, untyped locus. Two of the SNPs significantly associated in our study, rs975278 and rs729631, have been associated with COPD or FEV₁/FVC ratio in previous studies.^{6,9} Regarding our findings of an association between SFTP variants and PASP in the NETT subjects, the two associated SNPs in our studies are intronic SNPs in tight LD, spanning a region around exon 8, which has not previously been associated with disease.

The principal strength of our study is that we used two well-phenotyped cohorts to test for genetic associations with three, clinically important, COPD-related phenotypes. To our knowledge, the NETT Genetics Ancillary Study cohort is the largest population of COPD patients in which arterial blood gas values, pulmonary artery pressures, and genetic polymorphisms have been characterized. In addition, we were able to test our genetic associations while adjusting for potential confounding factors such as the level of FEV₁, smoking history, and Pao₂ level (in the PASP analyses).

Our study has the following limitations:

1. Both study populations were selected for the presence of severe COPD, so our findings may not be generalizable to patients with mild-to-moderate COPD.
2. We attempted to replicate our associations with PaO_2 using a similar, but not identical, phenotype. The phenotype used in the Boston Early-Onset COPD Study population is the self-reported use of continuous supplemental oxygen, which is a less accurate measure of hypoxemia than PaO_2 . However, the use of continuous supplemental oxygen is tightly regulated in the United States by the Center for Medicare and Medicaid Services and private payers, with the principle criteria for the coverage of continuous oxygen therapy being documented room air oxygen saturation $\leq 88\%$ or $\text{PaO}_2 \leq 55$ mm Hg.⁴⁰
3. Our replication population has a limited sample size. Given the sample size of our replication population, our study is at risk of false-negative failure to identify small-but-real SNP effects as well as false-positive association results.
4. No individual SNP was significantly associated with hypoxemia in both study populations. However, there are reasons to anticipate cases of gene-level, but not SNP-level, replication in complex disease genetics. In monogenic disorders, such as cystic fibrosis and α_1 -antitrypsin deficiency, numerous disease-causing mutations have been elucidated in the cystic fibrosis transmembrane regulator (or CFTR) and SERPINA1 genes. Some authors⁴¹ have suggested that gene-level analyses are more appropriate in complex-disease genetics than SNP-level analyses due to the presence of multiple important variations within a single gene. Nonetheless, until specific functional variants are identified in EPHX1 and SERPINE2 and are associated with hypoxia in multiple populations, we present these results as suggestive, not definitive, evidence of a causal relationship between these genes and hypoxemia in patients with severe COPD.
5. We were unable to attempt replication of our association between SFTPB and PASP as we did not have a suitable replication population with pulmonary artery pressure measurements. This association should be tested in a well-powered replication cohort when such data become available.

In summary, our study used two extensively phenotyped cohorts of subjects with severe COPD to identify genetic associations with three important COPD-related traits. Our findings suggest that in subjects with severe COPD, polymorphisms in EPHX1 and SERPINE2 contribute to the development of hypoxemia, and that polymorphisms in SFTPB contribute to pulmonary arterial hypertension. This provides additional information regarding the complex network of genes underlying the variety of clinical COPD-related phenotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

EPHX1	microsomal epoxide hydrolase
GSTP1	glutathione S-transferase P1
LD	linkage disequilibrium
NETT	National Emphysema Treatment Trial
PASP	pulmonary artery systolic pressure
SERPINE2	serpin peptidase inhibitor, clade E, member 2
SFTPB	surfactant protein B
SNP	single-nucleotide polymorphism
TGFB1	transforming growth factor- β 1

