Spirometric measures of pulmonary function are easily obtainable and reproducible indices of the physiological state of the lung and airways commonly used in clinical medicine. Two major spirometric measures in clinical practice are the forced expiratory volume in 1 second (FEV1) and the ratio of FEV1 to the forced vital capacity (FVC). The FEV1 reflects airflow obstruction and lung size. Reduction in FEV1 out of proportion to the FVC leads to a reduced FEV1/FVC ratio. The FEV1/FVC provides an index of airflow obstruction that is relatively independent of lung size and is the primary criterion for the diagnosis of airway obstruction and chronic obstructive pulmonary disease. The FEV1 is used to assess the severity of the airflow obstruction and monitor the progression of lung diseases including chronic obstructive pulmonary disease; asthma, and cystic fibrosis. In addition to its essential role in the diagnosis and monitoring of respiratory disease, lower pulmonary function has been shown in numerous studies to be related to increased cardiovascular morbidity and mortality in the general population, including among nonsmokers without lung disease and independent of standard risk factors.1–6

Cross-sectional measures of FEV1 and FEV1/FVC in adults reflect the maximal values attained at the conclusion of growth and the inevitable decline with age thereafter. Environmental factors, most notably smoking, influence both maximal growth and the rate of decline. However, genetics also influence pulmonary function: >40% of the variability in pulmonary function has been attributed to genetic factors.7 For decades, the role of the uncommon genetic deficiency of α-1 antitrypsin in reduced pulmonary function has been appreciated.8 However, other genes involved in pulmonary function remained elusive before the era of genome-wide association studies (GWASs). Recent GWASs have identified common genetic variants

**Background**—The pulmonary function measures of forced expiratory volume in 1 second (FEV1) and its ratio to forced vital capacity (FVC) are used in the diagnosis and monitoring of lung diseases and predict cardiovascular mortality in the general population. Genome-wide association studies (GWASs) have identified numerous loci associated with FEV1 and FEV1/FVC, but the causal variants remain uncertain. We hypothesized that novel or rare variants poorly tagged by GWASs may explain the significant associations between FEV1/FVC and 2 genes: **ADAM19** and **HTR4**.

**Methods and Results**—We sequenced **ADAM19** and its promoter region along with the ±21-kb portion of **HTR4** harboring GWAS single-nucleotide polymorphisms for pulmonary function and analyzed associations with FEV1/FVC among 3983 participants of European ancestry from Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. Meta-analysis of common variants in each region identified statistically significant associations (316 tests; \( P < 1.58 \times 10^{-4} \)) with FEV1/FVC for 14 **ADAM19** single-nucleotide polymorphisms and 24 **HTR4** single-nucleotide polymorphisms. After conditioning on the sentinel GWASs hit in each gene (**ADAM19** rs1422795, minor allele frequency=0.33 and **HTR4** rs11168048, minor allele frequency=0.40], 1 single-nucleotide polymorphism remained statistically significant (**ADAM19** rs13155908, minor allele frequency=0.12; \( P = 1.65 \times 10^{-6} \)). Analysis of rare variants (minor allele frequency <1%) using sequence kernel association test did not identify associations with either region.

**Conclusions**—Sequencing identified 1 common variant associated with FEV1/FVC independent of the sentinel **ADAM19** GWAS hit and supports the original **HTR4** GWAS findings. Rare variants do not seem to underlie GWAS associations with pulmonary function for common variants in **ADAM19** and **HTR4**. (Circ Cardiovasc Genet. 2014;7:350-358.)

**Key Words:** Airway Obstruction  •  genome-wide association study  •  lung  •  polymorphism, genetic  •  pulmonary disease, chronic obstructive  •  respiratory function tests  •  sequence analysis, DNA

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**ADAM19** and **HTR4** Variants and Pulmonary Function

**Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Targeted Sequencing Study**

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**Background**—The pulmonary function measures of forced expiratory volume in 1 second (FEV1) and its ratio to forced vital capacity (FVC) are used in the diagnosis and monitoring of lung diseases and predict cardiovascular mortality in the general population. Genome-wide association studies (GWASs) have identified numerous loci associated with FEV1 and FEV1/FVC, but the causal variants remain uncertain. We hypothesized that novel or rare variants poorly tagged by GWASs may explain the significant associations between FEV1/FVC and 2 genes: **ADAM19** and **HTR4**.

