Loci Identified by Genome-wide Association Studies Influence Different Disease-related Phenotypes in Chronic Obstructive Pulmonary Disease

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***Rationale*: Genome-wide association studies have shown significant associations between variants near hedgehog interacting protein *HHIP*, *FAM13A*, and cholinergic nicotinic acetylcholine receptor *CHRNA3/5* with increased risk of chronic obstructive pulmonary disease (COPD) in smokers; however, the disease mechanisms behind these associations are not well understood.**

***Objectives*: To identify the association between replicated loci and COPD-related phenotypes in well-characterized patient populations.**

***Methods*: The relationship between these three loci and COPD-related phenotypes was assessed in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-point (ECLIPSE) cohort. The results were validated in the family-based International COPD Genetics Network (ICGN).**

***Measurements and Main Results*: The *CHRNA3/5* locus was significantly associated with pack-years of smoking ($P = 0.002$ and 3×10^{-4}), emphysema assessed by a radiologist using high-resolution computed tomography ($P = 2 \times 10^{-4}$ and 4.8×10^{-5}), and airflow obstruction ($P = 0.004$ and 1.8×10^{-5}) in the ECLIPSE and ICGN populations, respectively. However, variants in the *IREB2* gene were only significantly associated with FEV_1. The *HHIP* locus was not associated with smoking intensity but was associated with FEV_1/FVC ($P = 1.9 \times 10^{-4}$ and 0.004 in the ECLIPSE and ICGN populations). The *HHIP* locus was also associated with fat-free body mass ($P = 0.007$) and with both retrospectively ($P = 0.015$) and prospectively ($P = 0.024$) collected COPD exacerbations in the ECLIPSE cohort. Single-nucleotide polymorphisms in the *FAM13A* locus were associated with lung function.**

***Conclusions*: The *CHRNA3/5* locus was associated with increased smoking intensity and emphysema in individuals with COPD, whereas the *HHIP* and *FAM13A* loci were not associated with smoking intensity. The *HHIP* locus was associated with the systemic components of COPD and with the frequency of COPD exacerbations. *FAM13A* locus was associated with lung function.**

Keywords: COPD exacerbations; nicotine addiction; high-resolution CT; genetic association analysis; emphysema

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

After more than a decade of inconsistent genetic association analysis results in chronic obstructive pulmonary disease (COPD), recent studies have identified three genetic loci that are unequivocally associated with COPD. These results provide a major step forward in the understanding of COPD; however, the disease mechanisms behind these associations are not well understood.

What This Study Adds to the Field

Our study in well-characterized COPD cohorts demonstrated that the CHRNA3/5 locus was associated with increased smoking intensity, airflow obstruction, and emphysema in individuals with COPD. The HHIP locus was associated with the systemic components of COPD and with the frequency of COPD exacerbations. The FAM13A locus was associated with airflow obstruction in patients with COPD. These results suggest that these three genetic loci influence different biologic processes in COPD.

Chronic obstructive pulmonary disease (COPD) is a common condition that is intimately linked with cigarette smoking and is predicted to be the third leading cause of mortality and the fifth leading cause of morbidity in the world by the year 2020 (1). COPD is characterized by the presence of airflow limitation resulting from airway inflammation and remodelling or lung parenchymal destruction with emphysema. Although COPD severity is typically determined by spirometric measures (2), there is extensive heterogeneity in the amount of emphysema, degree of functional impairment, and frequency of exacerbations among patients with COPD with the same level of airflow obstruction (3). COPD can be associated with systemic manifestations, such as cachexia, which can effectively result in impaired functional capacity, worsening dyspnea, reduced health-related quality of life, and increased mortality (4).

Familial aggregation studies suggest a strong genetic component to the risk of developing COPD (5–10). However, the only proven genetic risk factor for COPD is a severe deficiency of α_1-antitrypsin (11), which is present in only 1–2% of individuals with COPD. Major COPD susceptibility loci have recently been identified on chromosomes 4 (near hedgehog interacting protein [*HHIP*] and *FAM13A*) and 15 (in a linkage disequilibrium block including components of the cholinergic nicotinic acetylcholine receptor, *CHRNA3/5*, and the *IREB2* gene) in genome-wide association studies (GWAS) of COPD and related phenotypes (12–14). We hypothesized that different COPD-related phenotypes would be influenced by the *HHIP*, *FAM13A*, and *CHRNA3/5* loci. We have evaluated these loci in individuals recruited to the Evaluation of COPD Longitudinally to Identify Predictive Sur-

(*Received in original form February 1, 2010; accepted in final form August 2, 2010*)

Supported by GlaxoSmithKline and by National Institutes of Health grants R01 HL084323 and R01 HL075478 (E.K.S.).

This work was funded by GlaxoSmithKline.

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 182. pp 1498–1505, 2010
Originally Published in Press as DOI: 10.1164/rccm.201002-0151OC on July 23, 2010
Internet address: www.atsjournals.org

rogate End-point (ECLIPSE) cohort (15) and have replicated the findings in the International COPD Genetics Network (ICGN) population.

