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Genome-Wide Association Studies Identify *CHRNA5/3* and *HTR4* in the Development of Airflow Obstruction

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***Rationale*: Genome-wide association studies (GWAS) have identified loci influencing lung function, but fewer genes influencing chronic obstructive pulmonary disease (COPD) are known.**

***Objectives*: Perform meta-analyses of GWAS for airflow obstruction, a key pathophysiologic characteristic of COPD assessed by spirometry, in population-based cohorts examining all participants, ever smokers, never smokers, asthma-free participants, and more severe cases.**

***Methods*: Fifteen cohorts were studied for discovery (3,368 affected; 29,507 unaffected), and a population-based family study and a meta-analysis of case-control studies were used for replication and regional follow-up (3,837 cases; 4,479 control subjects). Airflow obstruction was defined as FEV_1 and its ratio to FVC (FEV_1/FVC) both less than their respective lower limits of normal as determined by published reference equations.**

***Measurements and Main Results*: The discovery meta-analyses identified one region on chromosome 15q25.1 meeting genome-wide significance in ever smokers that includes *AGPHD1*, *IREB2*, and *CHRNA5/CHRNA3* genes. The region was also modestly associated among never smokers. Gene expression studies confirmed the presence of *CHRNA5/3* in lung, airway smooth muscle, and bronchial epithelial cells. A single-nucleotide polymorphism in *HTR4*, a gene previously related to FEV_1/FVC, achieved genome-wide statistical significance in combined meta-analysis. Top single-nucleotide polymorphisms in *ADAM19*, *RARB*, *PPAP2B*, and *ADAMTS19* were nominally replicated in the COPD meta-analysis.**

***Conclusions*: These results suggest an important role for the *CHRNA5/3* region as a genetic risk factor for airflow obstruction that may be independent of smoking and implicate the *HTR4* gene in the etiology of airflow obstruction.**

Keywords: chronic obstructive pulmonary disease; single-nucleotide polymorphism; genes

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Genome-wide association studies of pulmonary function in population-based studies have discovered numerous loci, but association to a standardized definition of airflow obstruction has not yet been evaluated within population-based studies.

What This Study Adds to the Field

This is the largest study to date to evaluate genetic predictors of airflow obstruction. We confirm the association to the chromosome 15 *CHRNA5/CHRNA3* gene cluster and demonstrate nominal association to the region in never smokers with airflow obstruction. We also implicate the *HTR4* gene in the pathogenesis of airflow obstruction.

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide, and cigarette smoking is the most widely recognized risk factor for this disease. COPD is defined based on spirometry as airflow obstruction that is not fully reversible after administration of a bronchodilator. Airflow obstruction is a key pathophysiologic characteristic of COPD that is assessed by spirometry. Both COPD and spirometry measures of lung function have been demonstrated to have a genetic component. Family studies have reported an increased risk for COPD in relatives of a COPD proband (1) as well as significant heritability of pulmonary function measured by spirometry in population-based cohorts (2). The α_1-antitrypsin gene (*SERPINA1/A1AT*) is known to be associated with COPD and leads to increased risk for early-onset disease among individuals carrying the susceptibility alleles, but few other genes have such a conclusive relationship to COPD.

Recent genome-wide association studies (GWAS) have examined two spirometry measures of lung function, FEV_1 and its ratio to FVC (FEV_1/FVC). Two large-scale GWAS meta-analyses identified a total of 11 loci related to FEV_1 or FEV_1/FVC (3, 4), and a larger meta-analysis incorporating these studies along with new studies identified an additional 16 loci (5). Two genetic loci identified by the above studies, *HHIP* and *FAM13A*, have been demonstrated to influence risk of COPD at genome-wide levels of statistical significance (6–9). GWAS of COPD have also identified associations with SNPs in a region on chromosome 15q25.1 that includes cholinergic nicotinic receptor genes (*CHRNA5-CHRNA3-CHRNB4*) and the iron-responsive element binding protein 2 (*IREB2*) (7), but some questions remain as to the underlying genetic signal because of substantial linkage disequilibrium in the region. This region has also been associated with lung cancer (10, 11) and nicotine dependence (12–15), leading to the hypothesis that the association with the various disease endpoints may be mediated through the nicotinic receptor genes and thus smoking, smoking intensity, and cessation (16). In a meta-analysis of lung cancer among never smokers, no association to the *CHRNA* genes was observed, supporting the hypothesis that association was mediated through smoking behavior (17).

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However, the observation of increased *IREB2* protein and mRNA expression in COPD lung tissue compared with controls supports its potential involvement as well (18).

The standard definition of COPD is based on the presence of airflow obstruction that persists after administration of bronchodilator (19). In large population-based cohorts, post-bronchodilator spirometry is not generally available, so we have studied prebronchodilator airflow obstruction as a proxy for COPD. In this study, we performed GWAS using a standardized definition of airflow obstruction and control subjects across 15 population-based cohort studies and conducted a meta-analysis. We then sought replication of our top single-nucleotide polymorphisms (SNPs) and regions in a set of four COPD case-control studies previously included in a meta-analysis and in a population-based family study that used the same airflow obstruction phenotype definitions used in the discovery analyses.

METHODS

Discovery Phase

Most of the cohorts used in the discovery phase of this meta-analysis were included in meta-analyses of cross-sectional quantitative pulmonary function measures in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium (3), the SpiroMeta consortium (4), and/or their joint analysis (5). Cohorts not included in previous GWAS discovery sets for pulmonary function include Rotterdam Study III (RS3), Swiss Study on Air Pollution and Lung and Heart Disease in Adults (SAPALDIA), Lothian Birth Cohort (LBC1936), Multi-Ethnic Study of Atherosclerosis (MESA), and COPD Pathology: Addressing Critical gaps, Early Treatment and diagnosis, and Innovative Concepts (COPACETIC). All of the included participants are white and of European descent.

Standardized definitions of airflow obstruction based on the lower limit of normal of FEV_1 and FEV_1/FVC from the National Health and Nutrition Examination Survey III prediction equations (20) were used across all cohorts. The presence of airflow obstruction was defined as an FEV_1 and FEV_1/FVC both less than the lower limit of normal (21) based on prediction equations that include age, age^2, and height^2 calculated separately by sex. Unaffected participants were defined by FEV_1, FVC, and FEV_1/FVC all above the lower limit of normal. Individuals below the lower limit of normal for FEV_1 or FEV_1/FVC but not both were excluded from these analyses. Logistic regression models were adjusted for current and former smoking dummy variables, pack-years of smoking, age, sex, standing height, center/cohort as needed, and principal components for genetic ancestry as needed.