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related to FEV1/FVC or FEV1 in ≥27 loci. Most of these loci were identified for FEV1/FVC by 5-hydroxytryptamine (serotonin) receptor 4 (HTR4) and a disintegrin and metallopeptidase domain 19 (ADAM19). Both were subsequently associated with the clinical phenotypes of airflow obstruction and chronic obstructive pulmonary disease in GWAS. Interestingly, ADAM19 also plays an essential role in cardiac development, and copy number variants have recently been identified in patients with congenital heart disease.

Although segregation analyses suggest that genetics contribute a substantial portion of the variability in pulmonary function, the combined effects of all GWAS-identified loci seem to explain <8% of the predicted genetic variance. The issue of unexplained heritability in GWASs has been the subject of much recent interest. One of the explanations invoked is that rare functional variants linked to the common variants identified by GWAS platforms may be important. To this end, in-depth resequencing efforts to systematically follow up GWAS hits were undertaken in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Targeted Sequencing Study.

We analyzed deep sequencing data for ADAM19 and HTR4 in relation to FEV1/FVC and FEV1. The goal was to identify whether variants, common or rare, that were not included in previous GWAS data sets might underlying the observed single-nucleotide polymorphism (SNP) associations from earlier GWASs of these outcomes.

Methods

Study Samples

The CHARGE Targeted Sequencing Study includes participants enrolled in 3 cohorts: the Atherosclerosis Risk in Communities (ARIC) study, the Cardiovascular Health Study (CHS), and the Framingham Heart Study (FHS). All of the study participants were of European ancestry and had been included in previous GWAS meta-analyses of the pulmonary function parameters FEV1 and FEV1/FVC. The participants included those randomly selected from the cohort (the Cohort Random Sample) and those selected for different severe phenotypes (the Phenotype Groups). The Cohort Random Sample contains ~2000 unrelated individuals representing the distribution of phenotypes in the general population. The Phenotype Groups contain individuals with extreme values for ≥1 of 14 phenotypes; each group has ~200 participants. The phenotype Group for FEV1 and FEV1/FVC included participants selected from the ARIC study cohort based on meeting the following criteria at both visits 1 and 2: FEV1 <65% predicted and FEV1/FVC less than the lower limit of normal based on meeting the following criteria at both visits 1 and 2: FEV1 <65% predicted and FEV1/FVC less than the lower limit of normal based on GWAS findings for FEV1/FVC: ADAM19 and HTR4. We analyzed only these 2 target regions. For ADAM19 (chr5), the following regions were submitted for sequencing based on National Center for Biotechnology Information (NCBI) build 36: regulatory region (CTCF-binding site) between chromosomal locations 156831095 and 156832298 (hg18) and the gene region ±1 kb (locations 156835890–156936346). For HTR4 (chr5), we submitted for sequencing the 21-kb block containing all of the high-signal SNPs from previous GWASs plus 1-kb upstream and downstream (locations 147815802–147837526).

The methods of the CHARGE Targeted Sequencing Study have been described fully in a separate manuscript (H. Lin et al., unpublished data). Briefly, ~2 Mb of target regions were captured by a customized NimbleGen Capture array and sequenced using the ABI SOLiD V4.0 platform. The raw short reads were aligned to the reference human genome (NCBI Genome Build 36, hg18) by BFAST. SAMtools was used to pile up aligned reads and call variants with quality filters. The resulting data were then subjected to quality control procedures. Variants were categorized as known or novel by comparison with the dbSNP database and the 1000 Genomes project. The functional impact of identified variants on the encoded proteins was predicted by the ANNOVAR software package.