METHODS

ECLIPSE is a longitudinal prospective study being conducted at 46 clinical centers in 12 countries (15). All consenting subjects with COPD (n = 1,719) underwent spirometry and a low-dose multislice computed tomography (CT) scan of the chest (120-kVp, 40 mAs, 1-mm contiguous images). Extent of emphysema was assessed qualitatively by two independent radiologists and quantitatively using the Pulmonary Workstation 2.0 Software (VIDA Diagnostics, Iowa City, IA). The airway wall measurements were undertaken using the Pulmonary Workstation software. Reversibility to salbutamol was defined as greater than or equal to 12% improvement from the baseline FEV_1. The body mass index, airflow obstruction, and exercise capacity (BODE) index was calculated using the method previously described (16). Whole-body impedance was measured using the bioelectrical impedance method (Bodystat 1500; Bodystat Ltd, Isle of Man, UK) and fat-free body mass (FFM) was calculated (17).

COPD exacerbation frequency in ECLIPSE was assessed in two ways. For retrospective exacerbations, a questionnaire was administered at the time of recruitment and representing COPD-related exacerbations in the previous 12 months (subject-reported episodes that required antibiotics, systemic corticosteroids, or hospitalization). For prospective exacerbations, subjects were followed for 2 years based on monthly telephone calls (exacerbations over the first 2 years of the study were defined by the requirement for antibiotics, systemic corticosteroids, or hospitalization).

Individuals with COPD were recruited as probands to the ICGN study (18), and siblings and available parents were ascertained through the probands. In total, 1,891 whites from 606 pedigrees were included in the current analysis. High-resolution CT (120-kVp, 200-mAs, 1-slice thickness, 20-mm slice interval) measurements were available on 561 probands (48.5%) and 663 siblings (34.2%) using custom software developed at the University of British Columbia, Vancouver, Canada (18). All of the CT scans from the ECLIPSE and ICGN studies were processed at University of British Columbia. The ICGN population was originally used to identify the *HHIP*, *FAM13A*, and *CHRNA*3/5 single-nucleotide polymorphisms (SNP) using the COPD affection status phenotype in follow-up to genome-wide association analysis (12, 14), and a previously reported association analysis of FEV_1 in the ICGN is included for comparison in this manuscript. The ECLIPSE population was not a part of the original GWAS that identified those loci, but was included in the collaborative study that identified *FAM13A* as an additional COPD susceptibility locus (12). Additional information about these cohorts is available in the online supplement. A total of 110 subjects from the ICGN study used in these analyses participated in the ECLIPSE cohort, and these subjects were eliminated in the analyses involving both ECLIPSE and ICGN cohorts.

Genotyping

The Illumina 550Kv3 SNP array was used for SNP genotyping in the ECLIPSE subjects. Details on the procedures used for genotyping and quality control evaluation are described elsewhere (14). Data from two SNPs in the *CHRNA*3/5 locus (rs8034191 and rs1051730) and two SNPs in the *HHIP* locus (rs1828591 and rs13118928) and one SNP from *FAM13A* locus (rs7671167) were used in the current analysis. The ICGN subjects were genotyped using Sequenom's (San Diego, CA) iPLEX SNP genotyping protocol developed for measurement with the MassARRAY mass spectrometer (19). *FAM13A* SNP in the ICGN population was genotyped by the 5′ to 3′ exonuclease TaqMan (Applied Biosystems, Foster City, CA) method using Applied Biosystems Pre-Designed SNP Genotyping assays as per standard protocol.

Statistical Analyses

In the ECLIPSE population, logistic regression models for the binary phenotypes and linear regression models for the quantitative phenotypes were used; covariates included age, sex, pack-years of smoking, current smoking status, and EIGENSTRAT principal components for control of population stratification. Multivariate regression models were evaluated to identify the contribution of the *HHIP*, *FAM13A*, and *CHRNA*3/5 SNPs to phenotypes including spirometric measures, FFM, body mass index (BMI), bronchodilator responsiveness, and quantitative and qualitative measures of emphysema. The associations of the *HHIP*, *FAM13A*, and *CHRNA*3/5 SNPs with COPD exacerbations in ECLIPSE were assessed with negative binomial regression models.

Genotype imputations in the ECLIPSE population were done using the genotypes from the Illumina HumanHap550 Beadchip (Illumina, San Diego, CA) and phased chromosomes for the 60 HapMap Phase 2 CEU founders. Genotypes were imputed using a hidden Markov model as programmed in MACH (20).

Family-based association analysis was conducted in the ICGN families using PBAT version 3.6 (Golden Helix, Bozeman, MT) (21). In the analysis of quantitative and qualitative phenotypes, adjustments for age, gender, pack-years of smoking, and current smoking status were performed. Height was also included as a covariate in lung function analyses.

RESULTS

The demographics and clinical characteristics of the subjects assessed in the ECLIPSE and ICGN cohorts are shown in Table 1. Five SNPs from three loci (*HHIP* and *FAM13A* on chromosome 4 and *CHRNA*3/5/*IREB2* on chromosome 15) that were previously significantly associated with COPD (12, 14) were analyzed in this study. The SNPs in the *HHIP* region on chromosome 4 (rs1828591 and rs13118928) were in strong LD (D' = 0.98; r^2 = 0.97), so the results from only SNP rs13118928 are reported. Likewise, the SNPs on chromosome 15 (rs8034191 and rs1051730) were in high LD (D' = 0.99; r^2 = 0.9) and hence only the results from one SNP (rs8034191) are reported. The most significantly associated SNP in the FAM13 locus rs7671167 was analyzed and the results are reported. A detailed LD plot of the three loci in the ECLIPSE population is shown in the online supplement (*see* Figure E1) for *CHRNA3/5*, *HHIP*, and *FAM13A* loci, respectively.