Genome-wide imputation and analyses were performed by the cohort investigators, and results were shared for meta-analysis. Details of individual cohorts' imputation and GWAS methods are provided in the online supplement text and Table E1 in the online supplement. Genome-wide and regional meta-analyses were performed using METAL software (22) with inverse variance weighting to combine effect size estimates after applying a genomic control correction (23).

Five discovery analyses were performed. GWAS were performed in (*1*) all cohorts with both ever and never smokers, (*2*) ever smokers, (*3*) never smokers, (*4*) asthma-free participants, and (*5*) the subset of more severe airflow obstruction with FEV_1 less than 65% predicted (excluding milder cases from analysis). Never smoking GWAS were performed in eight cohorts. In the 10 cohorts that collected self-reported asthma data, an analysis was performed excluding all participants reporting a history of asthma with diagnosis before age 40 years or missing onset age.

Regional Meta-analysis and Replication

Two strategies were implemented for follow-up of top results. In two regions with association signals spanning multiple genes in discovery meta-analyses, results across the whole region were requested from the replication studies, and combined meta-analyses were performed to refine the association signal. These regions were located on chromosome 6 (27,599,278–32,787,304 bp) and chromosome 15 (76,499,754–76,711,042 bp). In addition, 60 SNPs with *P* values less than or equal to 1×10^{-5} in any of the five discovery meta-analyses were selected for replication. Combined meta-analysis was performed with the Family Heart Study (FamHS), which evaluated the same airflow obstruction phenotype as used in the discovery phase (331 affected and 2,550 unaffected). Replication was further evaluated in a meta-analysis of studies with clinically ascertained COPD (3,499 cases and 1,922 control subjects) (24). Gene expression in lung tissues was evaluated for two genes on chromosome 15. Additional details are included in the online supplement.

RESULTS

Descriptive characteristics of the 15 discovery cohorts are provided in Tables 1 and 2. The mean FEV_1 % predicted for participants with airflow obstruction ranged from 48.9 to 68.7% across cohorts, and for unaffected participants the means were generally around 100%. The mean FEV_1/FVC ratio ranged from 49.5 to 62.5% among affected participants and 74.1 to 81% among unaffected participants across the cohorts. The mean ages at measurement of spirometry across the cohorts ranged from 45 to 76 years. The number of participants contributing to each of the five discovery GWAS meta-analyses are provided in Table 3.

TABLE 1. DESCRIPTIVE CHARACTERISTICS OF COHORTS INCLUDED IN DISCOVERY META-ANALYSIS

	ARIC	FHS	CHS	COPACETIC	B58C	EPIC	MESA
No. affected,	914	571	402	312	264	127	104
No. unaffected	6,602	5,866	2,183	996	4,374	1,023	979
Age, yr	54.3 (5.7)	51.6 (14.6)	72.3 (5.3)	60.2 (5.6)	45.2 (0.39)	58.2 (9.0)	66.1 (9.8)
Male, %	47.2	46.4	39.4	100	49.6	46.8	49.6
Height, cm	169 (9)	168 (10)	165 (9)	179 (6)	169 (9)	167 (9)	169 (10)
BMI, kg/m^2	27.0 (4.7)	27.2 (5.2)	26.2 (4.3)	—	27.4 (4.9)	26.4 (3.9)	28.0 (5.2)
Current smoker, %	21.8	14.2	9.3	55.1	21.3	10.1	7.9
Former smoker, %	35.8	38.1	39.7	44.9	49.1	44.6	50.8
Pack-years smoking*	27.5 (21.4)	21.9 (21.2)	32.2 (26.7)	39.6 (17.0)	14.7 (11.7)	17.6 (16.0)	29.4 (28.5)
FEV_1/FVC							
Affected	58.1 (8.6)	58.6 (8.3)	52.0 (10.7)	51.0 (9.1)	62.5 (7.0)	57.0 (8.5)	56.6 (9.2)
Unaffected	76.6 (4.4)	77.6 (5.3)	74.1 (5.6)	75.5 (4.8)	80.9 (5.6)	81.0 (6.4)	75.5 (5.8)
FEV_1 % predicted							
Affected	62.2 (13.4)	63.2 (12.8)	52.1 (15.5)	58.9 (11.1)	68.7 (9.5)	57.1 (13.8)	61.0 (12.7)
Unaffected	99.5 (11.1)	100.4 (11.8)	98.4 (13.5)	105.8 (12.8)	100.7 (10.7)	96.6 (9.9)	98.2 (12.0)

Definition of abbreviations: ARIC = Atherosclerosis Risk in Communities; B58C = British 1958 Birth Cohort; CHS = Cardiovascular Health Study; COPACETIC = COPD Pathology: Addressing Critical gaps, Early Treatment and diagnosis, and Innovative Concepts; EPIC = European Prospective Investigation into Cancer and Nutrition; FHS = Framingham Heart Study; MESA = Multi-Ethnic Study of Atherosclerosis.

Data are presented as mean (SD) unless otherwise indicated.

* Pack-years calculated among current and former smokers.

TABLE 2. DESCRIPTIVE CHARACTERISTICS OF ADDITIONAL COHORTS INCLUDED IN DISCOVERY META-ANALYSIS

	AGES	Health ABC	RS1	SAPALDIA	BHS	RS3	LBC1936	RS2
No. affected,	109	108	99	98	89	70	61	40
No. unaffected	1,562	1,129	1,003	833	661	1,001	627	668
Age, yr	76.2 (5.6)	73.8 (2.8)	74.4 (5.7)	51.0 (11.1)	54.6 (16.3)	56.6 (5.5)	69.6 (0.9)	67.1 (6.3)
Male, %	40.6	52.1	42.9	48.1	44.0	42.3	49.4	43.8
Height, cm,	167 (9)	167 (9)	167 (9)	169 (9)	168 (9)	171 (9)	166 (9)	168 (9)
BMI, kg/m^2	27.1 (4.5)	26.5 (4.1)	27.4 (4.0)	25.7 (4.3)	25.8 (4.0)	27.4 (4.6)	27.4 (4.1)	27.6 (4.0)
Current smoker, %	9.7	6.6	10.7	20.4	16.4	19.0	11.1	13.4
Former smoker, %	42.2	49.0	56.2	32.9	26.8	47.0	40.7	51.7
Pack-years smoking*	24.5 (22.0)	36.0 (32.4)	25.8 (23.0)	21.5 (23.0)	20.1 (19.6)	18.5 (18.0)	34.3 (23.1)	23.8 (22.9)
FEV_1/FVC								
Affected	49.5 (18.1)	56.3 (6.1)	56.0 (7.2)	59.2 (7.3)	58.2 (9.8)	59.2 (8.4)	54.6 (8.6)	56.7 (6.9)
Unaffected	75.6 (7.2)	76.0 (5.1)	75.6 (5.4)	76.8 (5.0)	78.4 (5.4)	80.2 (5.8)	80.4 (6.7)	78.2 (5.9)
FEV_1 % predicted								
Affected	48.9 (20.2)	55.3 (11.6)	56.9 (11.0)	67.6 (10.4)	57.3 (16)	60.4 (13.3)	50.8 (10)	58.8 (10.5)
Unaffected	93.2 (18.3)	100.2 (14.0)	102.8 (18.7)	103.5 (11.3)	98.9 (11.7)	104.6 (13.1)	100.4 (12.2)	105.5 (17.3)

Definition of abbreviations: AGES = Age, Gene, Environment Susceptibility; BHS = Bussleton Health Study; Health ABC = Health, Aging and Body Composition; LBC1936 = Lothian Birth Cohort; RS1 = Rotterdam Study I; RS2 = Rotterdam Study II; RS3 = Rotterdam Study III; SAPALDIA = Swiss Study on Air Pollution and Lung and Heart Disease in Adults.