Statistical Analysis

Common Variants

Because of the study design of CHARGE Targeted Sequencing Study, participants with extreme phenotypes were over-represented in the sequenced samples compared with those selected for the random cohort. To account for this sampling bias, we performed analyses with individuals weighted by the inverse of their sampling probabilities to obtain population-based effect estimates. We tested each common SNP for association with FEV1 and FEV1/FVC using unweighted analyses with linear regression models with robust SE estimates in ARIC study and CHS and linear mixed-effects models in FHS to account for family relatedness. All analyses assumed an additive effect of the alternate allele and were adjusted for the same factors as in the original discovery GWAS: age, sex, standing height, smoking status (current, past, or never), and pack-years of smoking. Additional study-specific covariates included recruitment cohort (FHS), recruitment center (ARIC study and CHS), and principal component eigenvalues for population stratification adjustments (ARIC study and FHS). For the weighted association analysis, we used a weighted linear regression in ARIC study and CHS and weighted linear mixed model in FHS. To account for known GWAS loci, we compared analyses with and without conditioning on the sentinel SNPs in each GWAS locus from our earlier discovery GWASs of FEV1/FVC: ADAM19 rs1422795 and HTR4 rs11168048. The summary statistics (β, SE) from each cohort were then meta-analyzed using an inverse-variance meta-analysis approach. We report the P values from the unweighted analysis and the magnitude of effects from the weighted analysis (Lumley et al., http://stattech.wordpress.fos.auckland.ac.nz/files/2012/05/design-paper.pdf). To account for multiple comparisons, we applied a Bonferroni correction for the number of common variants (n=316 with minor allele frequency [MAF] >1%) analyzed. We consider common variants with association P values <1.6×10^-4 (0.05/316) as statistically significant.

Rare Variants

Single-marker–based association analysis has low power for rare variants. Therefore, we jointly analyzed all rare variants (MAF <1%; 2166 in ADAM19 and 454 in HTR4) occurring in each of the 2 target regions. We tested association of rare variants with FEV1 and FEV1/FVC using linear regression models with robust SE estimates in ARIC study and CHS and weighted linear mixed-effects models in FHS to account for family relatedness. All analyses assumed an additive effect of the alternate allele and were adjusted for the same factors as in the original discovery GWAS: age, sex, standing height, smoking status (current, past, or never), and pack-years of smoking. Additional study-specific covariates included recruitment cohort (FHS), recruitment center (ARIC study and CHS), and principal component eigenvalues for population stratification adjustments (ARIC study and FHS). For the weighted association analysis, we used a weighted linear regression in ARIC study and CHS and weighted linear mixed model in FHS. To account for known GWAS loci, we compared analyses with and without conditioning on the sentinel SNPs in each GWAS locus from our earlier discovery GWASs of FEV1/FVC: ADAM19 rs1422795 and HTR4 rs11168048. The summary statistics (β, SE) from each cohort were then meta-analyzed using an inverse-variance meta-analysis approach. We report the P values from the unweighted analysis and the magnitude of effects from the weighted analysis (Lumley et al., http://stattech.wordpress.fos.auckland.ac.nz/files/2012/05/design-paper.pdf). To account for multiple comparisons, we applied a Bonferroni correction for the number of common variants (n=316 with minor allele frequency [MAF] >1%) analyzed. We consider common variants with association P values <1.6×10^-4 (0.05/316) as statistically significant.
Predicted Functional Variants

Because the power of SKAT can be sensitive to the inclusion of non-functional variants, we performed additional analyses restricted to those rare variants predicted to be functional. In the ADAM19 gene region, variants were restricted to nonsynonymous and splice site SNPs (61 missense, 1 nonsense, and 5 splice site). The region of sequencing around the HTR4 top GWAS hits fell in a largely intronic region. The HTR4 rare variants most likely to be functional were selected by using noncoding annotation from ENCODE and TransFac tracks from the UCSC browser. In addition to the 4 rare exonic variants, SNPs in ENCODE regions annotated as DNase hypersensitivity sites or ChIP-Seq transcription factor binding sites and variants falling in conserved transcription factor binding motifs were selected. Applying these criteria, the 454 rare variants in the HTR4 region were refined to a set of 122 potentially functional variants.