Smoking Behavior

We first assessed the association of the three loci with smoking behavior in the ECLIPSE cohort, and we found a significant association of the *CHRNA*3/5 SNP with number of cigarettes smoked per day and the number of pack-years smoked, but not the age of starting to smoke (Table 2). Subjects with COPD who were current or former smokers that are homozygous for the rs8034191 risk allele smoked on average 3.47 more cigarettes per day (P = 0.0009) and 7.5 more cumulative pack-years (P = 0.0016) than did individuals with COPD who did not carry the risk allele. These findings were replicated in the ICGN family-based association analysis (P = 0.0003 for pack-years smoked) (Table 3). In contrast, the SNPs in the *HHIP* and *FAM13A* loci were not associated with any of these smoking phenotypes in either the ECLIPSE or ICGN populations.

Lung Function Measurements

The *CHRNA*3/5 SNP was significantly associated with FEV_1 and FEV_1/FVC ratio within COPD cases in the ECLIPSE cohort (Table 2). The *HHIP* SNP was also significantly associated with FEV_1/FVC ratio in ECLIPSE, and there was a trend toward association with FEV_1. The ICGN family-based association analyses showed similar results with significant associations between the *CHRNA*3/5 and *HHIP* SNPs with FEV_1/FVC ratio (Table 3). We have previously reported the significant association of *CHRNA3/5* SNPs with post-bronchodilator FEV_1 in the ICGN population (14).

TABLE 1. BASELINE DEMOGRAPHICS AND CLINICAL CHARACTERISTICS OF THE ECLIPSE AND ICGN POPULATIONS

	ECLIPSE COPD Cases	ICGN	
		Probands	Siblings
Subjects, n	1,609	606	1,285
Age, ± SD	63.8 (± 7.1)	58.4 (± 5.4)	58 (± 9.8)
Female, %	32.5	40.3	49.8
Post-bronchodilator FEV_1, % predicted, ± SD	48.1 (± 15.6)	36.2 (± 12.9)	77.5 (± 25.9)
Post-bronchodilator FEV_1/FVC ratio*, ± SD	0.45 (± 0.11)	0.37 (± 0.12)	0.61 (± 0.15)
Pack-years of smoking, ± SD	50.9 (± 28)	51.5 (± 26.7)	40.5 (± 24.6)
Current smoker, %	35.5	33.83	50.82
Body mass index, ± SD	26.7 (± 5.6)	26.1 (± 6.2)	27.2 (± 6.5)
Fat-free body mass, ± SD†‡	50 (± 10.2)		
BODE index, ± SD†§	3.2 (± 2.1)		
Frequency of exacerbation in the past 12 mo, ± SD†	0.83 (± 1.20)		
Frequency of exacerbation (2-yr follow-up), ± SD†	2.12 (± 2.62)		
Quantitative emphysema, −950 HU, ± SD‖	18 (± 12)	22.3 (± 14.4)	16.08 (± 10.9)
Radiologist score, % subjects with >5% emphysema	66.7	80.8	39.3
Airway wall thickness, mm, Pi_{10}, ± SD¶	3.96 (± 0.21)	4.86 (± 0.43)	4.78 (± 0.44)

Definition of abbreviations: BODE = body mass index, airflow obstruction, and exercise capacity; COPD = chronic obstructive pulmonary disease; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-point; ICGN = International COPD Genetics Network.

A total of 110 subjects from ICGN who also participated in the ECLIPSE study were eliminated from the summary of phenotypes analyzed in both ECLIPSE and ICGN cohorts.

* In the ICGN, FEV_1/VC was analyzed.

† Phenotypes not available or analyzed in ICGN study. All 1,719 ECLIPSE subjects were included in the mean and SD estimates.

‡ Expressed in Ω calculated using the method previously described (18).

§ BODE index calculated using the method previously described (17).

‖ Percentage of lung voxels with x-ray attenuation values less than −950 HU assessed by image analysis of high-resolution computed tomography scans.

¶ Square root of the wall area at a luminal perimeter of 10 mm.

FAM13A was not associated with FEV_1 in the ECLIPSE population, although it showed significant association in the ICGN cohort (12). This locus showed a trend toward association for FEV_1/FVC in the ECLIPSE cohort and significant association in the ICGN cohort (Table 3).

BMI, FFM, and BODE Index

There were no significant associations between BMI or FFM and the *CHRNA*3/5 SNP, but this SNP was associated with an increased BODE index in the ECLIPSE cohort ($P = 0.03$). In contrast, the *HHIP* risk allele showed a trend toward lower BMI ($P = 0.07$) and significant association with FFM ($P = 0.007$) in subjects with COPD in the ECLIPSE cohort. In the ICGN study, no significant association was observed between the *HHIP* SNP and BMI ($P = 0.18$); however, the direction of association was the same in both populations. The *FAM13A* SNP was not associated with BMI, FFM, or BODE index in the ECLIPSE population, whereas a borderline association with BMI in the ICGN population was observed ($P = 0.05$). FFM measurements and BODE index were not available in the ICGN population.