Data are presented as mean (SD) unless otherwise indicated.

* Pack-years calculated among current and former smokers.

The genomic control (λ_{GC}) values ranged from 0.946 to 1.045 for each cohort's GWAS and from 1.011 to 1.060 in the meta-analysis (Table E2). Figures E1 to E5 present the Manhattan and quantile-quantile (QQ) plots for the five discovery meta-analyses.

Discovery Meta-analyses

One region on chromosome 15 had 11 SNPs with genome-wide significant results (P values $< 5 \times 10^{-8}$) in discovery meta-analysis of ever smokers (Table 4). An SNP in the *AGPHD1* gene between the *IREB2* gene and *CHRN* gene cluster was the top association with airflow obstruction among ever smokers (rs8031948, P value $= 2.8 \times 10^{-9}$) with the minor allele conferring a 22% higher risk of airflow obstruction. Among 14 cohorts with both smoking and never-smoking participants, the top SNP results for all subjects combined were found in the same chromosome 15 region but localized to the *CHRNA5* gene (rs17486278, P value $= 1.9 \times 10^{-7}$). For comparison, results among never smokers (504 affected, 10,690 unaffected from eight cohorts) are included in Table 4, and the smallest P value in the region (8.4×10^{-5}) occurs at a synonymous SNP (rs1051730) in *CHRNA3*. The odds ratios (OR) shown in Table 4 demonstrate consistency in the effect size for the tested allele across the analyses of all cohorts with both ever and never smoking participants (14 cohorts), ever smokers (15 cohorts), and never smokers (8 cohorts). The results in Table 4 were based on meta-analyses

TABLE 3. SAMPLE SIZES CONTRIBUTED BY EACH COHORT FOR THE FIVE DISCOVERY META-ANALYSES OF AIRFLOW OBSTRUCTION

	All Participants		Ever Smokers		Never Smokers		Asthma-Free*		$FEV_1 < 65\%$†	
	Affected	Unaffected	Affected	Unaffected	Affected	Unaffected	Affected	Unaffected	Affected	Unaffected
ARIC	914	6,602	821	3,510	93	3,092	814	6,355	452	6,602
FHS	571	5,866	457	2,909	114	2,957	391	5,210	274	5,866
CHS	402	2,183	317	950	85	1,233	363	2,135	292	2,183
RS1	99	1,003	87	650	12‡	353‡	97§	967§	68	1,003
RS2	40	668	37	424	3‡	244‡	NA	NA	29	668
RS3	70	1,001	57	650	13‡	351‡	NA	NA	39	1,001
Health ABC	108	1,129	94	593	14‡	536‡	70	1077	80	1,129
AGES	109	1,562	81	787	28	775	NA	NA	34	1,562
EPIC	127	1,023	79	490	48	533	110	992	88	1,023
BHS	89	661	46	278	43	383	20	421	53	661
SAPALDIA	98	833	59	437	39	396	42	620	38	833
LBC1936	61	627	50	306	11‡	321‡	60	622	56	627
B58C	264	4,374	210	3,053	54	1,321	183	4,036	75	4,374
COPACETIC	0	0	312	996	NA	NA	NA	NA	142	996
MESA	104	979	89	533	15‡	531‡	85	923	54	979
Total	3,056	28,511	2,796	16,566	504	10,690	2,138	22,391	1,774	29,507

Definition of abbreviations: AGES = Age, Gene, Environment Susceptibility; ARIC = Atherosclerosis Risk in Communities; B58C = British 1958 Birth Cohort; BHS = Bussleton Health Study; CHS = Cardiovascular Health Study; COPACETIC = COPD Pathology: Addressing Critical gaps, Early Treatment and diagnosis, and Innovative Concepts; EPIC = European Prospective Investigation into Cancer and Nutrition; FHS = Framingham Heart Study; Health ABC = Health, Aging and Body Composition; LBC1936 = Lothian Birth Cohort; MESA = Multi-Ethnic Study of Atherosclerosis; RS1 = Rotterdam Study I; RS2 = Rotterdam Study II; RS3 = Rotterdam Study III; SAPALDIA = Swiss Study on Air Pollution and Lung and Heart Disease in Adults.

* Asthma-free: no history of an asthma diagnosis before age 40 years; participants reporting asthma with missing data on age at diagnosis were also excluded.

† $FEV_1 < 65\%$: cases were restricted to those with $FEV_1 < 65\%$ and FEV_1/FVC less than the lower limit of normal; the definition of control subjects was the same as used for all participants.

‡ Not analyzed due to low number of cases.

§ Results were not available for discovery meta-analyses.