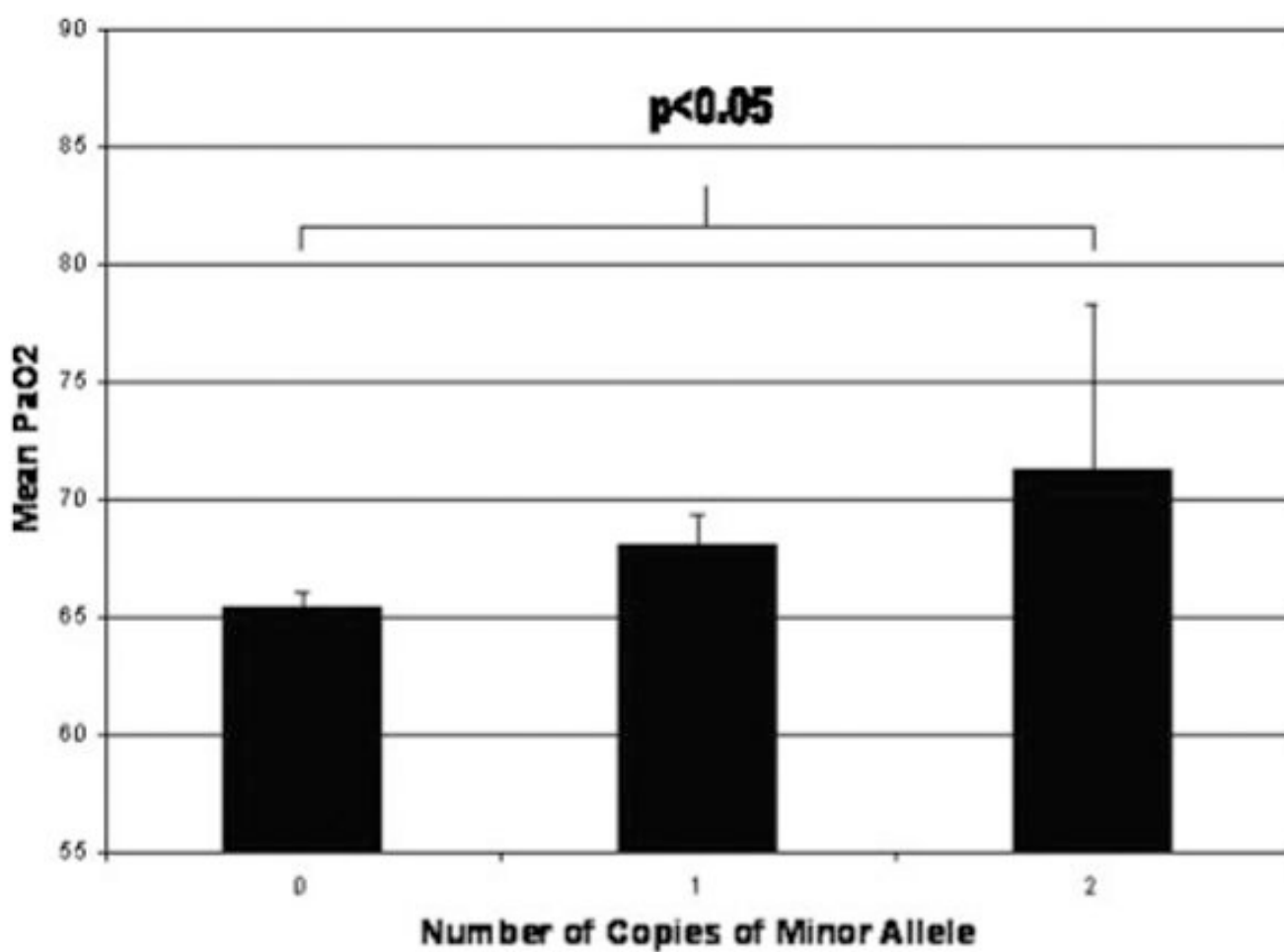


Figure 1. Unadjusted mean PaO₂ levels by EPHX1 SNP rs1051741 genotype in NETT Genetics Ancillary Study participants. Error bars represent the SEM. Twenty-four Denver participants were excluded.

Table 1
Characteristics of the Participants in the NETT Genetics Ancillary Study*

Characteristics	Values
Age, yr	67 ± 6
Female gender	141 (36)
Smoking history, pack-yr (n = 386)	66 ± 30
Postbronchodilator FEV ₁ , % predicted	28 ± 7
Emphysema by CT scan at – 950 HU, % (n = 358)	17 ± 11
Pao ₂ , mm Hg (n = 362, excluding Denver site)	65 ± 11
Paco ₂ , mm Hg (n = 362, excluding Denver site)	43 ± 6
PASP, mm Hg (n = 298, excluding Denver site)	33 ± 6

* Values are mean ± SD or No. (%). n = 389, except as noted. HU = Hounsfield units.