CT-defined Emphysema and Airway Wall Thickness

The *CHRNA*3/5 ($P = 0.002$) and *FAM13A* ($P = 0.04$) SNPs were associated with quantitative emphysema (−950 HU) in the ECLIPSE cohort, whereas the *HHIP* SNP only showed a trend toward significance ($P = 0.07$). We then evaluated the SNPs for association with the presence or absence of clinically significant emphysema based on the radiologist's assessment of the CT scans. The *CHRNA*3/5 SNP ($P = 0.0002$) and the *HHIP* SNP ($P = 0.006$) showed significant association with the radiologist's score of emphysema, whereas the *FAM13A* SNP did not show

TABLE 2. EFFECT OF SNPS IN THE *HHIP* (RS13118928), *FAM13A* (RS7671167), AND *CHRNA*3/5/*IREB2* (RS8034191) LOCI IN COPD SUBJECTS FROM THE ECLIPSE STUDY

	HHIP rs13118928				*CHRNA* rs8034191				*FAM13A* rs7671167			
	GG (*n = 270*)	GA (*n = 749*)	AA (*n = 590*)	*P* Value	CC (*n = 270*)	CT (*n = 804*)	TT (*n = 535*)	*P* Value	CC (*n = 343*)	CT (*n = 804*)	TT (*n = 457*)	*P* Value
Post-bronchodilator FEV_1	1.399 (0.51)	1.364 (0.53)	1.398 (0.49)	0.058	1.280 (0.47)	1.343 (0.52)	1.383 (0.53)	0.01	1.341 (0.52)	1.338 (0.50)	1.364 (0.54)	0.51
Percent-predicted FEV_1	49.13 (15.41)	48.54 (15.61)	47.06 (15.53)	0.077	46.63 (15.26)	47.43 (15.46)	49.35 (15.88)	0.02	48.3 (14.96)	48.17 (15.59)	47.77 (15.97)	0.53
FEV_1/FVC	46.33 (11.85)	45.66 (11.56)	43.14 (11.18)	1.9×10^{-4}	43.89 (11.44)	44.39 (11.34)	46.03 (11.83)	0.004	45.67 (11.44)	44.83 (11.56)	44.25 (11.58)	0.08
Number of cigarettes smoked per day	25.67 (13.04)	25.96 (12.57)	25.42 (12.43)	0.46	27.95 (12.95)	25.78 (12.62)	24.48 (12.24)	0.0009	25.7 (12.28)	25.63 (12.80)	25.86 (12.5)	0.48
Age started smoking cigarettes	17.54 (4.88)	16.94 (4.32)	16.78 (4.08)	0.18	16.81 (4.08)	17.08 (4.56)	16.59 (4.17)	0.09	16.8 (4.23)	16.99 (4.12)	17.08 (4.78)	0.43
Pack-years of smoking	51.92 (31.27)	50.40 (27.35)	50.94 (27.26)	0.34	56.36 (31.08)	50.35 (27.46)	48.84 (26.84)	0.0016	52.02 (28.05)	50.26 (28.43)	51.02 (27.22)	0.94
Body mass index	26.91 (5.48)	26.96 (5.74)	26.34 (5.59)	0.070	26.54 (5.96)	26.61 (5.69)	26.98 (5.43)	0.15	26.68 (5.58)	26.76 (5.74)	26.69 (5.54)	0.90
Fat-free body mass	51.34 (10.34)	50.40 (10.38)	49.26 (9.70)	0.007	49.28 (9.76)	50.15 (10.42)	50.56 (9.93)	0.09	49.65 (9.67)	50.02 (10.07)	50.71 (10.63)	0.41
Emphysema, −950 HU	17.05 (11.69)	17.66 (12.21)	18.99 (11.95)	0.07	20.34 (12.86)	18.02 (11.79)	16.89 (11.84)	0.0018	16.85 (10.95)	18.17 (12.23)	18.69 (12.42)	0.04
Clinically significant emphysema, radiologist score	64.2	65.1	70	0.006	*75.4*	*67*	*62*	*0.0002*	*75.3*	*74*	*74*	*0.82*
BODE index	*3.13 (1.95)*	*3.18 (2.16)*	*3.17 (2.11)*	*0.78*	*3.45 (2.14)*	*3.21 (2.13)*	*3.00 (2.05)*	*0.03*	3.101 (2.034)	3.13 (2.10)	3.277 (2.15)	*0.08*
Pi_{10}	*3.97 (0.19)*	*3.97 (0.21)*	*3.95 (0.21)*	*0.26*	*3.96 (0.20)*	*3.96 (0.21)*	*3.96 (0.21)*	0.58	3.969 (0.21)	3.967 (0.217)	3.951 (0.20)	0.19

Definition of abbreviations: BODE = body mass index, airflow obstruction, and exercise capacity; COPD = chronic obstructive pulmonary disease; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-point.