TABLE 4. GENOME-WIDE SIGNIFICANT RESULTS ON CHROMOSOME 15 FROM DISCOVERY META-ANALYSIS OF AIRFLOW OBSTRUCTION

						All*		Never Smokers		Ever Smokers	
				Coded Allele		14 Cohorts		8 Cohorts		15 Cohorts	
SNP	Position	Gene	Function	Allele	Frequency	OR	*P* Value	OR	*P* Value	OR	*P* Value
rs8031948	76603112	*AGPHD1*	Intronic	t	0.35	1.17	4.76×10^{-7}	1.15	1.53×10^{-3}	1.22	2.78×10^{-9}
rs931794	76613235	*AGPHD1*	Intronic	a	0.65	0.86	6.18×10^{-7}	0.87	1.46×10^{-3}	0.82	4.69×10^{-9}
rs10519203	76601101	*AGPHD1*	Intronic	a	0.65	0.86	7.16×10^{-7}	0.86	1.27×10^{-3}	0.82	5.67×10^{-9}
rs9788721	76589924	*AGPHD1*	Intronic	t	0.65	0.86	1.04×10^{-6}	0.86	1.17×10^{-3}	0.82	9.76×10^{-9}
rs2036527	76638670			a	0.34	1.18	3.51×10^{-7}	1.18	2.85×10^{-4}	1.22	1.72×10^{-8}
rs17486278	76654537	*CHRNA5*	Intronic	a	0.66	0.85	1.91×10^{-7}	0.84	1.06×10^{-4}	0.83	2.43×10^{-8}
rs7180002	76661048	*CHRNA5*	Intronic	a	0.66	0.85	2.23×10^{-7}	0.84	1.20×10^{-4}	0.83	2.68×10^{-8}
rs1051730	76681394	*CHRNA3*	Synonymous	a	0.33	1.17	3.29×10^{-7}	1.20	8.36×10^{-5}	1.21	3.36×10^{-8}
rs16969968	76669980	*CHRNA5*	Missense	a	0.34	1.17	3.46×10^{-7}	1.19	1.25×10^{-4}	1.20	3.47×10^{-8}
rs951266	76665596	*CHRNA5*	Intronic	a	0.34	1.17	3.64×10^{-7}	1.19	1.41×10^{-4}	1.21	3.47×10^{-8}
rs1317286	76683184	*CHRNA3*	Intronic	a	0.66	0.85	3.93×10^{-7}	0.84	1.18×10^{-4}	0.83	4.74×10^{-8}

Definition of abbreviation: OR = odds ratio.

*All cohorts with both ever and never smoking participants.

that included different cohorts as presented in Table 3, and thus the ever and never smoker results do not reflect a straightforward stratified analysis of all participants. The inclusion of the COPACETIC study in the ever smokers meta-analysis contributed to the improved association signal in the region. In the GWAS of a more severe airflow obstruction phenotype defined by FEV_1 less than 65% predicted, a missense SNP in the *CHRNA5* gene (rs16969968, Asp398Asn) had the third ranked *P* value (5×10^{-7}) with an OR of 1.22.

No other genome-wide significant associations were identified among discovery meta-analyses. In the meta-analysis of never smokers (504 affected and 10,690 control subjects), several top SNPs were observed 570 kb away from *ADARB2* (the closest gene), and results from the FEV_1 less than 65% meta-analysis also implicated this locus. Among never smokers, the chromosome 6 major histocompatability locus (MHC) region was among top results. The discovery meta-analysis results for the 60 SNPs selected for replication are included in Table 5. Genome-wide results for all five definitions of airflow obstruction are available in the online supplement.

Meta-analysis of Chromosome 6 and 15 Regions with Replication Studies

Regional meta-analyses were performed to further evaluate the regions on chromosomes 6 and 15 with the additional two replication studies. In discovery analysis of all airflow obstruction, the chromosome 6 MHC locus at 6p21.33 was among the top results (smallest *P* value = 6.8×10^{-7}, rs3094013). The closest gene to the top SNP was HLA complex P6 (*HCP5*), although the extensive linkage disequilibrium in the region makes interpretation difficult. When discovery results were meta-analyzed with the replication studies, the previous associations were attenuated. The top SNP from the meta-analysis of discovery and replication results had an OR of 1.13 for the common allele (66%) and a *P* value of 6.03×10^{-6} near the *HLA-A* gene. Thirty-six SNPs in chromosome 6 with combined meta-analysis *P* values less than 1×10^{-4} are provided in Table E3.

On chromosome 15, after meta-analysis of airflow obstruction in ever smokers from discovery populations with replication studies, the order of the top hits was generally unchanged and *P* values improved, reaching 2.6×10^{-15}. The COPD case-control studies meta-analysis included only ever smokers, so the FamHS served as a sole replication study for the never smoker regional results. Figure 1 depicts the chromosome 15 regional association of the meta-analysis of combined discovery and replication cohorts for the separate groups of ever smokers (A) and never smokers (B), created using LocusZoom (25). Figure 2 is a forest plot presenting the study-specific results among never smokers that demonstrates similar effect sizes across the cohorts.

Replication of Top 60 SNPs and Combined Meta-analysis

Table 5 presents the 60 SNPs selected for replication studies (not including the chromosome 6 and 15 SNPs included in the regional meta-analyses). A *P* value of 8×10^{-4}, representing Bonferroni correction for 60 tests at the $\alpha = 0.05$ level, was selected *a priori* as the threshold for statistically significant replication. No SNPs achieved the replication criterion. In a meta-analysis combining the discovery results with the FamHS, one SNP achieved genome-wide statistical significance (rs7733088 in *HTR4*) with a 38% frequent minor allele conferring an OR of 0.81 ($P = 4.09 \times 10^{-9}$). Of the top 60 SNPs, four had nominal association (*P* values < 0.05) in the COPD meta-analysis and a consistent risk allele; these SNPs were located in *ADAM19*, *RARB*, *PPAP2B*, and *ADAMTS19* (Table 6).

Association of Spirometry-associated SNPs with Airflow Obstruction

Previous meta-analyses in the CHARGE and SpiroMeta consortia (3–5) identified 75 SNPs associated with either FEV_1 or FEV_1/FVC at genome-wide significance (*P* value $\leq 5 \times 10^{-8}$). We examined the association *P* values for airflow obstruction for these 75 SNPs in the meta-analysis results from all subjects and from ever smokers. Association for these 75 SNPs represents 58 independent tests using a multiple-testing correction that incorporates the linkage disequilibrium structure derived from HapMap European (CEU) samples (26). Accordingly, we considered a *P* value of 8.6×10^{-4} as the criterion for statistically significant association with airflow obstruction (Bonferroni correction for 58 tests at the $\alpha = 0.05$ level) given the *a priori* association with spirometry. Among all participants, SNPs in *RARB*, *GPR126*, *HTR4*, *C10orf11*, near *HHIP*, and near *HLA-DRA* were statistically significantly associated with airflow obstruction. Among smokers, *HTR4*, *RARB*, *GPR126*, and *THSD4* were associated with airflow obstruction. Results for the 75 SNPs are presented in Tables E4 and E5. When only cohorts that did not contribute to the published spirometry findings (3–5) were considered (RS3, SAPALDIA, LBC1936, MESA, and COPACETIC) as an independent sample, power was reduced,

TABLE 5. ODDS RATIOS AND *P* VALUES FOR THE 60 SINGLE-NUCLEOTIDE POLYMORPHISMS IDENTIFIED IN THE DISCOVERY META-ANALYSIS AND SELECTED FOR REPLICATION AND COMBINED META-ANALYSIS WITH THE FAMILY HEART STUDY

