# A Genome-Wide Association Study of Spirometric Measures in the COPDGene Study

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# Abstract

## Rationale

Measures of pulmonary function are highly heritable and define chronic obstructive pulmonary disease (COPD). We conducted a genome-wide association analysis (GWA) for spirometry measures in the COPDGene study, a multicenter study of current and former smokers. We also conducted a GWA meta-analysis of lung function in the COPDGene, ECLIPSE, and GenKOLS studies.

## Methods

We performed a genome-wide association study in 9,919 COPDGene participants (6,659 non-Hispanic Whites [NHW] and 3,260 African Americans [AA]) to identify single nucleotide polymorphisms (SNPs) associated with FEV1, FEV1 percent of predicted and FEV1/FVC ratio. In addition, we examined these pulmonary function phenotypes in COPD cases only (2,820 NHW and 821 AA). All analyses were run separately for NHW and AA controlling for age, gender, height, pack-years of smoking and genetic ancestry using principal components. We also conducted a GWA meta-analysis of FEV1, FEV1 percent of predicted and FEV1/FVC ratio in the COPDGene, ECLIPSE, and GenKOLS cohorts.

## Results

In NHW, all three measures of pulmonary function were significantly associated with several SNPs in one genomic region on chromosome 15 [CHRNA3, CHRNA5, AGPHD1, IREB2, CHRNB4], 2 genomic regions on chromosome 4 [FAM13A, HHIP] and several SNPs in gene EEFSEC on chromosome 3. There were no genome wide significant SNPs associated with pulmonary function in the NHW COPD cases only. In AA, FEV1 and FEV1 percent of predicted were associated with SNPs on chromosome 22 in MGAT3 and FEV1/FVC was associated with 4 SNPs on chromosome 12 in FAM19A2. In AA COPD cases, FEV1 and FEV1 percent of predicted were associated with a SNP on chromosome 19 in TSHZ3 and a SNP on chromosome 2 in SNTG2. In AA COPD cases, FEV1/FVC was associated with 3 SNPs on chromosome 6 in BTBD9 and 3 SNPs on chromosome 16 in PRKCB.

## Conclusions

We confirmed genome wide association for pulmonary function traits and SNPs on chromosomes 15 and 4 in COPDGene and the meta-analysis. We identified novel SNPs in gene EEFSEC on chromosome 3 in NHW associated with FEV1 and novel SNPs in gene FAM19A2 on chromosome 12 in AA associated with FEV1/FVC.

# Background

In the United States, Chronic obstructive pulmonary disease (COPD) is the third leading cause of death.[1] A major environmental determinant of COPD is cigarette smoking, but only a minority of smokers will develop COPD. [2-3] COPD risk is most likely the cumulative result of genetic factors, environmental factors such as cigarette smoking, and gene-by-environment interactions. [4]

A diagnosis of COPD is based on spirometric measures: forced expiratory volume in the first second (FEV1) and forced vital capacity (FVC), the volume of air expired after a maximal inhalation. [5] The ratio of FEV1/FVC is a widely used measure of airflow obstruction. [3] Understanding the genetics underlying these spirometric measurements will help increase our knowledge of the genetics of COPD and pulmonary function.

Recent genome-wide association studies (GWAS) have examined spirometric measures in general population samples. [6-13] Large-scale GWAS meta-analyses have identified new loci related to FEV1 or FEV1/FVC. [14-15] Incorporating these studies and new studies, a larger meta-analysis identified additional novel loci. [17] Another large meta-analysis attempted to replicate and validate these previous findings. [18] However, GWAS of spirometric measures in populations containing large numbers of COPD subjects, and in COPD cases only, have not been reported.

In order to replicate known findings and identify new loci affecting pulmonary function, we performed a GWA of spirometric measures in the COPDGene study, a multi-center observational study designed to identify genetic factors associated with COPD. We also performed a meta-analysis of FEV1, FEV1 percent of predicted and FEV1/FVC ratio in the COPDGene, ECLIPSE, and GenKOLS studies. We hypothesized that different genomic regions would be associated with COPD in NWH and AA subjects.

