Polymorphisms in Surfactant Protein–D Are Associated with Chronic Obstructive Pulmonary Disease

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Chronic obstructive pulmonary disease (COPD) is characterized by alveolar destruction and abnormal inflammatory responses to noxious stimuli. Surfactant protein–D (SFTPD) is immunomodulatory and essential to host defense. We hypothesized that polymorphisms in SFTPD could influence the susceptibility to COPD. We genotyped six single-nucleotide polymorphisms (SNPs) in surfactant protein D in 389 patients with COPD in the National Emphysema Treatment Trial (NETT) and 472 smoking control subjects from the Normative Aging Study (NAS). Case-control association analysis was performed using Cochran–Armitage trend tests and multivariate logistic regression. The replication of significant associations was attempted in the Boston Early-Onset COPD Study, the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) Study, and the Bergen Cohort. We also correlated SFTPD genotypes with serum concentrations of surfactant protein–D (SP-D) in the ECLIPSE Study. In the NETT–NAS case-control analysis, four SFTPD SNPs were associated with susceptibility to COPD: rs2245121 ($P = 0.01$), rs911887 ($P = 0.006$), rs6413520 ($P = 0.004$), and rs721917 ($P = 0.006$). In the family-based analysis of the Boston Early-Onset COPD Study, rs911887 was associated with prebronchodilator and postbronchodilator FEV_1 ($P = 0.003$ and $P = 0.02$, respectively). An intronic SNP in SFTPD, rs7078012, was associated with COPD in the ECLIPSE Study and the Bergen Cohort. Multiple SFTPD SNPs were associated with serum SP-D concentrations in the ECLIPSE Study. We demonstrated an association of polymorphisms in SFTPD with COPD in multiple populations. We demonstrated a correlation between SFTPD SNPs and SP-D protein concentrations. The SNPs associated with COPD and SP-D concentrations differed, suggesting distinct genetic influences on susceptibility to COPD and SP-D concentrations.

Keywords: COPD; surfactant protein–D; single-nucleotide polymorphisms; genetics

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CLINICAL RELEVANCE

Genetic variants in surfactant protein–D (SFTPD), a gene essential to host defense, exert a dual impact. Polymorphisms in SFTPD are associated with susceptibility to chronic obstructive pulmonary disease and influence serum concentrations of SP-D, a pulmonary biomarker.

The alveolar lining is the principal mucosal surface in contact with the external environment (1). As a result, the lungs are constantly exposed to microbes and particulate material. The ability to detect and distinguish dangerous substances precedes the resistance to pathogenic invasion. Innate immunity, an essential facet of host defense, is the first line of contact, through the recognition of microbe-induced molecular patterns or pathogen-induced alterations in host receptors that trigger a cascade of inflammatory events. Pulmonary surfactant, which lines the alveolar epithelium, is highly active in host defense through bacterial agglutination, opsonization, and viral neutralization (2, 3). Surfactant protein–D bridges the innate and adaptive immune systems, with the ability to bind several classes of immunoglobulins, in addition to recognizing carbohydrate arrays on microbial cell surfaces (4). Surfactant protein–D also enhances the clearance of apoptotic cells (5), a potentially important mechanism in the pathogenesis of chronic obstructive pulmonary disease (COPD) (6, 7). Surfactant protein–D has proinflammatory and anti-inflammatory signaling functions (8).

Increasing evidence implicates surfactant protein–D in the pathogenesis of COPD. Elevated serum concentrations of surfactant protein–D are a biomarker for COPD (9), and may ultimately have clinical relevance in the evaluation and design of novel drug therapies. Although the genetic association of three surfactant protein–D single-nucleotide polymorphisms (SNPs) with surfactant protein–D serum concentrations was analyzed, and demonstrated that the Met11Thr variant (rs721917) was associated with the assembly, function, and concentration of surfactant protein–D (10), the effects of SFTPD SNPs on the susceptibility to COPD are unknown. We hypothesized that SNPs in SFTPD, a mediator of primordial immune responses, could influence the susceptibility to COPD.