TABLE 3. RESULTS OF THE FAMILY-BASED ASSOCIATION ANALYSES IN THE ICGN POPULATION

	Number of Info Families	Risk Allele	*P* Value
CHRNA rs8034191			
FEV_1/FVC	287	C	**1.85×10^{-5}**
Number of cigarettes smoked per day	287	C	**0.01**
Age started smoking cigarettes	287	C	0.16
Pack-years of smoking	301	C	**0.0003**
Body mass index	286	C	0.18
Emphysema, −950 HU*	119	C	**0.0082**
Emphysema radiologist score, %	198	C	**4.82×10^{-5}**
Pi 10*	105	C	0.94
HHIP rs13118928			
FEV_1/FVC	301	A	**0.0042**
Number of cigarettes smoked per day	301	A	0.06
Age started smoking cigarettes	301	A	0.651
Pack-years smoking	301	A	0.102
Body mass index	301	A	0.301
Emphysema, −950 HU*	144	A	**0.01**
Emphysema radiologist score, %	219	A	**0.004**
*Pi 10**	131	A	0.60
FAM13A rs7671167			
FEV_1/FVC	224	A	**0.010**
Number of cigarettes smoked per day	224	A	0.354
Age started smoking cigarettes	224	A	0.633
Pack-years smoking	224	A	0.746
Body mass index	301	A	**0.050**
Emphysema, −950 HU*	123	A	0.172
Emphysema radiologist score, %	80	A	0.200
*Pi 10**	224	A	0.759

Definition of abbreviation: ICGN = International COPD Genetics Network.

* The subjects from Holland and Liverpool centers were eliminated from these analyses, because these centers used different computed tomography scanner models.

The significant *P* values are shown in bold.

Association of FEV_1 in the ICGN population was reported in the genome-wide association studies publications (12, 14).

any association. In the ICGN population, the associations were significant for the quantitative and radiologist's classification of emphysema ($P = 0.0082$ and 4.82×10^{-5}, respectively) at the *CHRNA*3/5 locus. The *HHIP* SNP also showed association with quantitative ($P = 0.01$) and radiologist's score ($P = 0.004$) of emphysema in the ICGN population. *FAM13A* SNP did not show any association to emphysema phenotypes in the ICGN population. There was no association with airway wall thickness measurements (P_{i10} and wall area percent) with any of these SNPs in either population.

Multiple Regression Analyses With *HHIP*, *FAM13A*, and *CHRNA*3/5 Loci

The results of multivariate analyses, including the *HHIP*, *FAM13A*, and *CHRNA*3/5/*IREB2* SNPs, are shown in Table 4. These analyses were conducted to determine whether the *HHIP*, *FAM13A*, and *CHRNA*3/5 loci were independently associated with different COPD-related phenotypes. The results show that *CHRNA*3/5 and *HHIP* loci contribute significantly and independently to the airflow obstruction phenotypes (FEV_1 and FEV_1/FVC) in individuals with COPD. The *FAM13A* SNP did not show any association with FEV_1, but showed a trend toward association with FEV_1/FVC ($P = 0.059$). The effect of the *HHIP* variant was statistically more significant than the *CHRNA*3/5 variant in contributing to airflow obstruction in these multivariate models. All three loci contributed to the quantitative emphysema measurements. The *CHRNA*3/5 variant had the most significant association to quantitative emphysema, but the *FAM13A* SNP also had evidence for association to emphysema in this multivariate model. The *HHIP* variant had a significant effect on FFM and BMI.

The results of the multiple regression analyses for the outcome of COPD exacerbations in the ECLIPSE population are shown in Table 5. The *HHIP* SNP was associated with previous patient-reported exacerbations ($P = 0.015$) and exacerbations prospectively collected over 2 years of follow-up in subjects with COPD ($P = 0.024$). There was no association of exacerbations with the *CHRNA* or *FAM13A* SNPs. Because exacerbations are more frequent in subjects with COPD with more severely reduced FEV_1 values, we also conducted an analysis adjusting for baseline percent predicted FEV_1. The results from the *HHIP* SNP were statistically significant for prior exacerbations ($P = 0.025$; incidence ratio, 0.895). The trends were similar for prospectively collected exacerbations, but were not statistically significant ($P = 0.397$; incidence ratio, 0.966). We also analyzed the data by dividing the subjects into frequent exacerbators (two or more exacerbations per year) versus nonfrequent exacerbators (no more than one exacerbation per year), and the results were again significant for prior exacerbations ($P = 0.013$; odds ratio, 0.77). In the prospective exacerbation data, the association of the *HHIP* SNP with exacerbation frequency was not statistically significant ($P = 0.66$; odds ratio, 0.97). Because COPD exacerbations have been reported to be associated with BODE index (22) and serum surfactant protein D levels (23), we explored a multiple regression model including serum surfactant protein D level, BODE index, and

TABLE 4. MULTIVARIATE ANALYSES INCLUDING *HHIP*, *FAM13A*, AND *CHRNA*3/5 SINGLE-NUCLEOTIDE POLYMORPHISMS OF THE COPD-RELATED PHENOTYPES IN ECLIPSE COPD PATIENTS*