	Coded Allele					Discovery Meta-analysis			Family Heart Study*		Combined Meta-analysis	
SNP	Allele	Freq	Chr	Position	Closest Gene	OR	*P* Value	Analysis	OR	*P* Value	OR	*P* Value
rs7733088	A	0.38	5	147836526	*HTR4*	0.82	6.53×10^{-8}	Smoker	0.75	0.015	0.81	4.09×10^{-9}
rs2044029	A	0.4	15	69467013	*THSD4*	1.16	4.95×10^{-7}	All	1.20	0.078	1.17	1.06×10^{-7}
rs181654	A	0.28	10	119369646	*EMX2*	0.82	1.24×10^{-6}	No asthma	0.76	0.030	0.81	1.31×10^{-7}
rs4597955	A	0.59	5	147827466	*HTR4*	0.84	3.12×10^{-6}	Smoker	0.76	0.011	0.83	1.57×10^{-7}
rs12905014	T	0.95	15	90684844	*ST8SIA2*	0.58	5.24×10^{-6}	FEV_1 65%	0.47	0.020	0.57	3.83×10^{-7}
rs11744671	T	0.92	5	156853809	*ADAM19*	0.72	8.25×10^{-7}	No asthma	0.77	0.264	0.72	4.51×10^{-7}
rs8033889	T	0.21	15	69467134	*THSD4*	1.18	2.49×10^{-6}	All	1.23	0.066	1.19	4.67×10^{-7}
rs6684428	A	0.16	1	56132401	*PPAP2B*	1.24	3.60×10^{-7}	Smoker	1.08	0.574	1.23	4.73×10^{-7}
rs4767234	A	0.59	12	113122231	*TBX5*	1.18	1.28×10^{-6}	Smoker	1.15	0.242	1.17	6.32×10^{-7}
rs4534959	A	0.97	18	60028136	*SERPINB8*	0.57	4.19×10^{-7}	FEV_1 65%	0.91	0.844	0.58	6.90×10^{-7}
rs715921	A	0.31	13	23693489	*SPATA13*	1.22	5.18×10^{-7}	No asthma	1.07	0.551	1.20	7.60×10^{-7}
rs16889038	T	0.92	6	24414366	*DCDC2*	0.7	4.15×10^{-7}	FEV_1 65%	0.95	0.845	0.72	7.94×10^{-7}
rs9536318	A	0.83	13	52392695	*PCDH8*	0.81	5.47×10^{-6}	No asthma	0.75	0.051	0.80	8.24×10^{-7}
rs1997352	A	0.26	3	25513321	*RARB*	0.85	4.29×10^{-6}	All	0.82	0.076	0.84	8.64×10^{-7}
rs10759102	A	0.33	9	9900123	*PTPRD*	0.81	4.66×10^{-6}	FEV_1 65%	0.77	0.083	0.81	1.04×10^{-6}
rs13144621	T	0.32	4	109437378	*LEF1*	0.85	5.87×10^{-7}	All	0.98	0.850	0.86	1.20×10^{-6}
rs1982234	C	0.63	15	69478345	*THSD4*	1.16	4.80×10^{-6}	All	1.16	0.136	1.16	1.55×10^{-6}
rs7799265	C	0.95	7	28399001	*CREB5*	0.63	8.84×10^{-7}	FEV_1 65%	0.91	0.742	0.65	1.71×10^{-6}
rs181652	A	0.54	10	119369077	*EMX2*	1.18	8.12×10^{-6}	No asthma	1.19	0.097	1.18	1.94×10^{-6}
rs11766496	C	0.12	7	71026786	*CALN1*	1.34	3.90×10^{-7}	All	0.97	0.855	1.30	2.01×10^{-6}
rs2263638	A	0.37	10	94158777	*IDE*	0.78	4.68×10^{-6}	Never smoker	0.80	0.275	0.78	2.53×10^{-6}
rs7850092	A	0.21	9	9899119	*PTPRD*	0.79	4.00×10^{-6}	FEV_1 65%	0.85	0.324	0.79	2.60×10^{-6}
rs1329705	A	0.2	6	142795031	*GPR126*	0.79	3.75×10^{-6}	FEV_1 65%	0.85	0.342	0.79	2.63×10^{-6}
rs11209261	A	0.76	1	68557801	*GPR177*	0.81	6.76×10^{-6}	FEV_1 65%	0.80	0.191	0.81	2.78×10^{-6}
rs7607316	A	0.21	2	237186581	*CXCR7*	1.28	9.19×10^{-6}	Never smoker	1.35	0.152	1.29	3.21×10^{-6}
rs9975851	T	0.57	21	26638525	*CYYR1*	1.18	4.92×10^{-6}	No asthma	1.11	0.332	1.17	3.66×10^{-6}
rs12505749	C	0.92	4	57028869	*SRP72*	0.76	3.06×10^{-7}	All	1.22	0.258	0.79	4.69×10^{-6}
rs1207393	C	0.36	22	24983362	*SEZ6L*	0.83	8.69×10^{-6}	FEV_1 65%	0.85	0.283	0.84	4.78×10^{-6}
rs12744110	T	0.25	1	56168897	*PPAP2B*	1.18	4.51×10^{-6}	Smoker	1.06	0.681	1.17	5.95×10^{-6}