MATERIALS AND METHODS

Study Populations

Genetics Ancillary Study of the National Emphysema Treatment Trial. In this case–control association analysis, participants with COPD were derived from the Genetics Ancillary Study in the National Emphysema Treatment Trial (NETT), previously described in detail

(11, 12). For randomization into the NETT, eligible participants met a variety of inclusion criteria, such as severe obstructive airflow limitation ($FEV_1 \leq 45\%$ predicted), bilateral emphysema according to high-resolution computed tomography scanning, and hyperinflation according to pulmonary function testing. For this study, 389 non-Hispanic white NETT subjects out of the population of 1,218 NETT participants were analyzed.

Normative Aging Study. The Normative Aging Study (NAS) is a population-based, prospective, longitudinal study of aging in men. This multidisciplinary cohort was initiated in Boston by the Veterans Administration in 1963 (13). At the onset of the study, participants were free of chronic medical conditions, were predominantly of Northern European descent, and underwent extensive evaluations every 3–5 years. The 472 male, non-Hispanic white NAS control subjects in this analysis had normal lung function during their last study visit and a 10 pack-year minimum smoking history. The largest pack-year value reported at any follow-up visit was used for this analysis.

Boston Early-Onset COPD Study. The Boston Early-Onset COPD Study (EOCOPD) is a family-based study of the heritable determinants of COPD. Eligibility criteria for probands include: (*1*) age equal to or less than 52 years, (*2*) FEV_1 at less than 40% predicted, (*3*) no evidence of severe α-1 antitrypsin deficiency, and (*4*) physician-diagnosed COPD. Detailed descriptions concerning the inception of the cohort, enrollment criteria, and physiologic evaluations are available elsewhere (14, 15). For this study, we analyzed data from 949 individuals in 127 pedigrees.

Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints Study. The Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) Study (16) is a multinational, 3-year, noninterventional investigation of patients with COPD (GOLD Stages II–IV) and control subjects, designed to identify factors predictive of the progression of COPD in various COPD subtypes and to identify clinically relevant biomarkers for the prediction of disease progression (17). Only white subjects from the ECLIPSE cohort were included in this analysis, comprising 1,719 patients with COPD and 172 smoking ($>$ 10 pack-year) control subjects.

Bergen cohort. Eight hundred and twenty-three patients with COPD and 810 control subjects were analyzed in a cohort from Bergen, Norway. Postbronchodilator spirometric criteria for patients with COPD included the ratio of forced expiratory volume in one second/forced vital capacity (FEV_1/FVC) of less than 0.7, and FEV_1 at less than 80% predicted. Eligible control subjects exhibited FEV_1/FVC of greater than 0.7, and FEV_1 greater than 80% predicted. Both patients and control subjects reported at least 2.5 pack-years of smoking. Additional details concerning this cohort were described previously (18, 19).

SNP Selection and Genotyping

NETT–NAS/EOCOPD. Genotypic data for individuals of European ancestry (CEU) from the International HapMap Project (20) and the Seattle SNPs Program for Genomic Applications (http://www.pga.mbt.washington.edu/) were used to select linkage disequilibrium (LD) tagging SNPs in SFTPD. Pairwise LD tagging was achieved with Tagger (http://www/broad.mit.edu/mpg/tagger/) for SNPs with a minimum minor allele frequency of 0.1 and an r^2 of at least 0.8. LD between the SNPs in SFTPD was calculated using Haploview (21). Hardy–Weinberg equilibrium (HWE) was tested in control subjects, using the χ^2 goodness-of-fit test. Two genotyping platforms were used: the 5′ to 3′ exonuclease assay (TaqMan; Applied Biosystems, Foster City, CA), and unlabeled minisequencing reactions and mass spectrometry (Sequenom, San Diego, CA) (22). Duplicate genotyping was performed on approximately 5% of the population for quality control. One discordant genotype occurred among the 525 duplicates (0.2%). Genotype call rates were greater than 95%. Whole-genome amplification was performed for 11 NAS participants, to avoid depletion of the source specimens (23).

ECLIPSE Study/Bergen Cohort. The Illumina 550Kversion 3 SNP array was used for SNP genotyping. Details on the genotyping and quality-control evaluation are described elsewhere (24). Samples with a call rate of less than 98% were deleted. SNPs in SFTPD were selected from this genome-wide association study (GWAS) dataset, including the flanking regions 100 kb upstream and downstream from SFTPD. The 28 haplotype-tagging SNPs and their genotype frequencies are listed in Tables E1 and E2, respectively, in the online supplement.