Results

After quality control, valid sequencing data for ADAM19 and HTR4 as well as data on pulmonary function data were available for 3983 participants from the 3 cohorts. This included 186 selected for severe airflow obstruction, 1830 selected as a random sample of cohort participants, and 1967 selected because of extreme values for nonpulmonary phenotypes. Pulmonary function parameters, age, sex, and smoking history of participants are shown by cohort in Table 1.

For HTR4, sequencing identified a total of 2630 SNPs, including 207 coding SNPs and 2046 that are novel, defined as not present in 1000 Genomes phase I. For ADAM19, we identified 3494 SNPs, including 52 coding SNPs and 2662 novel SNPs (from Table 2a in Methods, H. Lin et al, unpublished data).

Common Variants

For each of the 2 regions (ADAM19 and HTR4), in the Figure, the \( P \) values are plotted for FEV1/FVC and FEV1 in relation to the 316 common variants (MAF >1%) before and after conditioning on the sentinel GWAS SNP from our earlier discovery analysis in each of the 2 regions (ADAM19 rs1422795 and HTR4 rs11168048). After Bonferroni correction for 316 tests, analysis of individual SNPs identified statistically significant \((P<1.58\times10^{-5})\) associations with FEV1/FVC for 14 SNPs in ADAM19 and 24 SNPs in HTR4 and with FEV1 for 12 HTR4 SNPs (Table 2). Among the statistically significant SNPs, 11 in HTR4 and 7 in ADAM19 were not included in the original GWAS discovery data set. After conditioning on the sentinel GWAS SNP in each gene (ADAM19 rs1422795, HTR4 rs11168048), only 1 SNP surpassed the statistical significance threshold for association with FEV1/FVC (ADAM19 rs13155908, \(P=1.53\times10^{-5})\). The MAFs for this SNP rs13155908 (0.12), as well as the other linked SNPs that were significant in the unconditional analysis (range, 0.10–0.17), are much lower than that of the sentinel GWAS SNP rs1422795 in ADAM19 (0.33). SNP rs13155908 is not in high linkage disequilibrium with the sentinel SNP \((r^2=0.07)\), suggesting an independent signal. SNP rs13155908 did not give genome-wide statistically significant association with FEV1/FVC in the original GWAS discovery data set (\(P=1.53\times10^{-5})\).

Rare Variants

Meta-analysis of the cohort-specific SKAT estimates combining all variants with MAF <1% did not provide any evidence for a role of rare variants in either ADAM19 (2166 variants) or HTR4 (454 variants) in relation to either FEV1 or FEV1/FVC (\(P>0.95\) for all 4 analyses). Because the large number of rare variants examined in ADAM19 might dilute signals from the modest expected number of associated variants, we also created 5 windows of equal size (433 in windows 1–4 and 434 in the window 5) and repeated the SKAT meta-analysis within those. The smallest \( P \) value in any window was 0.52.

Potential Functional Variants

Meta-analysis of the cohort-specific SKAT estimates combining all potential functional rare variants (62 in ADAM19 and 122 in HTR4) did not reveal any evidence for association with either FEV1 or FEV1/FVC (\(P>0.68\) for all 4 analyses).

Discussion

Spirometry is the most commonly used assessment of lung function, and FEV1/FVC and FEV1 are critical physiological measurements in the diagnosis of airflow obstruction and monitoring of its severity and progression in clinical practice. In previous GWASs,6–12 we have identified several novel loci containing common SNPs related to the FEV1/FVC and FEV1. Two of the novel loci were ADAM19 and HTR4. In subsequent work, we found evidence that ADAM19 and HTR4 are related to airflow obstruction and chronic obstructive pulmonary disease.13 In the current article, we used targeted sequencing of ADAM19 and HTR4 to address the question of whether our previous GWAS findings for FEV1/FVC were attributable to additional functionally relevant variants or, alternatively, attributable to the combined burden of rare alleles not represented in the earlier GWAS data sets.

