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ADAM19 and *HTR4* Variants and Pulmonary Function: The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Targeted Sequencing Study

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Abstract

Background—The pulmonary function measures of forced expiratory volume in one 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 (GWAS) have identified numerous loci associated with FEV1 and FEV1/FVC but the causal variants remain uncertain. We hypothesized that novel or rare variants poorly

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tagged by GWAS may explain the significant associations between FEV1/FVC and two genes: *ADAM19* and *HTR4*.

Methods and Results—We sequenced *ADAM19* and its promoter region along with the approximately 21 kb portion of *HTR4* harboring GWAS SNPs for pulmonary function and analyzed associations with FEV1/FVC among 3,983 participants of European ancestry from Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE). Meta-analysis of common variants in each region identified statistically significant associations (316 tests, $P < 1.58\times10^{-4}$) with FEV1/FVC for 14 *ADAM19* SNPs and 24 *HTR4* SNPs. After conditioning on the sentinel GWAS hit in each gene [*ADAM19* rs1422795, minor allele frequency (MAF)=0.33 and *HTR4* rs11168048, MAF=0.40] one SNP remained statistically significant (*ADAM19* rs13155908, MAF = 0.12, $P = 1.56\times10^{-4}$). Analysis of rare variants (MAF < 1%) using Sequence Kernel Association Test did not identify associations with either region.

Conclusions—Sequencing identified one common variant associated with FEV1/FVC independently of the sentinel *ADAM19* GWAS hit and supports the original *HTR4* GWAS findings. Rare variants do not appear to underlie GWAS associations with pulmonary function for common variants in *ADAM19* and *HTR4*.

Keywords

genetic polymorphism; lung; population studies; DNA sequencing; Genome Wide Association Study

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Spirometric measures of pulmonary function are easily obtainable and reproducible indices of the physiologic state of the lung and airways commonly used in clinical medicine. Two major spirometric measures in clinical practice are the forced expiratory volume in one 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 (COPD). The FEV1 is used to assess the severity of the airflow obstruction and monitor the progression of lung diseases including COPD, 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 independently 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; over 40% of the variability in pulmonary function has been attributed to genetic factors.[7] For decades, the role of the uncommon genetic deficiency of alpha-1 antitrypsin in reduced pulmonary function has been appreciated.[8] However, other genes involved in pulmonary function remained elusive prior to the era of genome wide association studies (GWAS). Recent

GWAS have identified common genetic variants related to FEV1/FVC or FEV1 in at least 27 loci.[9–12] Most of these are novel loci not previously implicated in lung pathology. Two of the novel genes identified for FEV1/FVC are 5-hydroxytryptamine (serotonin) receptor 4 (*HTR4)* and A Disintegrin And Metallopeptidase Domain 19 (*ADAM19*).[10] Both were subsequently associated with the clinical phenotypes of airflow obstruction and COPD in GWAS.[13] Interestingly, *ADAM19* also plays an essential role in cardiac development[14] and copy number variants have recently been identified in patients with congenital heart disease.[15]

While segregation analyses suggest that genetics contribute a substantial portion of the variability in pulmonary function[7], the combined effects of all GWAS-identified loci appear to explain less than eight percent of the predicted genetic variance.[12] The issue of unexplained heritability in GWAS 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.[16] To this end, in-depth re-sequencing efforts to systematically follow-up GWAS hits were undertaken in the CHARGE 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 datasets, might underlie the observed SNP associations from earlier GWAS of these outcomes.