Serum Surfactant Protein D Concentrations

Serum surfactant protein D (SP-D) concentrations were determined in the ECLIPSE Study with an ELISA (25–27) (Biovendor, Inc, Heidelberg, Germany). Briefly, this sandwich enzyme immunoassay used two monoclonal antibodies specific to human SP-D for the quantitative measurement of SP-D in biologic specimens. Additional details concerning the measurement of serum surfactant protein D were reported by Lomas and colleagues (9).

Statistical Analysis

Quantitative data with a normal distribution are presented as means ± standard deviations. Variables with a non-normal distribution are presented as medians ± interquartile ranges. Discrete data were analyzed in 2 × 2 tables with χ^2 test statistics. The case–control association analysis was performed using SAS/Genetics (SAS, Inc., Cary, NC). Polymorphisms with significant results for trend ($P \leq 0.05$) in the Cochran–Armitage test (a genotype-based test for association) (28) were entered into logistic regression models. Logistic regression models were constructed for the probability of COPD. The logistic models, adjusted for age and pack-years, analyzed linear trends with additive genetic coding. Haplotype analysis was performed on haplotypes with at least 5% frequency, using the haplo.stats package in the R Project for Statistical Computing (http://www.r-project.org) (version 2.6.0). The sliding-window haplotype analysis involved the sequential testing of overlapping, adjacent two-loci, three-loci, or four-loci allelic combinations. Empiric values for significant global haplotypes and sliding-window analyses were obtained through the permutation of *P* values with 1,000 simulations.

Family-based association analyses for the EOCOPD Study were performed using the Pedigree-Based Association Test implemented in SVS Golden Helix (SVS 7.2.2, 2009-11-24, build 6685) (Golden Helix, Inc., Bozeman, MT). PedCheck (http://www.genomeutwin.org/member/cores/stat/linkage/pedcheck.html) was used as a quality-control measure of the genotyping used in the family-based association analysis (29). The additive genetic models were adjusted for age, age^2, height, height^2, pack-years of smoking, pack-years of smoking^2, current smoking, and sex.

Multivariate models were constructed in a subset of the ECLIPSE subjects with COPD to determine the independent effects of genotype and lung function (FEV_1 percent predicted) on surfactant protein D concentrations. These models were constructed with PLINK (30) (http://pngu.mgh.harvard.edu/purcell/plink), controlling for several additional covariates: age, sex, pack-years of smoking, smoking status, and principal components derived from a population stratification analysis using EIGENSTRAT (http://genepath.med.harvard.edu/~reich/EIGENSTRAT.htm) (31).

We performed logistic regression analysis in the Bergen Cohort with COPD as the outcome variable, adjusting for sex, age, pack-years of smoking, smoking status (former/current), and principal components for genetic ancestry.

RESULTS

NETT–NAS/EOCOPD Study

The demographic characteristics of participants in the NETT–NAS case–control analysis and the EOCOPD family-based replication population are presented in Table 1. The healthy smoking control subjects in NAS were slightly older and had less exposure to tobacco smoke. The NETT and NAS subjects were predominantly male. Of 389 NETT subjects, fewer than 40% were female and all NAS subjects were male. Individuals who actively smoked cigarettes were excluded from NETT. Approximately 7% of NAS control subjects were current smokers. Participants in the EOCOPD Study consisted of 127 probands: 98% had a history of smoking, and 13% were current smokers. In the relatives of probands, 63% had a history of smoking, and approximately one third were current smokers.