rs11097912	T	0.33	4	107219911	*MGC16169*	0.85	6.04×10^{-6}	Smoker	0.92	0.497	0.85	5.95×10^{-6}
rs17086172	T	0.94	18	68378001	*CBLN2*	0.73	7.13×10^{-6}	No asthma	0.86	0.492	0.74	7.23×10^{-6}
rs2322734	A	0.96	3	4608492	*ITPR1*	0.67	7.19×10^{-6}	FEV_1 65%	0.83	0.518	0.69	7.52×10^{-6}
rs8036030	A	0.39	15	72503662	*SEMA7A*	0.84	5.71×10^{-6}	No asthma	0.95	0.653	0.85	8.31×10^{-6}
rs892961	A	0.41	17	72911695	*SEPT9*	0.85	8.00×10^{-6}	No asthma	0.93	0.479	0.85	8.91×10^{-6}
rs7629245	T	0.15	3	186624551	*MAP3K13*	1.23	0.00001	Smoker	1.12	0.456	1.22	9.06×10^{-6}
rs2830165	T	0.59	21	26598463	*APP*	0.81	5.27×10^{-6}	Never smoker	0.98	0.892	0.82	9.55×10^{-6}
rs7686928	T	0.14	4	188970823	*ZFP42*	1.21	8.77×10^{-6}	Smoker	1.08	0.630	1.21	9.65×10^{-6}
rs9632471	C	0.72	5	128761894	*ADAMTS19*	0.76	5.54×10^{-6}	FEV_1 65%	0.95	0.776	0.77	1.07×10^{-5}
rs4837614	T	0.15	9	118350186	*ASTN2*	0.76	4.56×10^{-6}	FEV_1 65%	0.98	0.911	0.78	1.20×10^{-5}
rs12960805	A	0.41	18	7909707	*PTPRM*	1.27	6.22×10^{-6}	Never smoker	1.00	0.995	1.25	1.27×10^{-5}
rs1799257	A	0.12	19	53664351	*PSCD2*	1.37	2.08×10^{-6}	FEV_1 65%	0.78	0.337	1.33	1.38×10^{-5}
rs1868466	A	0.79	16	76301059	*KIAA1576*	0.85	5.45×10^{-6}	All	1.00	0.997	0.86	1.39×10^{-5}
rs10518948	C	0.93	15	69415023	*THSD4*	0.68	9.91×10^{-6}	Never smoker	0.97	0.931	0.69	1.51×10^{-5}
rs6901575	A	0.1	6	28358963	*PGBD1*	1.24	6.76×10^{-6}	All	0.97	0.853	1.22	1.55×10^{-5}
rs3790728	T	0.97	1	215737150	*GPATCH2*	0.57	3.12×10^{-6}	FEV_1 65%	4.24	0.060	0.60	1.58×10^{-5}
rs9511117	A	0.09	13	23660045	*SPATA13*	0.73	6.78×10^{-6}	Smoker	1.01	0.979	0.75	1.65×10^{-5}
rs4957070	A	0.63	5	600858	*SLC9A3*	0.85	9.09×10^{-6}	Smoker	0.98	0.874	0.86	1.65×10^{-5}
rs3814818	T	0.9	14	94263130	*GSC*	0.74	2.51×10^{-6}	FEV_1 65%	1.24	0.357	0.77	1.68×10^{-5}
rs1895493	C	0.09	16	78122404	*MAF*	1.41	5.51×10^{-6}	Never smoker	0.77	0.460	1.37	1.72×10^{-5}
rs12872078	A	0.91	13	64002324	*PCDH9*	1.43	4.81×10^{-6}	FEV_1 65%	0.96	0.876	1.37	1.75×10^{-5}
rs764593	T	0.11	3	3687236	*LRRN1*	0.76	3.89×10^{-6}	Smoker	1.11	0.563	0.79	2.64×10^{-5}
rs1408298	T	0.7	6	17199273	*RBM24*	0.81	8.19×10^{-6}	No asthma	1.00	0.972	0.83	2.95×10^{-5}
rs2164220	T	0.08	7	157447986	*PTPRN2*	1.42	9.80×10^{-6}	Never smoker	0.61	0.266	1.38	3.14×10^{-5}
rs10496694	A	0.09	2	133252637	*NAP5*	1.31	7.42×10^{-6}	Smoker	0.86	0.512	1.27	3.30×10^{-5}
rs11023434	C	0.23	11	15083724	*INSC*	1.33	7.78×10^{-6}	Never smoker	0.95	0.766	1.28	3.70×10^{-5}
rs1567398	T	0.43	8	8764214	*MFHAS1*	1.2	8.20×10^{-6}	FEV_1 65%	0.91	0.519	1.17	3.98×10^{-5}
rs1125729	T	0.81	8	93427586	*RUNX1T1*	0.8	1.63×10^{-6}	FEV_1 65%	1.59	0.014	0.83	4.89×10^{-5}
rs7163331	A	0.04	15	96260720	*ARRDC4*	1.55	6.81×10^{-6}	FEV_1 65%	0.56	0.132	1.46	6.34×10^{-5}
rs12265908	A	0.97	10	2339319	*ADARB2*	0.59	6.92×10^{-6}	FEV_1 65%	1.81	0.157	0.64	7.54×10^{-5}
rs7719062	T	0.08	5	1222044	*SLC6A19*	1.42	9.79×10^{-6}	Smoker	0.81	0.393	1.34	7.71×10^{-5}