Because HTR4 and ADAM19 were only recently identified as novel genes for pulmonary function and disease in GWASs,10–13 they have not been well studied in relation to these lung phenotypes. However, within the limited published data, there is biological plausibility for a role of both genes in lung function and lung pathobiology.

Table 1. Numbers of Subjects and Characteristics by Cohort

<table>
<thead>
<tr>
<th>Cohort</th>
<th>n</th>
<th>FEV1, L (Mean SD)</th>
<th>FEV1/FVC, % (Mean SD)</th>
<th>Age at Examination, y (Mean SD)</th>
<th>Female, %</th>
<th>Ever Smoked, %</th>
<th>Ever Smokers, Pack-Years (Mean SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARIC</td>
<td>1914</td>
<td>2.81(0.83)</td>
<td>71.8(9.9)</td>
<td>54.8(5.7)</td>
<td>48.8</td>
<td>64.4</td>
<td>32.1 (23.0)</td>
</tr>
<tr>
<td>CHS</td>
<td>1080</td>
<td>2.13(0.66)</td>
<td>70.0(10.9)</td>
<td>72.5(5.5)</td>
<td>53.7</td>
<td>55.3</td>
<td>36.6 (29.3)</td>
</tr>
<tr>
<td>FHS</td>
<td>989</td>
<td>2.68(0.81)</td>
<td>72.5(8.2)</td>
<td>59.8(11.0)</td>
<td>51.4</td>
<td>62.9</td>
<td>28.7 (23.7)</td>
</tr>
</tbody>
</table>

Age at examination is the age at which the FEV1 and FEV1/FVC values used in this analysis were measured. This is the baseline examination for ARIC and CHS and the latest examination with acceptable pulmonary function for FHS. ARIC indicates Atherosclerosis Risk in Communities; CHS, Cardiovascular Health Study; FEV1, forced expiratory volume in 1 second; FHS, Framingham Heart Study; and FVC, forced vital capacity.
ADAM19 is a member of the a disintegrin and metalloprotease family of membrane-tethered glycoproteins and is expressed in most tissues including the lung. ADAM19 is a key responder to stimulation by transforming growth factor-β in lung epithelial cells, transforming growth factor-β1 is a prominent mediator of the response to injury, including fibrosis. ADAM19 was found to be a key responder to stimulation by...
transforming growth factor-β1 in alveolar epithelial cells and a potentially critical effector of the fibrotic response to injury, an important step in the pathogenesis of pulmonary fibrosis and other lung diseases. ADAM19 can potentiate proinflammatory activity of tumor necrosis factor-α, a key modulator of airway inflammation. ADAM19 plays a crucial role in cardiac development; Adam19−/− mice have multiple cardiac developmental defects, and ADAM19 copy number variants have recently been identified in patients with congenital heart disease. Thus, genetic variation and differential expression of ADAM19 are linked to both pulmonary and cardiac disease pathogenesis.

HTR4 is a member of the serotoninergic signaling cascade and is expressed in the lung. Although serotonin (5-hydroxytryptamine) is best studied as a neurotransmitter, 5-hydroxytryptamine signaling plays an important role in many organ systems. In the lung, it is involved in control of breathing and smooth muscle contractility. Serotonin signaling including HTR4 is involved in human airway inflammation. In a primate asthma model, ozone exposure increased HTR4 expression in the airways, and this effect was accompanied by enhanced smooth muscle contractility. A recent study designed to follow up GWAS findings for HTR4 genetic variants in lung function identified evidence for greater expression of HTR4 in fetal
Table 2. SNPs in HTR4 and ADAM19 That Were Statistically Significant ($P<1.58\times10^{-4}$) for Association With Either FEV1 or FEV1/FVC in the Unconditional Analysis

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Target</th>
<th>SNP Position</th>
<th>dbSNP</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Frequency 1</th>
<th>$\beta$</th>
<th>SE</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1 HTR4</td>
<td>chr5:147822546 t c</td>
<td>rs11168048</td>
<td>0.6011</td>
<td>-0.0309</td>
<td>0.0140</td>
<td>5.47×10^{-4}</td>
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<td>Sentinel</td>
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<tr>
<td></td>
<td>chr5:147827466 a g</td>
<td>rs4597955</td>
<td>0.5670</td>
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<td>0.0138</td>
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<td>0.0141</td>
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<td>6.59×10^{-5}</td>
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Table 2. Continued