TABLE 1. DEMOGRAPHIC CHARACTERISTICS

	NETT	NAS	EOCOPD Probands	EOCOPD Relatives	ECLIPSE COPD	ECLIPSE Smoking Control Subjects	Bergen COPD	Bergen Smoking Control Subjects
Subjects (*n*)	389	472	127	822	1719	172	823	810
Age (years)	67 ± 6	70 ± 8	48 ± 5	46 ± 19	64 ± 7	57 ± 10	65 ± 10	56 ± 10
Pack-years	66 ± 30	40 ± 28	39 ± 22	19 ± 25	51 ± 28	32 ± 25	32 ± 19	19 ± 13
FEV_1 percent predicted, post-BD*	28 ± 7	100 ± 13	22 ± 8	87 ± 20	48 ± 16	108 ± 13	51 ± 17	95 ± 9
Sex (percent male)	64%	100%	25%	44%	67%	58%	54%	58%

Definition of abbreviations: COPD, chronic obstructive pulmonary disease; NETT, National Emphysema Treatment Trial; NAS, Normative Aging Study; ECLIPSE, Evaluation of Chronic Obstructive Pulmonary Disease Longitudinally to Identify Predictive Surrogate Endpoints Study; Bergen, Bergen Cohort from Bergen, Norway; EOCOPD, Boston Early-Onset Chronic Obstructive Pulmonary Disease Study; post-BD, postbronchodilator.

Values are mean ± SD unless noted otherwise.

* FEV_1 percent predicted values for the NETT and NAS are based on the prediction equations of Crapo and Morris; Normative Aging Study 1988 standards were used in the selection of the control group. Postbronchodilator spirometry measurements were available in 118 probands and 789 relatives from the EOCOPD Study. Postbronchodilator spirometry was not available in NAS.

The characteristics of the six SFTPD SNPS in the NETT–NAS case–control association analysis and the EOCOPD family-based replication analysis are listed in Table 2. Five SNPs were in Hardy–Weinberg equilibrium ($P > 0.05$). For SNP rs6415320, the HWE P value was 0.04, but this SNP was not excluded because of the marginal P value for the deviation from HWE. In NETT–NAS, pairwise LD was determined for the six SFTPD SNPs using r^2 (shown in the LD map in Figure 1). To maximize efficiency, we selected these six SNPs for genotyping that were not in strong LD. The highest r^2 between genotyped SNPs was 0.6, between rs2245121 and rs911887. For individuals of European–American ancestry, the six LD-tagging SNPs genotyped in SFTPD captured 100% of the HapMap SNPs, with a minor allele frequency of at least 10% and an r^2 greater than 0.8. The genotype frequencies for the ECLIPSE subjects with COPD are listed in Table E2.

Of the six SFTPD SNPs analyzed in the NETT–NAS case–control association analysis (Table 3), two intronic SNPs were significantly associated with susceptibility to COPD in the multivariate logistic regression models: rs2245121 (odds ratio [OR], 1.3; 95% confidence interval [CI], 1.1–1.7; $P = 0.01$) and rs911887 (OR, 1.4; 95% CI, 1.1–1.7; $P = 0.006$). In addition, significant associations were found for two nonsynonymous SNPs in SFTPD and susceptibility to COPD: rs6413520 (OR, 0.5; 95% CI, 0.3–0.8; $P = 0.004$) and rs721917 (OR, 1.4; 95% CI, 1.1–1.7; $P = 0.006$). In the family-based EOCOPD Study, rs911887 was associated with prebronchodilator FEV_1 ($P = 0.003$) and postbronchodilator FEV_1 ($P = 0.02$). In the EOCOPD Study, an exonic SNP, rs721917, revealed evidence suggestive of an association with prebronchodilator FEV_1, but this finding did not reach statistical significance ($P = 0.058$). An additional SNP, rs1051246, revealed evidence suggestive of an association with moderate or greater COPD as defined by GOLD stage, but this finding did not reach statistical significance ($P = 0.059$). In all these models, as the number of minor alleles increased, increased airflow limitation was manifested as a reduction in prebronchodilator FEV_1 and FEV_1/FVC.

Haplotype analysis was performed in NETT–NAS on SFTPD haplotypes of a least 5% frequency to extract additional information from the SNP data. The permuted and unadjusted global haplotype score P value for all six SNPs, based on 1,000 simulations, was $P = 0.02$. The A–G–T–A–G–A haplotype (rs1051246–rs2245121–rs911887–rs225601–rs6413520–rs721917) had the strongest statistical significance ($P = 0.002$) and a frequency of 7%. The empiric P value, as determined by simulation with sliding windows, was also significant ($P = 0.008$) for two SNP sliding windows (rs6413520–rs721917), three SNP sliding windows ($P = 0.02$) (rs911887–rs225601–rs6413520), and four SNP sliding windows ($P = 0.02$) (rs911887–rs225601–rs6413520–rs721917). Performing sliding-window simulations can be useful in prioritizing loci. Our strongest signal in the sliding-windows analysis involved the two-SNP sliding window that included rs6413520 and rs721917. After adjustment for age and pack-years of smoking, the global haplotype P value, based on 1,000 simulations, was 0.03.