Definition of abbreviations: Chr = chromosome; Freq = frequency; OR = odds ratio; SNP = single-nucleotide polymorphism.

SNPs are ordered by the combined meta-analysis *P* value.

* Family Heart Study results are generated from phenotypes consistent with the discovery analysis indicated, with affected/unaffected sample sizes: 331/2,550 all, 248/1,003 smoker, 83/1,547 never smoker, 266/2,350 no asthma, 155/2,550 FEV_1 65%.

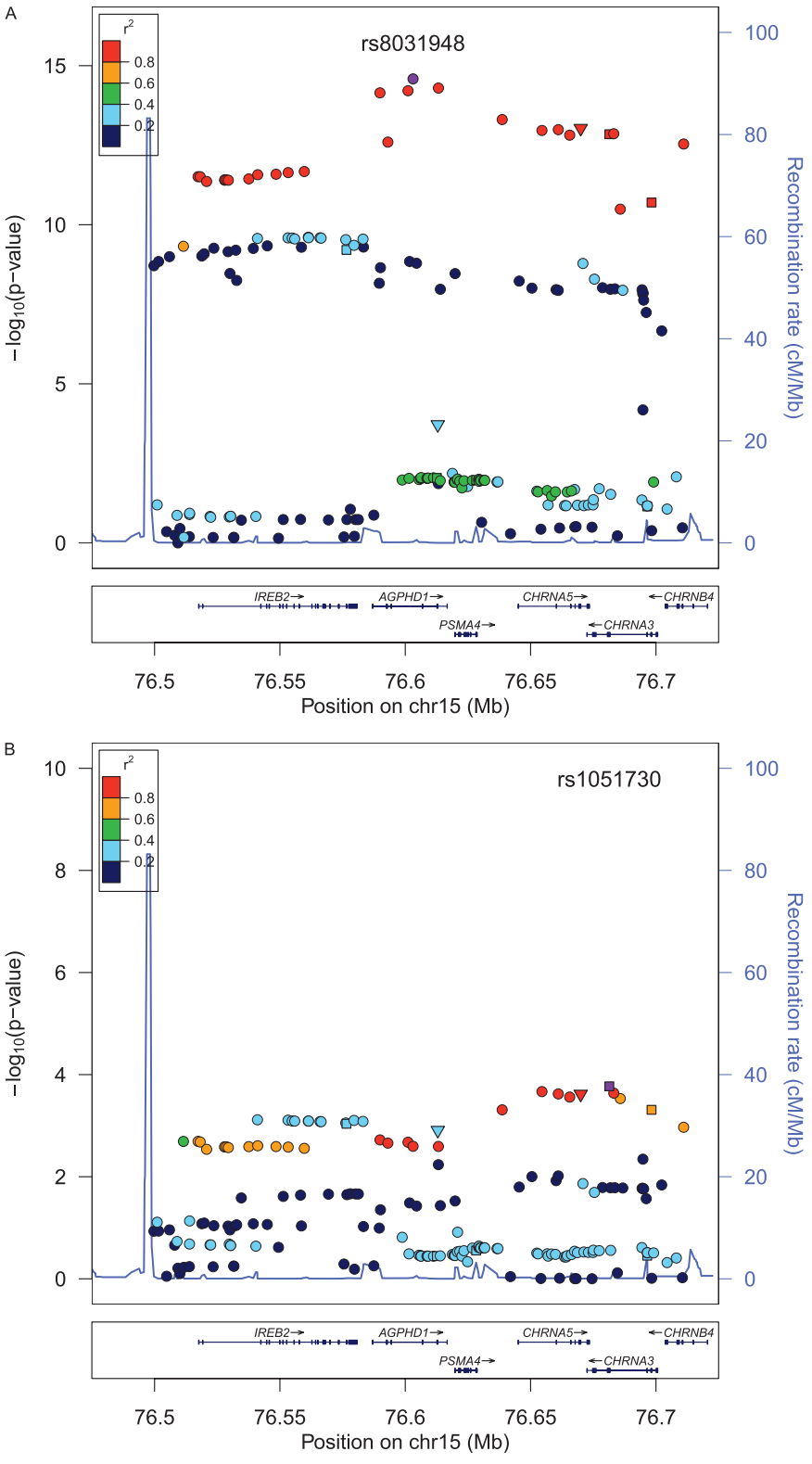

Figure 1. Regional association plot for chromosome 15 presenting results from combined meta-analysis of discovery and replication studies. X-axis is megabase (Mb) position. Y-axis is negative log of the *P* values. Linkage disequilibrium to the named single-nucleotide polymorphism (SNP) (*purple*) is depicted by degree of color according to the legend. Nonsynonymous SNPs are depicted by an *inverted triangle* and other coding SNPs by a *square*. (*A*) Ever smokers. (*B*) Never smokers.

and only the *ADAM19* SNP in smokers achieved the Bonferroni cutoff for significance (Table E6).

Gene Expression Results

Expression of *CHRNA3* and *CHRNA5* was evaluated in cDNA from human whole lung, peripheral blood mononuclear cells, and primary cultures of bronchial epithelial cells and airway myocytes, together with control tissues (kidney, brain, and placenta: see online supplement for methods). Both genes were expressed in all lung-derived tissues examined. Within the lung, expression of both *CHRNA3* and *CHRNA5* appeared strongest in airway myocytes and epithelial cells. The identity of reverse transcriptase–polymerase chain reaction products was confirmed by direct sequencing of bands of the relevant size from at least one tissue type for each gene.

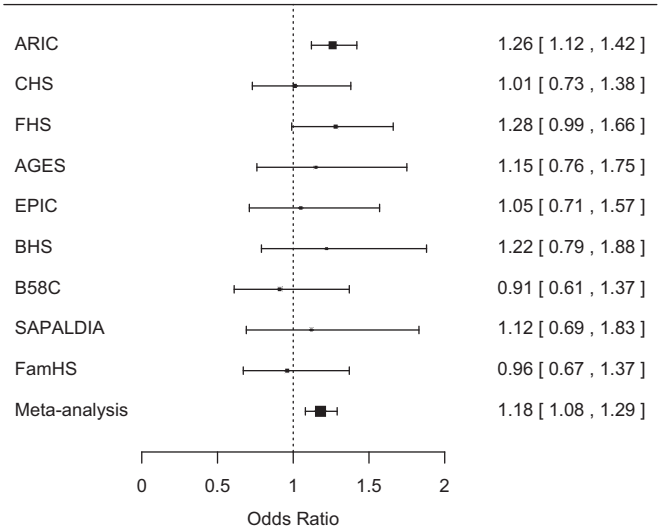

Figure 2. Forest plot depicting the association results for rs1051730 (*CHRNA3*) and airflow obstruction among never smokers in each cohort and the meta-analysis. AGES = Age, Gene, Environment Susceptibility; ARIC = Atherosclerosis Risk in Communities; B58C = British 1958 Birth Cohort; BHS = Bussleton Health Study; CHS = Cardiovascular Health Study; EPIC = European Prospective Investigation into Cancer and Nutrition; FamHS = Family Heart Study; FHS = Framingham Heart Study; SAPALDIA = Swiss Study on Air Pollution and Lung and Heart Disease in Adults.

DISCUSSION

These meta-analyses included 32,875 participants from population-based studies for discovery of loci associated with airflow obstruction. In addition, we attempted to replicate results in 2,881 participants from a population-based family study and a meta-analysis of 5,421 participants from case-control studies of clinically ascertained COPD. The present study confirms the previously identified (7) association between the chromosome 15q25 region and airflow obstruction among smokers. Although the number of participants with airflow obstruction among never smokers was low (504 affected), and statistical power is therefore limited, analyses of airflow obstruction among never smokers also showed a nominal association to the Asp398Asn missense SNP in *CHRNA5* and to a synonymous SNP in *CHRNA3*.

Results from gene expression studies demonstrated that both *CHRNA5* and *CHRNA3* were expressed in whole lung, airway smooth muscle, and bronchial epithelial cells. In a publication reporting *CHRNA5* gene expression in normal lung tissue samples, the Asp398Asn genotype was strongly related to mRNA levels, with homozygosity of the risk allele (A) associated with 2.5-fold lower mRNA levels compared with homozygosity for the G allele (27). A similar pattern was observed for rs1051730 in the sputum of COPD cases, in which the minor allele was associated with lower expression of *CHRNA5* (28). The correlation between associated SNP genotypes and *CHRNA5* expression levels in the lung and sputum combined with our finding of increased risk for airflow obstruction in never smokers suggests that the variants in this region may have an influence on risk of airflow obstruction that is not simply mediated by an influence on nicotine dependence. Supporting a direct influence of variants in this region on lung phenotypes, a *CHRNA3/5* variant was recently found to be associated with bronchial hyperresponsiveness in children not exposed to cigarette smoke (29). Silencing *CHRNA5* in bronchial epithelial cells was found to reduce expression of adhesion molecules, thereby increasing cell motility, which may influence the repair and remodeling processes that lead to COPD (30). Our results suggest that the A allele of rs16969968 confers as much as a 20% increased odds of airflow obstruction, and based on the prior report, this increased risk may be mediated by lower mRNA levels in lung tissue (27).

In addition to the chromosome 15q region, SNPs in *HTR4* met genome-wide statistical significance in ever smokers. The *HTR4* (5-hydroxytryptamine [serotonin] receptor 4) gene was originally identified with association to FEV_1/FVC in CHARGE (3) and SpiroMeta (4), and subsequently showed a statistically significant association with COPD in a targeted gene analysis of six loci in the SpiroMeta cohorts (31). Serotoninergic receptors have been demonstrated to regulate cytokine and chemokine release in human airway epithelial cells and have been implicated in the pathogenesis of asthma (32). The reduced risk of airflow obstruction was strongest when limited to ever smokers, suggesting that variation in *HTR4* may contribute to the inflammatory response to cigarette smoke.