	FEV_1 Percent Predicted			FEV_1/FVC			Quantitative Emphysema		
	Estimate	SE	*P* Value	Estimate	SE	*P* Value	Estimate	SE	*P* Value
rs13118928 (*HHIP*)	1.29	0.56	0.021	1.921	0.412	<0.001	−1.195	0.475	0.012
rs8034191 (*CHRNA*)	−1.341	0.574	0.02	−1.212	0.423	0.004	1.422	0.492	0.004
rs7671167 (*FAM13A*)	0.263	0.558	0.638	0.776	0.411	0.059	−0.963	0.476	0.043
Sex (female)	3.811	0.855	<0.001	2.231	0.63	<0.001	−1.63	0.724	0.024
Pack-years smoking	−0.006	0.014	0.688	−0.007	0.011	0.538	−0.007	0.013	0.57
Age	0.094	0.056	0.093	−0.09	0.041	0.028	0.188	0.048	<0.001
	Fat-Free Body Mass			Body Mass Index					
rs13118928 (*HHIP*)	0.952	0.289	0.001	0.441	0.2	0.028			
rs8034191 (*CHRNA*)	−0.49	0.296	0.098	−0.302	0.206	0.142			
rs7671167 (*FAM13A*)	−0.197	0.287	0.492	0.059	0.2	0.766			
Sex (female)	−13.17	0.441	<0.001	−0.34	0.306	0.267			
Pack-years smoking	0.01	0.008	0.206	0.014	0.005	0.006			
Age	−0.136	0.028	<0.001	−0.033	0.02	0.091			

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-point.

* A total of 1,719 subjects from ECLIPSE cohort were used in this analysis.

TABLE 5. ANALYSES OF PRIOR AND PROSPECTIVE 2-YEAR COPD EXACERBATIONS IN THE SUBJECTS WITH COPD FROM THE ECLIPSE COHORT*

Effect	Incidence Rate Ratio	95% Confidence Interval	*P* Value
Prior Exacerbations			
rs13118928 (*HHIP*)	0.877	0.78–0.975	0.015
rs8034191 (*CHRNA*)	0.971	0.869–1.084	0.598
rs7671167 (*FAM13A*)	1.081	0.978–1.195	0.129
Female	1.173	1.004–1.372	0.044
Pack-years smoked	1	0.997–1.002	0.763
Age	1.002	0.992–1.011	0.754
Prospective Exacerbations (2-yr data)			
rs13118928 (*HHIP*)	0.906	0.832–0.987	0.024
rs8034191 (*CHRNA*)	1.017	0.930–1.113	0.709
rs7671167 (*FAM13A*)	1.028	0.943–1.22	0.528
Female	1.202	1.061–1.36	0.004
Pack-years smoked	1	0.997–1.002	0.726
Age	1.01	1.001–1.018	0.021

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points.

* All 1,719 subjects from the ECLIPSE cohort were used in this analysis. The associations of the *HHIP*, *FAM13A*, and *CHRNA* single-nucleotide polymorphisms with prior exacerbations (exacerbations during the 12 mo before study entry that required antibiotics, systemic corticosteroids, or hospitalization) and prospective exacerbations (exacerbations over the first 2 yr of the study that required antibiotics, systemic corticosteroids, or hospitalization) were assessed by negative binomial regression models. Robust SE for the model coefficients were determined by generalized estimating equations. An offset variable based on the log of the number of days on study was included in the model for prospective exacerbations.

HHIP SNP genotypes in predicting exacerbations. The results indicated that the effect of *HHIP* in predicting exacerbations is independent of BODE index and serum surfactant protein D levels (data not shown).

Results from the Genotype Imputation Analysis

Because the causative variants in these loci are not known, we conducted an imputation analysis using the HAPMAP Phase 2 data (200 kb upstream and downstream of the variant of interest at each of the three loci) in the ECLIPSE population and conducted association analyses for the key phenotypes. The results from the imputation analyses are provided in the online supplement (*see* Table E1) for *CHRNA*, *HHIP*, and *FAM13A*, respectively, and summarized next.

*CHRNA*3/5/*IREB2* Locus

The most significant association with pack-years of smoking and cigarettes smoked per day were in an LD block containing the *CHRNA*3/5 genes. A nonsynonymous coding SNP in the *CHRNA5* gene (rs16969968) and a synonymous coding SNP in the *CHRNA3* showed the most significant associations. The quantitative and radiologist score of emphysema were also significantly associated with this LD block with the most significantly associated SNP in the *CHRNA* 3 gene (rs1051730) followed by the nonsynonymous SNP in *CHRNA*5 (rs16969968). The FEV_1/FVC analyses also showed significant association with this LD block, whereas the most significant association for FEV_1 was in the intronic region in the *IREB2* gene (rs17483929; $P = 0.0056$).

FAM13A Locus

Imputation analysis of the ECLIPSE data showed that one of the SNPs in *FAM13A* (rs10007590; $P = 0.02$) was associated with FEV_1. This SNP was 33 kb away from the SNP shown to be associated with COPD in the GWAS study (rs7671167), which did not show any association with FEV_1 in the ECLIPSE population. Three SNPs in the *FAM13A* gene showed significant association with FEV_1/FVC (rs2869966, rs2869967, and rs2045517). Quantitative emphysema analyses identified SNPs 118 kb upstream of rs7671167 ($P = 0.035$) with significant associations. Radiologist score of emphysema analysis found one SNP with marginally significant association.

HHIP Locus

Imputation analysis of FEV_1 did not identify SNPs that are more significantly associated than the one identified in the GWAS study (rs13118928). However, the FEV_1/FVC analyses identified a genomic SNP (rs6817273; $P = 0.0001$) that maps 5 kb downstream of rs13118928. The analyses of quantitative and radiologist score of emphysema identified a significant genomic SNP (rs10519717; $P = 0.018$) that maps 6 kb upstream of rs13118928.