Methods

Study samples

The CHARGE Targeted Sequencing Study includes participants enrolled in three cohorts: the Atherosclerosis Risk in Communities Study (ARIC), the Cardiovascular Health Study (CHS), and the Framingham Heart Study (FHS).[17] All of the study participants were of European ancestry and had been included in previous GWAS meta-analysis of the pulmonary function parameters FEV1 and FEV1/FVC.[10] The participants included those randomly selected from the cohort (the Cohort Random Sample) and those selected for several different extreme phenotypes (the Phenotype Groups). The Cohort Random Sample contains approximately 2,000 unrelated individuals representing the distribution of phenotypes in the general population. The Phenotype Groups contain individuals with extreme values for at least one of 14 phenotypes; each group has approximately 200 participants (Lin H, et al. *Circ Cardiovasc Genet*, submitted). The Phenotype Group for FEV1 and FEV1/FVC included participants selected from the ARIC cohort based on meeting the following criteria at both visits 1 and 2: FEV1 < 65% predicted and FEV1/FVC < the lower limit of normal based on NHANES III prediction equations.[18] Institutional review boards at participating centers approved the study and participants provided informed consent.

Sequencing data

In the CHARGE Targeted Sequencing Study, a total of 77 target regions were sequenced to follow-up selected GWAS findings across multiple phenotypes. Two of these target regions were selected based on GWAS findings for FEV1/FVC: *ADAM19* and *HTR4*. We analyzed only these two target regions. For *ADAM19* (chr 5), the following regions were submitted for sequencing based on NCBI build 36: regulatory region (CTCF binding site) between chromosomal locations 156831095 and 156832298 (hg18) and the gene region +/− 1 kb (locations 156835890 to 156936346). For *HTR4* (chr 5), we submitted for sequencing the 21 kb block containing all of the high signal SNPs from our previous GWAS plus 1 kb up and downstream (locations 147815802 to 147837526).

The methods of the CHARGE Targeted Sequencing Study have been described fully in a separate manuscript (Lin H, et al. *Circ Cardiovasc Genet*, submitted). Briefly, approximately 2Mb 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.[19] SAMtools[20] 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.[21]

Statistical analysis

Common variants—Due to 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. In order 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 (Lin H, et al. *Circ Cardiovasc Genet*, submitted). We tested each common SNP for association with FEV1 and FEV1/FVC using un-weighted analyses with linear regression models with robust standard error estimates in ARIC 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[10]: age, sex, standing height, smoking status (current, past or never-smoker) and pack-years of smoking. Additional study-specific covariates included recruitment cohort (FHS), recruitment center (ARIC and CHS) and principal component eigenvalues for population stratification adjustments (ARIC and FHS). For the weighted association analysis, we used a weighted linear regression in ARIC and CHS, and weighted linear mixed model in FHS. In order 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 GWAS of FEV1/FVC: *HTR4* rs11168048 and *ADAM19* rs1422795.[10] *HTR4* rs11168048 gave the smallest P value among SNPs at this locus in the discovery GWAS.[10] *ADAM19* rs1422795 was chosen as the sentinel SNP for conditional analyses because among the several highly correlated ($r^2 > 0.95$) genome-wide significant SNPs in the discovery GWAS, it is a non-synonymous missense SNP. Regression models were used to adjust trait values for all covariates in addition to the

genotype of the sentinel SNP. Residuals from these linear models were then used as the independent (outcome) variable in the conditional analysis.

The summary statistics (Beta, SE) from each cohort were then meta-analyzed using an inverse variance meta-analysis approach. We report the p-values from the un-weighted analysis, and the magnitude of effects from the weighted analysis (Lumley T, Dupuis J, Rice KM, Barbalic M, Bis JC, Cupples LA, et al. http://stattech.wordpress.fos.auckland.ac.nz/files/2012/05/design-paper.pdf). In order to account for multiple comparisons, we applied a Bonferroni correction for the number of common variants (N=316 with MAF>1%) analyzed. We consider common variants with association p-values less than 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 two target regions. We tested association of rare variants with FEV1 and FEV1/FVC using the Sequence Kernel Association Test (SKAT).[22] Single variant summary statistics and genotype covariance matrices were pooled for meta-analysis (Lumley T, Brody J, Dupuis J, Cupples LA http://stattech.wordpress.fos.auckland.ac.nz/files/2012/11/skat-meta-paper.pdf). We regarded statistical significance for either of the two regions tested based on P=0.05/2 = 0.025.