Several genes represented among the top SNP results were nominally replicated in the COPD case-control meta-analysis (*ADAM19*, *RARB*, *PPAP2B*, and *ADAMTS19*). Of them, both *ADAM19* and *RARB* have been previously implicated in GWAS of lung function as measured by spirometry (3–5). *ADAM19* (a disintegrin and metalloprotease domain 19) was originally shown to be associated with FEV_1/FVC in the CHARGE GWAS (3), and these SNPs were subsequently reported to be associated with COPD in a case-control study (33). Here, we demonstrate that *ADAM19* is associated with airflow obstruction in population-based cohort studies. *ADAM19* is expressed in bronchial epithelial cells, bronchial smooth muscle, and interstitial inflammatory cells and may have a role in immune defense and the inflammatory process (34). *ADAMTS19* (a disintegrin and metalloproteinase with thrombospondin motifs 19) has several of the same domains and has been shown to be expressed in fetal lung (35). *PPAP2B* is a lipid phosphate phosphohydrolase, which are generally believed to influence surfactant secretion and have a role in lung injury and repair (36).

TABLE 6. FOUR SINGLE-NUCLEOTIDE POLYMORPHISMS WITH NOMINAL ASSOCIATION TO CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND CONSISTENT RISK ALLELE OUT OF 60 SINGLE-NUCLEOTIDE POLYMORPHISMS SELECTED FOR REPLICATION FROM AIRFLOW OBSTRUCTION DISCOVERY GENOME-WIDE ASSOCIATION STUDIES

	Coded Allele					Airflow Obstruction Meta-analysis			COPD Meta-analysis	
SNP	Allele	Freq	Chr	Position	Closest Gene	Analysis	OR	*P* Value	OR	*P* Value
rs11744671	T	0.92	5	156853809	*ADAM19*	No asthma	0.72	4.51×10^{-7}	0.8	0.027
rs1997352	A	0.26	3	25513321	*RARB*	All	0.84	8.64×10^{-7}	0.88	0.038
rs12744110	T	0.25	1	56168897	*PPAP2B*	Smoker	1.17	5.95×10^{-6}	1.13	0.045
rs9632471	C	0.72	5	128761894	*ADAMTS19*	FEV_1 65%	0.77	1.07×10^{-5}	0.86	0.045

Definition of abbreviations: Chr = chromosome; COPD = chronic obstructive pulmonary disease; Freq = frequency; OR = odds ratio; SNP = single-nucleotide polymorphism.

RARB (retinoic acid receptor β) was recently demonstrated to be associated with lung function measures at genome-wide significance in the combined CHARGE and SpiroMeta meta-analysis (5). Retinoic acid (RA) has been evaluated as a potential therapeutic agent for emphysema after results in rats demonstrated reversibility of experimentally induced emphysema with administration of RA (37); however, subsequent studies in animal models had conflicting results (38), and a small feasibility study of RA for the treatment of emphysema did not show significant improvement in lung function (39). The finding that *RARB* minor alleles were associated with lower risk of airflow obstruction may provide insight into which patients may benefit from RA therapy or suggest modifying the design of RA therapeutics to target the β receptor.

The *HHIP* region was associated with airflow obstruction in our look-up replication of spirometry-associated SNPs, which was expected given the prior findings of association with COPD in earlier GWAS (7, 9) and further replication in targeted studies of *HHIP* and COPD (8). This region of chromosome 4q31 including SNPs in *HHIP* and *GYPA* has also been shown to be associated with lung cancer (40). Recently, a COPD risk haplotype upstream of *HHIP* was identified to be associated with reductions in *HHIP* promoter activity (41). Our meta-analysis is able to confirm that rs6537296 is associated with airflow obstruction ($P = 3.2 \times 10^{-4}$), but the other SNP in the haplotype (rs1542725) was not studied. Also, previously identified SNPs in *GPR126*, *THSD4*, and near *HLA-DRA* were associated with airflow obstruction, and *GPR126* demonstrated a nominal association with COPD in a prior report focusing on clinically ascertained cases and control subjects (33). It should be noted that the look-up replication that supports the relation of these genes with airflow obstruction is not statistically independent from the original meta-analyses of spirometry traits because of overlap between the samples. When only the cohorts not included in the earlier published meta-analyses (3–5) were analyzed separately, in this reduced sample size (567 affected, 2,922 unaffected) only the *ADAM19* gene achieved the cutoff criterion for significant association with airflow obstruction.

The chromosome 6 region identified in discovery meta-analysis did not replicate when additional studies were included in the meta-analysis. The regional meta-analysis results demonstrated modest association (P values $< 1 \times 10^{-4}$) across five megabases in the HLA region, including 17 SNPs in the histone gene cluster at 27.9 Mb. Our results are not able to clarify which gene or combination of genes may give rise to the underlying association signal given the extensive linkage disequilibrium in the MHC. Recently, a meta-analysis of the COPD case-control cohorts that served as replication cohorts in our study implicated a locus on chromosome 19q13 (24) as a COPD susceptibility locus; however, the rs7937 SNP identified is not replicated in the discovery meta-analyses described here (P values ranged from 0.12 among never smokers to 0.87 among ever smokers).

Our study has several limitations. Our cohorts had only pre-bronchodilator spirometry, and thus we could not examine the formal definition of COPD. Our main analysis used a definition of airflow obstruction that includes persons with very mild ventilatory impairment, and the participants who meet this definition may not all have COPD. Our definition of more severe airflow obstruction is likely to be more comparable to clinically ascertained COPD in the replication studies, but the numbers of affected participants were reduced. In addition, our ability to address asthma in the context of airflow obstruction was limited to a subset of cohorts with self-reported asthma diagnoses. Last, as our study was limited to white participants of European descent, the generalizability of these findings to other ethnic groups is unknown.

In summary, we performed meta-analyses and replication studies using data from more than 40,000 study participants of European ancestry to identify genetic loci influencing airflow obstruction as a categorical disease phenotype. We identified the *CHRNA3/5* genes and *HTR4* at genome-wide significance, and several genes that were implicated by previous GWAS of single spirometry measures as quantitative phenotypes (*ADAM19*, *RARB*) were among top results. Here we show, for the first time, that a *CHRNA5* missense SNP is associated with airflow obstruction in never smokers, suggesting a main effect on risk of airflow obstruction that is independent of the influence mediated through effects on smoking habits. This was supported by gene expression findings demonstrating the *CHRNA3/5* genes in relevant lung and airway tissues. Thus, *CHRNA3/5* variants may mediate airflow obstruction in both ever and never smokers.

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