Variants in FAM13A are associated with chronic obstructive pulmonary disease

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**Abstract**

Substantial evidence suggests that there is genetic susceptibility to chronic obstructive pulmonary disease (COPD). To identify common genetic risk variants, we performed a genome-wide association study in 2940 cases and 1380 smoking controls with normal lung function. We demonstrate a novel susceptibility locus at 4q22.1 in FAM13A (rs7671167, OR=0.76, P=8.6×10\(^{-8}\)) and provide evidence of replication in one case-control and two family-based cohorts (for all studies, combined P=1.2×10\(^{-11}\)).

Chronic obstructive pulmonary disease (COPD) is characterized by a reduction in lung function, with airflow obstruction that is not fully reversible\(^1\). COPD is the fourth leading cause of mortality in the United States; however, COPD is underrecognized and underdiagnosed. While the major risk factor for COPD is cigarette smoking, the development of COPD among current and former smokers is highly variable, and substantial evidence suggests that genetic factors influence the risk of developing COPD\(^2\). A variety of approaches – including candidate gene association studies, linkage analysis, and rare variant studies – have been used to identify COPD susceptibility loci, but with the exception of a relatively rare monogenic disorder (alpha-1 antitrypsin deficiency), few have been consistently replicated\(^2\).

Recently, a genome-wide association study for COPD in a cohort from Norway\(^3\) found an association with SNPs at the CHRNA3/CHRNA5/IREB2 locus that replicated in several populations, including COPD cases from the National Emphysema Treatment Trial (NETT)\(^4\) and controls from the Normative Aging Study (NAS)\(^5\). A second locus near HHIP did not reach genome-wide significance, but replicated in a population-based genome-wide association study of forced expiratory volume in one second (FEV\(_1\)) to forced vital capacity (FVC) ratio – a lung function measurement that is part of the diagnostic criteria for COPD – and likely represents another COPD susceptibility locus\(^3,6\). We hypothesized that a larger genome-wide association study would reveal additional common variants that contribute to COPD susceptibility. Our study included white subjects from three populations: 1) the case-control population from Norway, 2) NETT cases and NAS controls, and 3) cases and controls from the multicenter Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE) Study\(^7\) (Supplementary Data). All of our controls were current or former smokers with normal lung function, and all of our COPD cases had moderate to very severe disease according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification\(^1\). We applied a uniform set of quality control procedures to each of the three raw Illumina data sets (Supplementary Data), and merged the cleaned data into one primary data set.

A total of 499,578 markers that passed quality control criteria in this primary data set were tested for association in 2940 cases and 1380 controls (Supplementary Tables 1 and 2). We performed logistic regression adjusting for age and pack-years of cigarette smoking (packs smoked per day multiplied by years of smoking), and we adjusted for population substructure using principal components as covariates in the regression. The genomic inflation factor after adjustment was 1.02, indicating minimal residual evidence of stratification (Supplementary Figure 2).
The most highly associated locus included SNPs in linkage disequilibrium ($r^2 = 0.85$) at 4q22.1 in the gene FAM13A – rs1903003 and rs7671167 (Table 1 and Figure 1). To address the possibility of false positive results due to intra-study heterogeneity, we additionally performed a stratified analysis in all three case-control cohorts. The results for this association were similar to the primary analysis (Supplementary Table 3); in addition, there was no evidence of between-study heterogeneity of effect for the FAM13A SNPs ($I^2 = 0$).

To replicate these findings, we first genotyped these two FAM13A SNPs, as well as the third-ranked SNP in this locus, rs2869967 ($r^2$ with rs7671167, 0.68), in 502 cases and 504 controls from the COPDGene Study, then tested the top two SNPs in two additional, family-based cohorts: the Boston Early-Onset COPD Study (EOCOPD) with 949 subjects in extended pedigrees and the International COPD Genetics Network (ICGN) with 2859 subjects in nuclear families (Supplementary Table 2b). The association with rs7671167 replicated in COPDGene and in ICGN; in EOCOPD, there was a trend towards effect in the same direction ($P = 0.11$). The lack of significant association in EOCOPD could be due to phenotypic differences (specifically, selection of probands for younger age of onset and greater severity), or due to decreased power from analyzing a relatively small number of pedigrees. As testing for a quantitative trait in the family-based studies could have greater power, we tested rs7671167 for association with forced expiratory volume in one second (FEV$_1$), a quantitative measurement of lung function and a key measurement of severity of COPD, and found an association in EOCOPD (pre- and post-bronchodilator $P = 0.017$ and 0.06, respectively) as well as ICGN (pre- and post-bronchodilator $P=5.3\times10^{-5}$ and $3.3\times10^{-4}$). For the replication populations alone, the $P$ value for association with COPD affection status was $1.07\times10^{-5}$ (Fisher’s method, $3.02\times10^{-5}$); the overall $P$ value for COPD affection status across all six populations was $1.22\times10^{-11}$ (Fisher’s method, $1.16\times10^{-10}$) (Table 1).