DISCUSSION

Recent GWAS identified three major loci that are associated with COPD susceptibility. Here we show that a genetic variant in the *CHRNA*3/5 locus is significantly associated with smoking intensity and airflow obstruction as defined by reduced FEV_1 and FEV_1/FVC. More detailed analysis demonstrated that this association was with emphysema rather than with airway wall thickness phenotypes of COPD. These data are explicable by the *CHRNA*3/5 locus influencing addiction to tobacco with the resulting consequence being smoking-related lung diseases (i.e., COPD and lung cancer). However, the association of the *CHRNA*3/5 locus with smoking behavior is controversial. It was not observed in either COPD cases or controls from the discovery cohort in our original GWAS (14), nor was it clearly seen in two of the three GWAS linking the *CHRNA*3/5 locus to lung cancer (24, 25). These lung cancer reports argued that the association of lung cancer is not related to nicotine dependence. Another lung cancer study reported a very strong association of this locus with smoking quantity and nicotine dependence (26). This locus has been shown to be associated with nicotine dependence by other investigators (27, 28). Moreover, our conclusion is supported by the recent demonstration that this locus also influences the risk for alcohol dependence (29). Recent fine-mapping efforts on smoking dependence have shown the involvement of functional variants in the *CHRNA*3/5 gene (30). To localize the functional variants associated with these loci, we undertook a detailed genotypic imputation analysis and found that the SNPs in the *CHRNA*3/5 gene showed significant association with smoking, FEV_1/FVC, and both quantitative and radiologist definition of emphysema. The most significant association with FEV_1 was in an intronic region in the *IREB2* gene. This raises the possibility of multiple functional genes in the locus controlling different aspects of COPD. Recent work suggests that the *IREB2* gene, which is also located in the *CHRNA*3/5 locus, may confer COPD susceptibility (13).

Cholinergic activity in the airways primarily induces tracheobronchial smooth muscle contraction and mucous secretion. However, there is an increasing body of literature showing the importance of extraneuronal cholinergic signaling (31) in the lung. It has been shown that nicotinic acetylcholine receptors are active in the nonneuronal cells in the lung, including bronchial epithelial cells and a variety of inflammatory cells (32). These nicotinic acetylcholine receptors in lymphocytes are partially composed of the $\alpha4\beta2$, $\alpha3\beta4$, and $\alpha7$ subtypes, whereas neutrophils express principally $\alpha3\beta4$ (33). Nicotine reduces the integrin expression in freshly isolated peripheral blood neutrophils (34) and prolongs the survival of neutrophils by suppressing apoptosis (35). Neutrophils are important cells in COPD because they are

increased in the conducting airways and are activated to release proteases and oxidants associated with the pathophysiology of the disease (36). It is possible that stimulation of nicotinic receptors by acetylcholine or tobacco-associated agonists could contribute to the pathogenesis of COPD by nicotinic regulation of cellular proliferation through the activation of Akt (37), by β-arrestin–mediated activation of Src and Rb-Raf-1 pathways (38), or by blocking apoptosis (39, 40). Of interest, we found the most significant association with emphysema within the *CHRNA3/5* locus, whereas the strongest evidence of association for FEV_1 was at the *IREB2* locus. The protein product of IREB2 is an RNA binding protein that, together with *IREB1*, is involved in maintaining human cellular iron metabolism (41). Alterations in iron maintenance in the presence of hypoxia could lead to increased oxidative stress resulting in tissue damage and reduced FEV_1.

The association of the *HHIP* locus with lung function measurements was convincingly demonstrated recently in two large general population lung function GWAS (42, 43). There was no significant association of the smoking-related phenotypes with the *HHIP* locus in our study. However, this locus was significantly associated with airflow obstruction and associated with emphysema defined by radiologist readings of chest CT scans. The association with quantitative measures of emphysema was not significant in the ECLIPSE population. The results from the multiple regression analyses also show that the contribution from the *CHRNA*3/5 locus is predominant in explaining the quantitative emphysema phenotype. The subjects with COPD with the *HHIP* risk genotypes had significantly lower FFM. Because the *HHIP* locus has been associated with height (44), we assessed the association of the *HHIP* SNP with height in our datasets but did not find an association (data not shown). A striking feature of our study was the association of variation at the *HHIP* locus and frequency of COPD exacerbations. The genetic factors underlying exacerbations in COPD are poorly understood. The only reports of genetic associations with COPD exacerbation are with variants in *SFTPB* and *CCL1* genes (45, 46). Capturing reliable data on exacerbations in large COPD populations has been a challenge. We analyzed the historical exacerbation assessments and the ongoing exacerbation assessments in the ECLIPSE population for possible association with the genetic variants under study. We identified significant associations between the *HHIP* variant and patient-reported prior exacerbations and prospectively collected exacerbations. This observation increases the reliability of the association of *HHIP* with COPD exacerbations.

The *FAM13A* locus has been shown to be associated with COPD (12, 30) and lung function (30, 42). This locus showed significant association with FEV_1 and FEV_1/FVC in the ICGN population. Our genotypic imputation analysis showed that this locus is also significantly associated with FEV_1 and FEV_1/FVC in the ECLIPSE population. Association of this locus with emphysema was not consistent in the two populations.