Table 2
Baseline Characteristics of Boston Early-Onset COPD Study Probands Values*

Variables	Continuous Oxygen Use (n = 70)	No Oxygen Use (n = 34)	p Value
Age, yr	48 ± 4	48 ± 4	0.99
Female gender	52 (74)	25 (74)	0.93
Postbronchodilator FEV ₁ , % predicted	18 ± 7	22 ± 8	0.001
Smoking history, pack-yr	43 ± 24	37 ± 19	0.26

* Values are given as mean ± SD or No. (%), unless otherwise indicated. A total of 35 participants were not included in either group due to intermittent oxygen use or missing data.

Table 3

Genetic Associations in NETT Participants*

Phenotype	Gene	SNP	Model 1 [†]		Model 2 [‡]	
			β (SD)	p Value	β (SD)	p Value
Pao ₂	EPHX1	rs1051741	3.2 (1.2)	0.01	3.0 (1.3)	0.02
		rs2292558	2.8 (1.3)	0.03	2.6 (1.3)	0.04
		rs3738042	- 1.6 (0.8)	0.04	- 1.9 (0.9)	0.03
	SERPINE2	rs729631	- 2.8 (1.1)	0.008	- 2.6 (1.1)	0.02
		rs7605945	- 3.0 (1.0)	0.004	- 2.9 (1.1)	0.008
PASP [§]	SFTPB	rs975278	- 2.0 (1.0)	0.04	- 1.8 (1.0)	0.08
		rs2118177	1.3 (0.5)	0.01	1.6 (0.5)	0.002
		rs2304566	0.9 (0.6)	0.1	1.4 (0.6)	0.01

* $p < 0.05$ in either regression model. There were no significant associations with Pao₂ and no significant associations with SNPs from GSTP1 and TGFB1.

[†] Adjusted for age, sex, pack-years of cigarette smoking, FEV₁, and Denver site.

[‡] Adjusted for above covariates plus percent of CT scan emphysema at - 950 HU.

[§] PASP models are also adjusted for Pao₂.

Table 4
Two SNP Sliding Window Haplotype Analysis Results in NETT Participants*

Gene	Phenotype	Starting SNP	End SNP	Global p Value
EPHX1	Pao ₂	rs1051741	rs360063	0.02
		rs3600063	rs2292558	0.05
SERPINE2	Pao ₂	rs33916072	rs729631	0.02
		rs729631	rs17195216	0.005
		rs7605945	rs6436449	0.03
SFTPB	PASP	rs2118177	rs2304566	0.05

* p Values ≤ 0.05 . Analyses were adjusted for age, sex, pack-years of cigarette smoking, FEV₁, and Denver site.

Table 5
Genetic Associations With Hypoxemia for EPHX1 and SERPINE2 in NETT Participants (Pao₂) and Boston Early-Onset COPD Study Proband (Oxygen Use)*

Gene	SNP	NETT†			Boston Early-Onset COPD Study‡		
		p Value	β (SE)	p Value	p Value	OR (95% CI)	
EPHX1	rs1051741	0.01	3.2 (1.2)	0.91		0.95 (0.37–2.42)	
	rs1877724	0.13	1.3 (0.8)	0.01		2.80 (1.24–6.34)	
	rs2292558	0.03	2.8 (1.3)	0.94		1.04 (0.40–2.68)	
	rs2740170	0.61	0.5 (0.9)	0.004		0.32 (0.14–0.70)	
	rs2854450	0.47	0.6 (0.9)	0.01		0.35 (0.15–0.78)	
SERPINE2	rs3738042	0.04	– 1.6 (0.8)	0.12		1.88 (0.85–4.16)	
	rs868966	0.72	0.3 (0.7)	0.005		2.73 (1.34–5.56)	
	rs1025734	0.52	0.6 (0.9)	0.01		0.40 (0.19–0.83)	
	rs729631	0.008	– 2.8 (1.1)	0.44		1.44 (0.57–3.60)	
	rs7605945	0.004	– 3.0 (1.0)	0.55		1.29 (0.56–2.99)	
	rs975278	0.04	– 2.0 (1.0)	0.22		1.75 (0.71–4.30)	

* OR = odds ratio; CI = confidence interval.

† Linear regression model; response is Pao₂. Adjusted for age, sex, pack-years of cigarette smoking, FEV₁, and Denver site.

‡ Logistic regression model; response is supplemental O₂ use. Adjusted for FEV₁.