ECLIPSE Study–Bergen Cohort

The ECLIPSE and Bergen participants in this study were slightly younger than the subjects in NETT–NAS. Demographic characteristics of the ECLIPSE and Bergen Cohort subjects are described in Table 1.

An intronic SNP, rs7078012, was associated with COPD susceptibility in the Bergen Cohort ($P = 0.049$; OR, 0.79; 95% CI, 0.62–0.999) and replicated in the ECLIPSE Study ($P = 0.004$; OR, 0.63; 95% CI, 0.4595–0.8622). This polymorphism was not genotyped in the NETT–NAS case–control study, and it was not in strong LD with SNPs in the NETT–NAS panel. Only two SNPs from NETT–NAS, rs911887 and rs721917, were among the 28 SNPs genotyped in the Illumina genome-wide panel in the ECLIPSE Study.

TABLE 2. MINOR ALLELE FREQUENCIES IN SURFACTANT PROTEIN–D

SNP Identification	Region	Alleles	Minor Allele	NETT ($n = 389$) Patients	NAS ($n = 472$) Control Subjects	EOCOPD ($n = 949$)
rs1051246	Coding exon	A/G	G	0.12	0.14	0.13
rs2245121	Intron	A/G	A	0.44	0.38	0.45
rs911887	Intron	C/T	C	0.42	0.36	0.46
rs2255601	Intron	A/G	A	0.40	0.45	0.36
rs6413520	Coding exon	A/G	G	0.05	0.09	0.07
rs721917	Coding exon	A/G	G	0.45	0.38	0.42

Definition of abbreviations: SNP, single-nucleotide polymorphism; NETT, National Emphysema Treatment Trial; NAS, Normative Aging Study; EOCOPD, Boston Early-Onset Chronic Obstructive Pulmonary Disease Study.

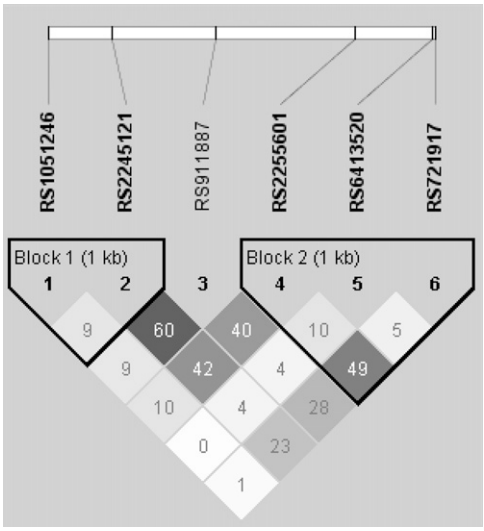

Figure 1. Linkage disequilibrium map for six surfactant protein–D (SFTPD) single-nucleotide polymorphisms (SNPs) in subjects from the National Emphysema Treatment Trial and Normative Aging Study. Values of r^2 (×100) are shown. *Solid squares*, an r^2 of 1; *open squares*, an r^2 of zero; *shaded squares* are proportional to r^2.

Serum SP-D Concentrations

Serum SP-D concentrations in ECLIPSE subjects were log-transformed, using the natural logarithm to approximate a normal distribution. In additive genetic models adjusting for age, sex, pack-years, and current smoking, several SNPs in SFTPD demonstrated strong associations with SP-D concentrations in current or former smokers (Table 4). Similar to the findings of Leth-Larsen and colleagues (10), we found an association between rs721917 and SP-D concentration. However, our strongest effect occurred with rs1885551, a promoter polymorphism (Figure 2). This finding suggests that the previously reported nonsynonymous SNP (rs721917) is not the most significant determinant of SFTPD gene regulation. Instead, rs1885551 or an SNP in LD with that polymorphism appears to exert the strongest impact on serum SP-D concentrations. In our multivariate genetic models, FEV_1 percent predicted and SNP genotypes for multiple SFTPD variants were significant and independent predictors of SP-D concentrations (Table 5), demonstrating that lung function and SFTPD SNP genotype significantly and independently influence serum SP-D concentrations.