Our study has limitations that require discussion. The associations that we identified with COPD-related phenotypes in the three replicated loci were restricted to the SNPs analyzed at these loci. None of the SNPs that we studied are known to be functional, and the functional variants related to these loci are still unknown. We conducted genotypic imputation analyses to localize potential functional variants, and we demonstrated the variants in *CHRNA3/5* genes were associated with smoking behavior, airflow obstruction, and emphysema. The identification of functional variants in *HHIP* and *FAM13A* loci need fine-mapping efforts using large populations, potentially with different genetic ancestry. Our samples were limited only to white subjects. Although ECLIPSE is a well-characterized population with rich phenotypic assessments, some of the associations that we identified in ECLIPSE could not be replicated in the ICGN population because these phenotypes were not available in the ICGN cohort. The CT acquisition algorithms and software used for measuring the airway wall thickness were different in ECLIPSE and ICGN as evidenced by the mean values in Table 1. This technical issue might have contributed to the lack of association with airway phenotypes. The association analysis results from the quantitative and radiologist score of emphysema were consistent for the *CHRNA* locus, but not for the *HHIP* and *FAM13A* locus in the ECLIPSE population. In the radiologist definition of emphysema, we dichotomized the phenotype based on the presence or absence of clinically significant emphysema, whereas in the quantitative emphysema analysis the quantitative measurement from the algorithm was used and this may have contributed to the differences in the results.

Conclusions

Our data show that the *CHRNA*3/5 locus affects smoking behavior and has a significant effect on COPD-related phenotypes; it is unclear whether there are additional influences of this locus on COPD, independent of smoking. The *HHIP* locus is associated with significant airflow obstruction, increased exacerbations, reduced BMI, and FFM suggesting a relationship with the systemic component in COPD. *FAM13A* locus was not associated with smoking behavior; the association with CT-defined emphysema was also not consistent. However, this locus showed significant association with airway obstruction in subjects with COPD. The three COPD susceptibility loci identified by GWAS may influence distinct subtypes of COPD.

Author Disclosure: S.G.P. was a full time employee of GlaxoSmithKline until September 2009 and is currently a full time employee of Roche Pharmaceuticals since September 2009, and owns stock in GlaxoSmithKline. X.K. is an employee of GlaxoSmithKline. L.D.E. is a full-time employee of GlaxoSmithKline and holds $5,001–$10,000 in stock ownership/options in GlaxoSmithKline. M.C. received more than $100,001 from the National Institutes of Health (NIH) as a NIH K12 grant. W.H.A. is an employee of GlaxoSmithKline and holds more than $100,001 in stock in GlaxoSmithKline. W.H.A.'s employer, GlaxoSmithKline, provides more than $100,001 in sponsored grants as educational support for American Thoracic Society meetings. H.O.C. received up to $1,000 from GlaxoSmithKline and up to $1,000 from AstraZeneca in consultancy fees; $1,001–$5,000 from GlaxoSmithKline in advisory board fees; more than $100,001 from GlaxoSmithKline, $50,001–$100,000 from Spiration Inc., and more than $100,001 from Wyeth in industry-sponsored grants; and more than $100,001 from the NIH and more than $100,001 from the CIHR in sponsored grants. D.A.L. received $10,001–$50,000 from GlaxoSmithKline, $10,001–$5,000 from Novartis, up to $1,000 from Genzyme, and up to $1,000 from Amicus in consultancy fees; $10,001–$50,000 from GlaxoSmithKline, $1,001–$5,000 from Talecris, and $1,001–$5,000 from Thorax in advisory board fees; $1,001–$5,000 from GlaxoSmithKline, up to $1,000 from AstraZeneca, $1,001–$5,000 from Boehringer Ingelheim, and $1,001–$5,000 from LFB in lecture fees; more than $100,001 from GlaxoSmithKline and more than $100,001 from Merck, Sharpe & Dohme in industry-sponsored grants; more than $100,001 from the Medical Research Council, more than $100,001 from the Wellcome Trust, more than $100,001 from the British Lung Foundation, and $10,001–$50,000 from the Alpha-1 Foundation in sponsored grants; and $1,001–$5,000 from the Medical Research Council in advisory board fees. E.K.S. received $10,001–$50,000 from GlaxoSmithKline and $10,001–$50,000 from AstraZeneca in consultancy fees; $1,001–$5,000 from GlaxoSmithKline, $5,001–$10,000 from AstraZeneca, and $1,001–$5,000 from Bayer in lecture fees; more than $100,001 from GlaxoSmithKline in industry-sponsored grants; and more than $100,001 from the NIH in sponsored grants.

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Acknowledgment: The authors are grateful to the efforts of Linda Briley, Mark Hall, Sara Alalouf, Andrew Slater, Jennifer Johnson, Pam St. Jean, David Yarnall, Bo Zheng, and Sanjay Sharma of GlaxoSmithKline for the sample management, data management, genotyping, and analysis. The authors thank Drs. Nestor Müller and Paola Nasute Fauerbach for reading the CT scans and Tara Candido, Sebastian Cogswell, Heather Davis, Nima Farzaneh, Lukas Holy, Natasha Krowchuk, Helena Lee, Evan Phillips, Claudine Storness-Bliss, Nerissa Tai, Anh-Toan Tran, Nghia Tran, Eugene Wang, and Tomonori Yokogawa for technical assistance with the CT analysis and data management.

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