# Methods

## COPDGene Study Subjects

COPDGene is a multicenter study of current and former smokers and has been described in detail previously. [18] The COPDGene study contains 10,192 current and ex-smoking participants. We excluded subjects from the analysis with severe alpha-1 antitrypsin deficiency, genotyping failure and those who had failed spirometry values. This resulted in 9,919 subjects (6,659 non-Hispanic Whites [NHW] and 3,260 African Americans [AA]) and 3641 COPD cases only (2,820 NHW and 821 AA)

## Meta Analysis Study Populations: ECLIPSE and GenKOLS

The Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) is a longitudinal prospective study being conducted at 46 clinical centers in 12 countries.[19] The GenKOLS cohort consists of 863 COPD cases and 808 controls from Bergen, Norway. Genotyping methods and study descriptions for the GenKOLS cohort have been described previously. [21]

## Spirometry Measurements

Spirometry was performed using a standardized spirometer (Easyone by ndd, inc). Spirometry was performed at baseline and repeated after two puffs (180 mcg) of albuterol administered through a spacer. The analyses in this manuscript focused on the post-bronchodilator spirometric values. Pulmonary function measurements were collected according to the American Thoracic Society guidelines [23]. Percent predicted values for FEV1 were calculated using equations of Hankinsonand colleagues [24].

## Genotyping, Quality Control and Imputation

Details concerning genotyping, quality control, and imputation are posted on the COPDGene website (http://www.copdgene.org).

## Statistical Analysis

Genome-wide association analyses were performed in PLINK [25]. Linear regression analyses of FEV1, FEV percent of predicted, and the ratio of FEV1/FVC were adjusted for age, gender, height, pack-years, genetic ancestry using principal components and stratified by race. All analyses were run on the whole cohort (including smoking controls with normal spirometry, individuals with unclassified spirometry (reduced FEV1 but FEV1/FVC > 0.7), and GOLD stages 1 to 4 COPD) and on moderate-to-severe COPD cases only (GOLD stage 2,3,4). While FEV1 percent predicted is adjusted for age, height, and gender in the general population, these adjustments do not account for all of the effects of COPD. As a result, we also analyzed FEV1 percent of predicted adjusting only for pack-years of smoking and genetic ancestry using principal components. Since these results were similar to those of FEV1 percent of predicted adjusting for all the covariates, we only present the results for FEV1 precent of predicted adjusting for the same set of covariates as FEV1 and the ratio FEV1/FVC.

A weighted z-score approach was used in the meta-analysis for FEV1 percent predicted, FEV1 and FEV1/FVC, adjusting for the same covariates as above (age, gender, height, pack-years, and genetic ancestry using principal components). The analyses were run for (1) the entire COPDGene cohort with the ECLIPSE and GenKOLS cohort, (2) only the Non-Hispanic White COPDGene participants with the ECLIPSE and GenKOLS cohort, (3) African American and Non-Hispanic White COPD cases in the COPDGene study, COPD cases in the ECLIPSE and GenKOLS cohort, (4) only the non-hispanic white COPD cases in the COPDGene study with the COPD cases in the ECLIPSE and GenKOLS cohort.

# Results

## COPDGene GWAS in Non-Hispanic Whites

Figure 1 shows the QQ-plots for FEV1, FEV1 percent of predicted, and FEV1/FVC in NHW subjects and NHW COPD cases. While tseveral genomic regions included SNPs with genome-wide significance in NHW subjects, the signal was diminished in the NHW COPD cases and no SNP reaches genome-wide significance. 292 SNPs reached genome-wide significance for FEV1 in the NHW COPDgene subjects. Table 1 shows the number of SNPs found in each gene with the minimum and maximum p-value for those SNPs found in that region. Note that most of these SNPs were found in genes CHRNA3, CHRNA5, CHRNB4, AGPHD1, and IREB2 on chromosome 15 which have previously been associated with COPD, emphysema, and smoking intensity. [26-35] One SNP reached genome-wide significance on gene HHIP which has previously been associated with spirometry measures and COPD. [36-38] One SNP reached genome-wide significance on gene FAM13A which has previously been associated with COPD [22,39] While SNPs on these genes have previously been associated with pulmonary function and COPD, a novel region including 148 SNPs near the EEFSEC gene on chromosome 3 reached genome-wide significance. Similar results were seen for the 299 SNPs that reached genome-wide significance for FEV1 percent of predicted as seen in Table 2 and the 384 SNPs that reached genome-wide significance for FEV1/FVC as seen in Table 3.