DISCUSSION

We demonstrated a significant association of several SNPs in SFTPD with susceptibility to COPD and COPD-related phenotypes. The strength of these associations was enhanced by the replication across multiple study designs and independent populations, although the same SFTPD SNP was not associated with COPD in all four study populations. We demonstrated that genetic variants in SFTPD correlate with quantifiable differences in serum protein concentrations, measured as serum SP-D.

A potential role for SFTPD in the pathogenesis of COPD is supported by murine models, where genetically altered mice lacking the production of SP-D spontaneously develop emphysema-like lesions, and have increased metalloproteinase activity and increased oxidant production (32–35). The attenuation of these effects was shown after treatment with truncated recombinant human SP-D (36). These murine models provide support for the concept that SP-D is necessary for the regulation of oxidant production, inflammatory responses in alveolar macrophages, and apoptotic cell clearance.

SP-D is predominantly produced by alveolar Type II cells, and is systemically released as a consequence of lung injury (37). SP-D production is constitutive and under genetic (38–40) and environmental control. In animal models, increased concentrations were demonstrated with exposure to tobacco smoke (40, 41), and decreased concentrations coincided with the resolution of ozone exposure (42). Serum SP-D was analyzed as a biomarker for COPD, a proxy for COPD pathology, or an unbiased measure of a pharmacologic response, in a study where inhaled corticosteroid therapy in conjunction with a long-acting β-agonist reduced circulating SP-D (43, 44). In multivariate analyses, COPD was an independent predictor of the presence of SP-D. The reduction in SP-D after treatment with corticosteroids was confirmed in a larger study, where SP-D was also demonstrated to be predictive of risk for a COPD exacerbation (9).

The Met11Thr variant (rs721917) of SFTPD was previously associated with lower serum concentrations of SP-D, and was found to inhibit the oligomerized state, with a reduction in binding of bacterial ligands (40). High SP-D concentrations were associated with an increased risk of COPD exacerbations (9). SP-D concentrations are reduced in the bronchoalveolar lavage fluid of patients with COPD (45), in contradistinction to

TABLE 3. ASSOCIATION OF SURFACTANT PROTEIN D AND COPD PHENOTYPES IN FOUR STUDIES

	NETT–NAS: 389 NETT Cases and 472 NAS Control Subjects			EOCOPD* (n = 949)		ECLIPSE: 1,719 COPD Cases, and 172 Smoking Control Subjects		Bergen Cohort: 823 COPD Cases, and 810 Control Subjects
SNP Identification	OR	95% CI	P Value	Spirometric Phenotype	P Value	OR	95% CI	P Value
rs1051246	0.8	0.5–1.1	0.1	Pre-BD FEV_1	P = 0.1		NG	NG
rs2245121	1.3	1.1–1.7	0.01	Pre-BD FEV_1	P = 0.08		NG	NG
rs911887	1.4	1.1–1.7	0.006	Pre-BD FEV_1	P = 0.003		NS	NS
				Post-BD FEV_1	P = 0.02			
rs2255601	0.8	0.7–1.1	0.1	Pre-BD FEV_1	P = 0.3		NG	NG
rs6413520	0.5	0.3–0.8	0.004	Pre-BD FEV_1	P = 0.7		NG	NG
rs721917	1.4	1.1–1.7	0.006	Pre-BD FEV_1	P = 0.058		NS	NS
rs7078012						P = 0.0039		P = 0.049

Definition of abbreviations: SNP, single-nucleotide polymorphism; NETT, National Emphysema Treatment Trial; NAS, Normative Aging Study; EOCOPD, Boston Early-Onset Chronic Obstructive Pulmonary Disorder Study; COPD, Chronic Obstructive Pulmonary Disorder; OR, odds ratio; CI, confidence interval; Pre-BD, prebronchodilator; NS, nonsignificant; NG, not genotyped.