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 utilizing non-coding annotation from ENCODE and TransFac tracks from the UCSC browser.[23] In addition to the four 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 3,983 participants from the three cohorts. This included 186 selected for severe airflow obstruction, 1,830 selected as a random sample of cohort participants and 1,967 selected because of extreme values for non-pulmonary 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 2,630 SNPs including 207 coding SNPs and 2,046 that are novel defined as not present in 1000 Genomes Phase I. For *ADAM19*, we identified 3,494 SNPs, including 52 coding SNPs and 2,662 novel SNPs (from Table 2a in

Lin H, Wang M, Brody JA, Bis JC, Dupuis J, Lumley T, et al. Methods manuscript submitted to *Circ Cardiovasc Genet* along with this manuscript).

Common variants

For each of the two regions (*ADAM19* and *HRT4*), in Figure 1, the P values are plotted for FEV1/FVC and FEV1in 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 two regions (*ADAM19* rs1422795 and *HTR4* rs11168048).[10] After Bonferroni correction for 316 tests, analysis of individual SNPs identified statistically significant ($P<1.58 \times 10^{-4}$) 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 dataset.[10] After conditioning on the sentinel GWAS SNP in each gene (*ADAM19* rs1422795, *HTR4* rs11168048), only one SNP surpassed the statistical significance threshold for association with FEV1/FVC (*ADAM19* rs13155908, $P = 1.56\times10^{-4}$). 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 LD 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 dataset ($P= 1.53\times10^{-5}$).

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 four 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 one to four and 434 in the fifth) 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 four analyses).

Discussion

Spirometry is the most commonly employed assessment of lung function and FEV1/FVC and FEV1 are critical physiologic measurements in the diagnosis of airflow obstruction and monitoring of its severity and progression in clinical practice. In previous GWAS[9–12], we have identified a number of 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 COPD.[13] In the current paper, we used targeted sequencing of *ADAM19* and *HTR4* to address the question of

whether our previous GWAS findings for FEV1/FVC were due to additional functionally relevant variants or, alternatively, due to the combined burden of rare alleles not represented in the earlier GWAS datasets.

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

ADAM19 is a member of the "A Disintegrin And Metalloprotease" (ADAM) family of membrane tethered glycoproteins and is expressed in most tissues including the lung.[24] In lung epithelial cells, TGF-β1 is a prominent mediator of the response to injury, including fibrosis. ADAM19 was found to be a key responder to stimulation by TGF- 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. [25] ADAM19 can potentiate pro-inflammatory activity of tumor necrosis factor alpha (TNFa), [26] a key modulator of airway inflammation. [27] ADAM19 plays a crucial role in cardiac development; *Adam19*$^{-/-}$ mice have multiple cardiac developmental defects[14] and *ADAM19* copy number variants have recently been identified in patients with congenital heart disease.[15] 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.[11] While serotonin (5-HT) is best studied as neurotransmitter, 5-HT signaling plays an important role in many organ systems.[28] In the lung it is involved in control of breathing [28] and smooth muscle contractility[29]. Serotonin signaling including HTR4 is involved in human airway inflammation. [30] In a primate asthma model, ozone exposure increased *HTR4* expression in the airways[31] and this effect was accompanied by enhanced smooth muscle contractility.[32] 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 compared with adult human lung suggesting an important role in lung development[33]. 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 US, 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 cohort, that contributed the largest number to this dataset, 49% reported that one or both biologic parents had a history of myocardial infarction. Family history of cardiovascular disease was not associated with airways obstruction [age, sex and smoking adjusted odds ratio = 1.07, 95% CI 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 epidemiologic findings that reduced pulmonary function is a risk factor for mortality in the general population independent of traditional risk factors for cardiovascular disease.[1–6]