FAM13A (also known as FAM13A1) has a putative role in signal transduction, and our most statistically significant SNPs lie in an intronic region downstream of a Rho GTPase-activating proteins (RhoGAP) domain. While little is known about FAM13A function, gene expression analyses in cell lines from several tissues (not including the lung) have demonstrated a consistent increase in response to hypoxia. Differences in respiratory epithelial cell expression of FAM13A have been noted during differentiation into pulmonary type II cells in vitro and in mild versus severe cystic fibrosis patients. Recently, a population-based genome-wide association study of lung function implicated the same locus in FEV$_1$/FVC (rs2869967, $P=1.57\times10^{-8}$; rs7671167, $P=6.30\times10^{-7}$). While the association with rs2869967, or another nearby SNP, rs6830970, failed to replicate in a second study of spirometric phenotypes in the general population, our findings suggest that the former study is not a false positive, and that variants in FAM13A may be important for both variation of FEV$_1$/FVC in the general population as well as susceptibility to COPD.

In addition to our findings at FAM13A, one additional locus was significant at a threshold of $5\times10^{-7}$, and a third was just below this threshold (8.4×10$^{-7}$) in our primary combined analysis (Supplementary Figure 3 and Supplementary Table 4a). Not surprisingly, these two loci were the previously reported CHRNA3/CHRNA5/IREB2 and HHIP loci. We genotyped a subset of these SNPs that were not in complete linkage disequilibrium in

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COPDGene and found nominal significance for replication (P < 0.05) at the CHRNA3/CHRNA5/IREB2 locus (Supplementary Table 4a). However, this finding was not as robust in our stratified analysis of individual cohorts (Supplementary Table 4b).

The association of diseases such as COPD and lung cancer with the CHRNA3/CHRNA5/IREB2 locus remains controversial because of the difficulty in determining whether this association is with the disease, or through a causal pathway with cigarette smoking behavior. The FAM13A SNPs were not associated with pack-years of cigarette smoking within cases or controls. We also tested for an interaction effect with these SNPs and pack-years of cigarette smoking; there was no statistically significant evidence of interaction in the primary or replication analyses (Supplementary Data).

Our study, the largest genome-wide association study in COPD, in no way diminishes the importance of control of cigarette smoking: despite using only current or ex-smoking controls for this study, the effect of the genetic variants in this study is dwarfed in comparison to smoking (in the primary analysis, for each 10 pack-years of smoking, odds ratio = 1.5, P value = 9.3x10^{-95}; Supplementary Data). However, in light of the millions worldwide currently affected with COPD and the millions who will likely develop COPD due to ongoing smoking or other environmental exposures, the discovery of genetic risk variants, like those in FAM13A, could contribute to the eventual development of truly novel therapeutic approaches to this disease.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Regional association plot for signal at the *FAM13A* locus

P values in the primary analysis are shown as circles; colors indicate $r^2$ of each SNP with rs7671167. Dotted lines connect the top two P values from the primary analysis with diamonds, showing the combined (primary plus replication) studies. Grey bars show the recombination rate based on CEU HapMap Build 22. The top of the figure shows UCSC genes at the corresponding location based on the March 2006 (hg18) assembly (genome.ucsc.edu).
Association results in FAM13A

The primary analysis includes 2940 cases and 1380 controls; replication results are shown for the case-control COPDGene and the family-based EOCOPD and ICGN studies. All analyses are adjusted for age and pack-years of cigarette smoking; the primary analysis is also adjusted for population stratification using principal components. Minor allele frequencies are given for the cohort.

Table 1

| Location* |
|-----------|
| Minor MAF | Beta  | OR   | P value | MAF  | Beta  | OR   | P value | MAF  | P value | MAF  | P value | MAF  | P value | MAF  | P value |
| rs1903003  | 4:90105320 allele 0.45 -0.28 0.76 7.18×10⁻⁸ | 0.46 -0.25 0.78 7.19×10⁻³ | 0.46 0.48 0.45 1.29×10⁻³ | 9.47×10⁻¹¹ |
| rs7671167  | 4:90103002 C 0.48 -0.28 0.76 8.59×10⁻⁸ | 0.49 -0.27 0.77 3.93×10⁻³ | 0.51 0.11 0.49 5.15×10⁻⁴ | 1.22×10⁻¹¹ |
| rs2869967  | 4:90088355 C 0.42 0.26 1.29 1.48×10⁻⁶ | 0.40 0.21 1.24 1.72×10⁻² |

*Chromosome:base position, referencing hg18