*Additive genetic model contained the covariates of age, age^2, height, height^2, packs, packs^2, current smoking, and sex.

rs911887 was associated with susceptibility to COPD in the NETT–NAS case–control study. rs911887 was associated with spirometric phenotypes in the EOCOPD family-based association analysis. rs7078012 was associated with susceptibility to COPD in the ECLIPSE Study and Bergen Cohort.

TABLE 4. EFFECTS OF SFTPD ON SERUM SURFACTANT PROTEIN–D CONCENTRATIONS IN THE ECLIPSE STUDY

SNP Identification	Beta Coefficient	*P* Value
rs12763012	−0.190	1.15×10^{-8}
rs7073842	−0.006	0.759
rs2250992	0.008	0.695
rs2256573	−0.071	1.11×10^{-4}
rs7911085	0.020	0.463
rs2819106	−0.010	0.605
rs11597219	−0.035	0.579
rs1923541	−0.071	1.02×10^{-4}
rs2758555	−0.010	0.605
rs1923539	0.107	4.06×10^{-7}
rs911887	0.128	3.85×10^{-12}
rs2243639	−0.008	0.686
rs7078012	0.145	4.32×10^{-8}
rs721917	−0.060	1.03×10^{-3}
rs2819096	−0.012	0.523
rs12770776	−0.031	0.512
rs1885553	0.164	3.01×10^{-17}
rs1885551	−0.344	2.11×10^{-30}
rs2146192	−0.344	2.11×10^{-30}
rs3923564	−0.436	3.49×10^{-19}
rs11201011	−0.017	0.354
rs7084667	0.185	5.94×10^{-22}
rs7904954	−0.030	0.106
rs1932571	−0.179	2.85×10^{-14}
rs7070394	−0.012	0.510
rs12220777	−0.191	1.19×10^{-7}
rs7083625	0.062	0.902×10^{-4}
rs2342606	−0.004	0.844

Definition of abbreviations: SFTPD, surfactant protein–D; SNP, single-nucleotide polymorphism; ECLIPSE, Evaluation of Chronic Obstructive Pulmonary Disorder Longitudinally to Identify Predictive Surrogate Endpoints.

Additive models controlled for age, sex, pack-years, smoking status, and principal components of population stratification.

the high concentrations measured in the peripheral blood of patients with COPD. According to a mechanism postulated to explain this discordance, inflammation in the lungs of patients with COPD results in the endothelial leakage of SP-D systemically, although other mechanisms may exist (46). In addition to its potential as a lung-specific biomarker, SP-D was correlated with body mass index (BMI) (47, 48). In a murine model, the overexpression of SFTPD was associated with atherosclerosis. Whether SP-D is related to systemic manifestations of COPD, such as low BMI or cardiovascular comorbidity (9), remains to be proven.

Few previous studies investigated the association between SFTPD and susceptibility to COPD or declines in lung function (49, 50). Although we demonstrated gene-level replication for an association of SFTPD SNPs with susceptibility to COPD in multiple populations, our study contains some limitations. In a previous candidate gene case–control association study by our research group, we found no association in NETT–NAS and EOCOPD participants with two SNPs in SFTPD (rs2243639 and rs721917) (51). However, in the present analysis of new genotyping data in these same populations, a significant association with rs721917 was evident in the NETT–NAS case–control analysis, with a trend toward the association of rs721917 with prebronchodilator FEV_1. The present and previous investigations contained substantial differences that likely account for the disparate results. The present study used an LD-tagging approach in an attempt to assess the entire SFTPD gene. This NETT–NAS analysis was larger, with a net accrual of 115 additional subjects (85 NETT and 30 NAS subjects). Slight differences were also evident in the results of the previous and present genotyping. In the family-based association analysis, advances were implemented in the more recent version of Pedigree Based Association Testing (PBAT), used in the present analysis, which incorporates an algorithm with greater efficiency in detecting associations in extended pedigrees (C. Lange, personal communication). In addition, the spirometric phenotypes differed between the two analyses of the EOCOPD Study. Although the present analysis from the EOCOPD Study used prebronchodilator FEV_1 and FEV_1/FVC, resulting in a larger sample size and allowing the inclusion of 35 additional participants, the result for rs721917 is concordant with the previous report of postbronchodilator spirometry.