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| ADAM19    | rs1422795 gave $P=0.02$ in the unconditional analysis and is thus not listed in this table. ARIC indicates Atherosclerosis Risk in Communities; CHS, Cardiovascular Health Study; FEV1, forced expiratory volume in 1 second; FHS, Framingham Heart Study; FVC, forced vital capacity; and SNP, single-nucleotide polymorphism.

*Unconditional analysis includes only the SNP listed. For FEV1, the $\beta$ value is the change in liters per copy of allele 1. For FEV1/FVC, the $\beta$ value is the change in FEV1/FVC scaled as a proportion (range, 0–1) per copy of allele 1. Conditional analysis model includes the SNP listed plus the sentinel SNP for the specific gene target ($HTR4$ rs11168048, ADAM19 rs1422795), and the $P$ value given is for the $\beta$ value for the SNP listed.

†Direction refers to the sign of the $\beta$ coefficient by cohort in the following order: FHS, ARIC, CHS. + indicates a positive $\beta$ coefficient; and −, a negative $\beta$ coefficient.

compared with adult human lung, suggesting an important role in lung development.13 The observation that $HTR4$ genetic variants are related to pulmonary function in both adults and children further supports a role in lung development.11,12

Given that family history of cardiovascular disease is common among older adults in the United States, our study sample included a large proportion of participants with a family history of cardiovascular disease. For example, in the participants in this analysis from the ARIC study cohort, which contributed the largest number to this data set, 49% reported that 1 or both biological parents had a history of myocardial infarction. Family history of cardiovascular disease was not associated with airway obstruction (age-, sex-, and smoking-adjusted odds ratio=1.07; 95% confidence interval, 0.79–1.42; $P=0.69$), a clinically relevant phenotype that showed association with both SNPs in ADAM19 and $HTR4$.13 This result is consistent with previous epidemiological findings that reduced pulmonary function is a risk factor for mortality in the general population independent of traditional risk factors for cardiovascular disease.1,4

In analyses of all common variants (MAF >1%) in ADAM19 and $HTR4$ without conditioning on our sentinel GWAS SNPs, we identified several SNPs significantly related to either FEV1/FVC or FEV1. However, these associations seemed to be explained by our previous GWAS findings because all but 1 (ADAM19 rs13155908, $P=1.56\times10^{-4}$; cutoff $P=1.58\times10^{-4}$) were no longer significant after adjusting for the sentinel GWAS SNP at each locus.

There is functional evidence supporting the potential etiologic role of the ADAM19 sentinel GWAS SNP (rs1422795). It is a nonsynonymous coding SNP resulting in a serine to glycine substitution. This change is predicted to be possibly damaging in Mutation Taster (www.mutationtaster.org/index.html) and PolyPhen-2.34 Evaluation of this SNP in the UCSC Genome Browser indicates that it is in the cis-regulatory region of this transcript. In addition, rs1422795 is close to the beginning of the translation start site (17 amino acids away) of another ADAM19 transcript variant. Because of this proximity, the amino acid change could influence the expression of this transcript. Furthermore, rs1422795 is located within a histone H3K27Ac mark—a region associated with regulatory control of gene expression.