## COPDGene GWAS in African Americans

Figure 2 displays the significant findings for FEV1 in AA COPDGene subjects, FEV1 in AA COPD cases, FEV1 percent of predicted in AA COPDGene subjects, and FEV1 percent of predicted in AA COPD cases in the COPDGene cohort. In AA, FEV1 and FEV1 percent of predicted was associated with SNPs on chromosome 22 in MGAT3. In AA COPD cases, FEV1 and FEV1 percent of predicted were associated with a SNP on chromosome 19 in TSHZ3 and a SNP on chromosome 2 in SNTG2. Figure 3 displays significant findings for FEV1/FVC in AA and FEV1/FVC in AA COPD cases in the COPDGene cohort. In AA, FEV1/FVC was associated with 4 SNPs on chromosome 12 in FAM19A2. In AA COPD cases, FEV1/FVC was associated with 3 SNPs on chromosome 6 in BTBD9 and 3 SNPs on chromosome 16 in PRKCB.

## Results for the Meta-Analysis

Figure 4 and 5 show the QQ-plots for FEV1, FEV1 percent of predicted, and FEV1/FVC in the ECLIPSE and GenKOLS cohorts, respectively. Note that these results are less significant than seen in the NHW COPDGene cohort. For FEV1 in both analyses (only NHW and NHW with AA), most of the significant results replicate previous findings on chromosome 15 in genes CHRNA3, CHRNA5, CHRNB4, AGPHD1, IREB2 and chromosome 4 in genes HHIP and FAM13A. Results of interest for FEV1 in the meta-analyses using both NHW and AA are shown in Figure 6 and results of interest for FEV1 in the meta-analyses using only NHW are shown in Figure 7. Note that several SNPs on chromosome 3 in EEFSEC were associated with FEV1 in the NHW cohorts.

Similarly, for FEV1 percent of predicted in both the NHW cohort and the AA and NHW cohort, most of the significant results replicate previous findings on chromosome 15 in genes CHRNA3, CHRNA5, CHRNB4, AGPHD1, IREB2 and chromosome 4 in genes HHIP and FAM13A. Results of interest for FEV1 percent of predicted in the meta-analyses using both NHW and AA are shown in Figure 8 and results of interest for FEV1 percent of predicted in the meta-analyses using only NHW are shown in Figure 9. Note that several SNPs on chromosome 3 in EEFSEC were associated with FEV1 percent of predicted in the NHW cohorts.

Figure 10 shows the significant findings of the meta-analysis in only COPD cases or FEV1 and FEV1 percent of predicted in both the AA and NHW cohort and the NHW only cohort. Several SNPs on chromosome 15 in IREB2 were associated with FEV1 percent of predicted in both the AA and NHW cohort and the NHW only cohort. In both cohorts of cases, a SNP on chromosome 13 in LINC00558 was associated with FEV1.

For FEV1/FVC in both the NHW cohort and the AA and NHW cohort, most of the significant results replicate previous findings on chromosome 15 in genes CHRNA3, CHRNA5, CHRNB4, AGPHD1, IREB2 and chromosome 4 in genes HHIP and FAM13A. Results of interest for FEV1/FVC in the meta-analyses using both NHW and AA are shown in Figure 11 and results of interest for FEV1/FVC in the meta-analyses using only NHW are shown in Figure 12. Note that several SNPs on chromosome 11 in MMP12, chromosome 1 in TGFB2, chromosome 15 in ADAMTS7 were associated with FEV1/FVC in both the NHW and AA cohort and the NHW only cohort. Several SNPs on chromosome 14 in RIN3 were associated with FEV1/FVC in only the NHW cohort.

# Discussion

## Summary of Results

In both COPDGene and the meta-analysis, most of the significant results replicated previous findings on chromosome 15 in genes CHRNA3, CHRNA5, CHRNB4, AGPHD1, IREB2 and chromosome 4 in genes HHIP and FAM13A. There were several novel findings, including the several SNPs on chromosome 3 in EEFSEC associated with all 3 measures of pulmonary function in the COPDGene cohort and in the meta-analysis.

## Novel Nature of COPDGene Study

The COPDGene study is novel in many ways: there are enough COPD cases to perform a COPD case only analysis, there are both AA and NHW subjects, there are standardized spirometry and post-bronchodilator spirometry, and all subjects are former or current smokers.

## Comparison to Previous Spirometry GWAS Studies

In CHARGE and SPIROMETA

## Discussion of Genes Located in Novel Association Regions

## Review of Potential Limitations

Ascertained sample with uncertainty about best approach for adjusting for ascertainment; modest sample size of AA subjects.

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