In regard to a further limitation, we do not replicate the association of the same SNP across all study populations. For instance, although rs721917 was associated with susceptibility to COPD in NETT–NAS, with lung function in the EOCOPD Study, and with SP-D concentrations in the ECLIPSE Study, this SNP was not associated with COPD in the ECLIPSE Study and Bergen Cohort. Moreover, although we demonstrated significant and independent effects of SFTPD SNPs and FEV_1 percent predicted in surfactant protein D concentrations, our strongest association with SP-D concentrations occurred for a promoter polymorphism (rs1885551) rather than the nonsynonymous SNP, rs721917, previously suggested to be functional (10).

Our data suggest the possibility that different SFTPD SNPs influence susceptibility to COPD versus SP-D concentrations. Although we did not perform functional studies to determine the effects of these polymorphisms in the lungs, Leth-Larsen and colleagues demonstrated that polymorphic variations in the N-terminal domain of SFTPD affect surfactant serum concentrations, polymerization, and function (10). Surfactant reduces sputum adherence to the epithelium and airway inflammation, and improves mucociliary clearance (52). In a cohort of patients with chronic bronchitis, the administration of exogenous surfactant (palmitoylphisphadidylcholine) resulted in improvements in lung function and an increased *in vitro* ciliary transport of sputum (52).

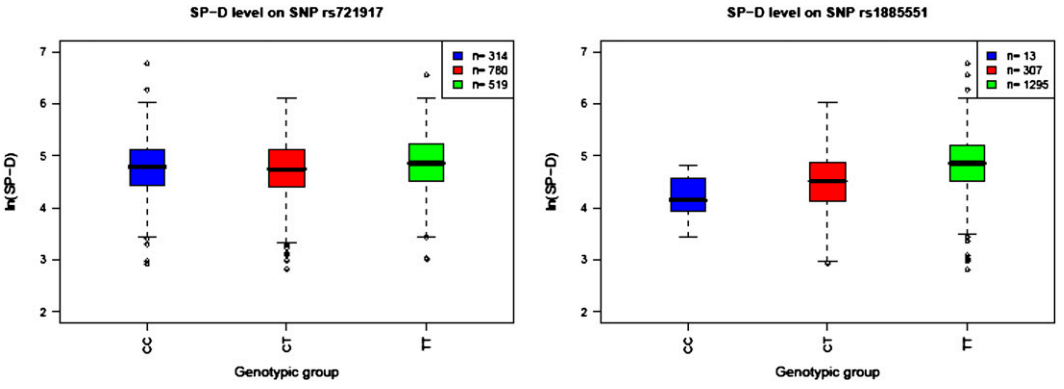

Figure 2. Boxplots of SFTPD genotypes versus SP-D concentration (unadjusted) in rs721917: CC = Thr/Thr CT = Met/Thr TT = Met/Met.

TABLE 5. PREDICTION OF SERUM SURFACTANT PROTEIN–D LEVEL BY SFTPD SNPS AND FEV_1 IN ECLIPSE CURRENT OR FORMER SMOKERS

SFTPD SNP	SNP *P* Value	FEV_1 *P* Value
rs2256573	0.0005	0.007
rs721917	0.0008	0.004
rs1885551	2×10^{-31}	0.02
rs1932571	2×10^{-15}	0.01

Definition of abbreviations: SFTPD, surfactant protein–D; SNP, single-nucleotide polymorphism; ECLIPSE, Evaluation of Chronic Obstructive Pulmonary Disease Longitudinally to Identify Predictive Surrogate Endpoints.

Models are adjusted for age, gender, pack-years, and current smoking.

In recognition of another potential limitation, we appreciate that corrections for multiple statistical testing are controversial. Our primary approach to the confirmation of genetic association was through replication. Lastly, spurious allelic associations attributable to population stratification are ameliorated in family-based designs. Previous genotyping for population stratification in the NETT–NAS cohort did not demonstrate significant population stratification (53).

We provide evidence that SFTPD is involved in the pathogenesis of COPD. In independent populations and across multiple study designs, we demonstrate that genetic variations in SFTPD influence serum concentrations and lung function, and are associated with susceptibility to COPD. Further work is required to understand the role of shared versus independent genetic influences on SP-D concentrations and susceptibility to COPD.

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