In analyses of all common variants (MAF>1%) in *ADAM19* and *HTR4* without conditioning on our sentinel GWAS SNPs, we identified a number of SNPs significantly related to either FEV1/FVC or FEV1. However these associations appeared to be explained by our previous GWAS findings because all but one (*ADAM19* rs13155908, P= 1.56×10^{-4}, cut-off P value = 1.58×10^{-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 (.mutationtaster.org/index.html) and PolyPhen-2 [34]. Evaluation of this SNP in the UCSC Genome Browser indicates that it is about 6kb upstream of the transcription start site (TSS) of an *ADAM19* transcript variant suggesting that it could be part of 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 (eQTL) 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's rank correlation coefficient= 0.23, P-value= 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) were associated with *ADAM19* gene expression in the CEU population (P-value = 0.001 for both SNPs) as well as several other ethnic groups. Furthermore, rs1422795 (and rs2277027) had significant *cis*-eQTLs in multiple other tissues including nerve (P-value = 5.3×10^{-7}), adipose (P-value = 1.1×10^{-6}), skeletal muscle (P-value = 3.0×10^{-5}), whole blood (P-value = 6.7×10^{-6}), artery (P-value = 1.2×10^{-4}), and suggestive *cis*-eQTLs in lung (P-value = 8.0×10^{-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 functional consequence for the protein. A search of transcription element binding sites (TESS, http://www.cbil.upenn.edu/cgi-bin/tess/tess) identified this SNP as located within a potential binding site of the transcription factor 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 SNP 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 LD in this region may help

inform follow-up functional studies to identify the causal variant. We acknowledge that we limited our sequencing effort to a 21 kb LD 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. While 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 more than one causal variant at this locus.

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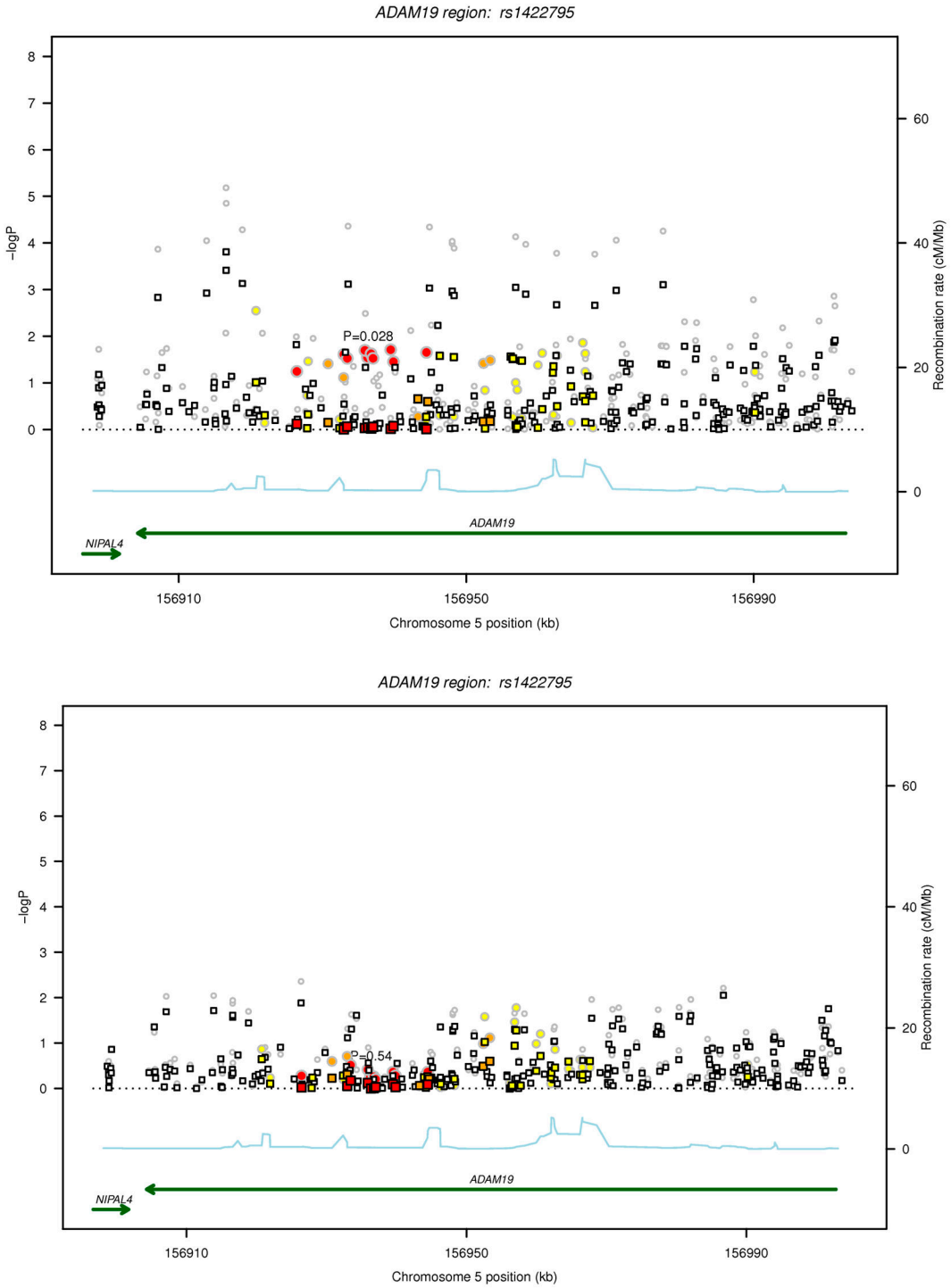