We interrogated the HapMap3 expression Quantitative Trait Loci database of lymphoblastoid cell lines to assess whether ADAM19 rs13155908 that remained statistically significant in the conditional analysis was related to gene expression.35,36 We found a significant cis-association between rs13155908 alleles and ADAM19 expression (Spearman rank correlation coefficient=0.23; $P=0.019$) in participants of European ancestry (CEU, n=109) but not in other ethnicities. The MAF of rs13155908 is very low in HapMap Asian populations and low in Africans. In contrast, the ADAM19 sentinel GWAS SNP rs1422795 as well as the other top SNP in the original GWAS to which it is closely linked (rs2277027) was associated with ADAM19 gene expression in the European ancestry population ($P=0.001$ for both SNPs) as well as several other ethnic groups. Furthermore, rs1422795 (and rs2277027) had significant cis-expression quantitative trait loci in multiple other tissues including nerve ($P=5.3\times10^{-5}$), adipose ($P=6.7\times10^{-4}$), and suggestive cis-expression quantitative trait loci in lung ($P=8.0\times10^{-4}$) based on the Genotype-Tissue Expression Portal.37 We did not find other evidence in public databases supporting a functional role for rs13155908.

For $HTR4$, our top GWAS SNP rs11168048 is intronic and not predicted to have a functional consequence for the protein. A search of transcription element binding sites (Transcription Element Search System [TESS], http://www.cbil.upenn.edu/cgi-bin/tess/tess) identified this SNP as located within a potential binding site of the transcription factor autonomously replicating sequence binding factor 1 (ABF1), which is abolished when the T allele is present. In a subsequent analysis of airflow obstruction, $HTR4$ rs11168048 gave the smallest $P$ value among top $HTR4$ SNPs identified previously by GWAS of pulmonary function,10 and in an analysis limited to smokers, it gave the smallest $P$ value overall among 75 SNPs from all previous GWAS loci for pulmonary function.13 Among the high-signal SNPs in the current analysis, 4 fall within regulatory regions identified through overlap with fetal lung DNAse I hypersensitivity sites.38 Functional annotation of variants in high linkage disequilibrium in this region may help inform follow-up functional studies to identify the causal variant.
acknowledge that we limited our sequencing effort to a 21-kb linkage disequilibrium block of HTR4 that harbored all of our high-signal SNPs. Thus, if our top GWAS SNPs are in high LD with variants outside of this area, we would not have captured them with our sequencing effort.

SKAT analysis of possibly functional rare variants or of all rare variants did not provide any evidence for association with FEV1 or FEV1/FVC. Although this suggests that our previous GWAS signals are not explained by rare variants in linkage disequilibrium with them, we acknowledge that our study and other sequencing efforts tend to be smaller than the discovery sample sizes and thus will be underpowered for rare variants.

Our analysis of targeted sequencing data for HTR4 gives support for the importance of the sentinel GWAS hit, although functional evidence in support of this SNP remains sparse. For ADAM19, the analysis conditioning on the sentinel GWAS SNP suggests the involvement of an additional SNP, implying that there might be >1 causal variant at this locus.

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Disclosures
J.B. Wilk is employed by and holds stock options in Pfizer Inc. Dr. Gibbs served as a consultant to GE Clarient. The other authors report no conflicts.

Appendix
From the Epidemiology Branch (S.J.L., D.B.H.) and Laboratory of Respiratory Biology (S.J.L., J.S.H.), Division of Intramural Research, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC; Department of Biostatistics (W.G., J.D., Y.Z.) Boston University School of Public Health; Center for Lung Biology, Division of Pulmonary and Critical Care Medicine, Department of Medicine (S.A.G.), Cardiovascular Health Research Unit, Department of Medicine (S.R.H., B.M.P., J.A.B.), Department of Epidemiology (B.M.P., S.R.H.), and Department of Health Services (B.M.P.), University of Washington, Seattle; Behavioral Health Epidemiology Program, Research Triangle Institute, Research Triangle Park, NC (D.B.H.); Precision Medicine, Pfizer Global Research & Development, Cambridge, MA (J.B.W.); Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX (R.A.G., D.M.M., C.L.K.); Department of Statistics, University of Auckland, Auckland, New Zealand (T.L.); Department of Epidemiology (N.F., K.E.N.) and Carolina Center for Genome Sciences (K.E.N.), University of North Carolina at Chapel Hill; Group Health Research Institute, Group Health Cooperative, Seattle, WA (B.M.P., S.R.H.); Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD (J.C.); and Human Genetics Center, School of Public Health, University of Texas Health Science Center at Houston (A.C.M.).

References
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