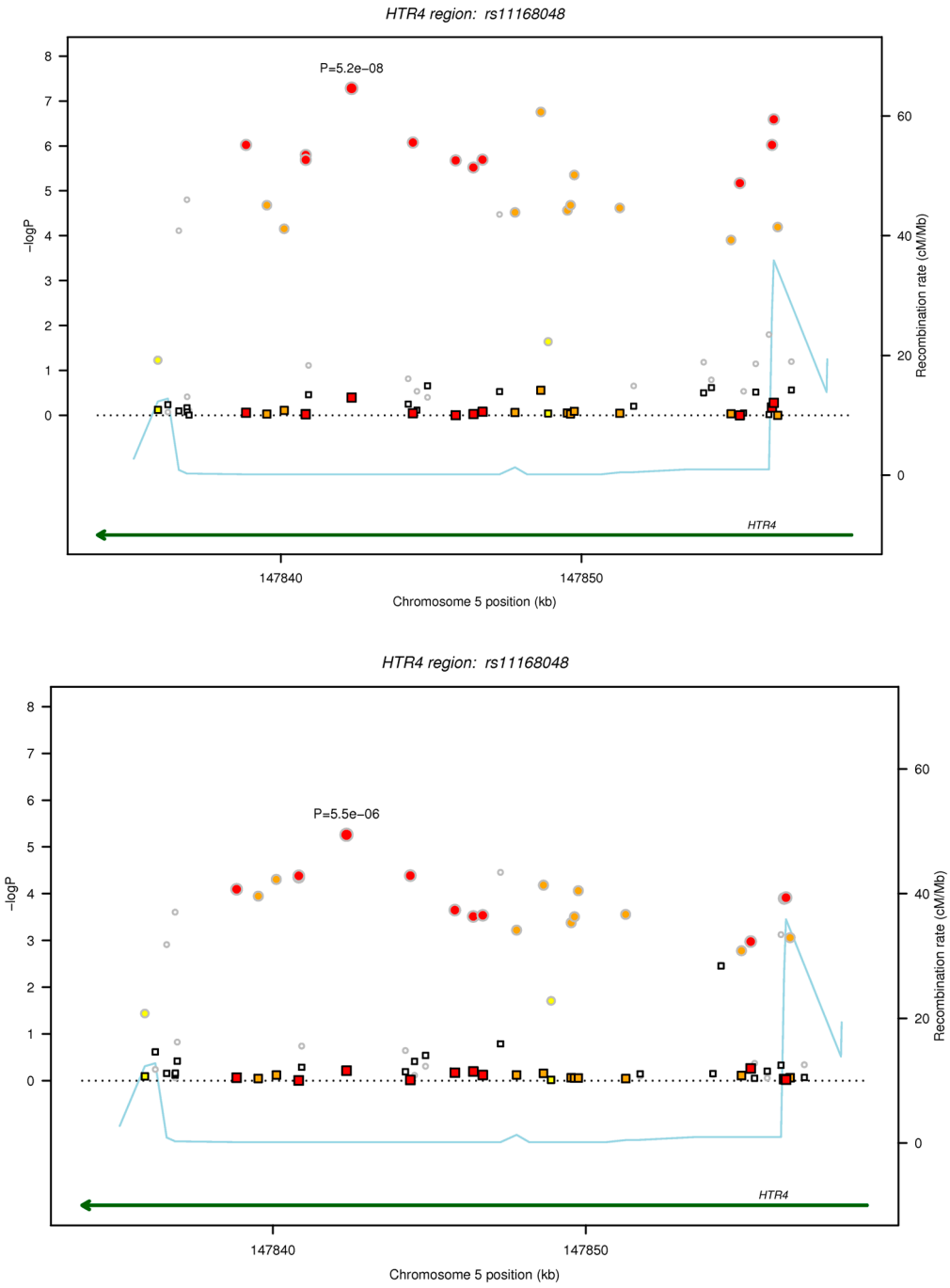

Figure 1.

Regional association plot for common SNPs identified by sequencing in loci investigated for FEV1/FVC and FEV1. The two loci are *ADAM19* on 5q33.3 and *HTR4* on 5q.31.1. The P values for association with the trait (FEV1/FVC or FEV1) from the unconditional analysis are represented by circles and those from the conditional analysis, accounting for the sentinel SNP in each locus, are denoted by squares. For each locus, correlations in the combined study sample between the sentinel SNP from the GWAS and other SNPs identified by sequencing in the region are depicted in red when $0.8 \leq r^2 < 1$, orange when

$0.5 \leq r^2 < 0.8$, yellow when $0.2 \leq r^2 < 0.5$ and white when $r^2 < 0.2$. Gene annotations are shown in green, and estimated recombination rates from HapMap are shown in light blue. The sentinel SNP (rs1422795 for *ADAM19* and rs11168048 for *HTR4*) is annotated with its unconditional association P value for the trait.

(**a**) FEV1/FVC, *ADAM19* locus

(**b**) FEV1, *ADAM19* locus

(**c**) FEV1/FVC, *HTR4* locus

(**d**) FEV1, *HTR4* locus

Table 1

Numbers of subjects and characteristics by cohort

Cohort	N	FEV1 – liters mean(SD)	FEV1/FVC -% mean(SD)	Age at exam mean(SD)	% Female	% Ever Smoked	Pack-years Ever Smokers mean(SD)
ARIC	1914	2.81(0.83)	71.8 (9.9)	54.8(5.7)	48.8	64.4	32.1(23.0)
CHS	1080	2.13(0.66)	70.0(10.9)	72.5(5.5)	53.7	55.3	36.6(29.3)
FHS	989	2.68(0.81)	72.5(8.2)	59.8(11.0)	51.4	62.9	28.7(23.7)

ARIC indicates Atherosclerosis Risk in Communities Study; CHS indicates Cardiovascular Health Study; FHS indicates Framingham Heart Study. Age at exam is the age at which the FEV1 and FEV1/FVC values used in this analysis were measured. This is the baseline exam for ARIC and CHS and the latest exam with acceptable pulmonary function for FHS.

Table 2

Single Nucleotide Polymorphisms 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

						Unconditional Analysis *					
Phenotype	**Target**	**SNP position**	**dbSNP**	**Allele1**	**Allele2**	**Freq1**	**Beta**	**SE**	**P value**	**DirectionFHS, ARIC, CHS** **	**Conditional Analysis P value**
FEV1	HTR4	chr5:147822546	rs11168048	t	c	0.6011	−0.0309	0.0140	5.47×10	−−−	Sentinel-NA
		chr5:147827466	rs4597955	a	g	0.5670	0.0165	0.0133	3.50×10^{-5}	+++	0.1630
		chr5:147824585	rs7735184	t	g	0.3962	0.0251	0.0139	4.11×10^{-5}	−++	0.9672
		chr5:147821023	rs6860089	a	t	0.3864	0.0317	0.0138	4.16×10^{-5}	−++	0.9888
		chr5:147821021	rs6860087	a	t	0.3914	0.0313	0.0138	4.45×10^{-5}	−++	0.9911
		chr5:147820305	rs10041517	t	c	0.3293	0.0307	0.0141	4.96×10^{-5}	−++	0.7584
		chr5:147828839	rs1989153	t	c	0.6767	−0.0300	0.0142	6.59×10^{-5}	−−−	0.6981
		chr5:147819037	rs10463406	t	c	0.3953	0.0235	0.0139	8.05×10^{-5}	−++	0.8637
		chr5:147829952	rs3995091	a	g	0.4292	0.0306	0.0135	8.66×10^{-5}	+++	0.8748
		chr5:147819730	rs10075211	t	c	0.5624	−0.0285	0.0135	1.13×10^{-4}	−−−	0.895
		chr5:147836585	rs7715901	a	g	0.6198	−0.0253	0.0140	1.22×10^{-4}	+−−	0.9497
		chr5:147836526	rs7733088	a	g	0.3792	0.0273	0.0140	1.27×10^{-4}	−++	0.9234
FEV1/FVC	HTR4	chr5:147822546	rs11168048	t	c	0.6025	−0.0093	0.0022	5.17×10^{-8}	−−−	Sentinel-NA
		chr5:147828839	rs1989153	t	c	0.6717	−0.0090	0.0022	1.75×10^{-7}	−−−	0.2784
		chr5:147836585	rs7715901	a	g	0.6216	−0.0090	0.0021	2.56×10^{-7}	−−−	0.5276
		chr5:147824585	rs7735184	t	g	0.3942	0.0081	0.0022	8.38×10^{-7}	+++	0.8955
		chr5:147819037	rs10463406	t	c	0.3936	0.0079	0.0022	9.50×10^{-7}	+++	0.8758
		chr5:147836526	rs7733088	a	g	0.3772	0.0091	0.0021	9.51×10^{-7}	+++	0.6665
		chr5:147821021	rs6860087	a	t	0.3891	0.0086	0.0021	1.59×10^{-6}	+++	0.9395
		chr5:147826900	rs6889822	a	g	0.6306	−0.0088	0.0022	2.02×10^{-6}	−−−	0.8349
		chr5:147821023	rs6860089	a	t	0.3840	0.0083	0.0021	2.04×10^{-6}	+++	0.9517
		chr5:147826008	rs3995090	a	c	0.6030	−0.0080	0.0022	2.10×10^{-6}	−−−	0.9947
		chr5:147826596	rs11742110	t	c	0.6055	−0.0078	0.0022	3.02×10^{-6}	−−−	0.9425
		chr5:147829952	rs3995091	a	g	0.4278	0.0071	0.0021	4.44×10^{-6}	+++	0.8141
		chr5:147835457	rs11168049	t	c	0.6187	−0.0076	0.0022	6.74×10^{-6}	−−−	0.997

						Unconditional Analysis *					Conditional Analysis
Phenotype	Target	SNP position	dbSNP	Allele1	Allele2	Freq1	Beta	SE	P value	DirectionFHS, ARIC, CHS **	P value
		chr5:147817073	rs12374521	t	c	0.5391	−0.0063	0.0021	1.58×10^{-5}	−−−	0.6832
		chr5:147829828	rs1988818	t	c	0.4307	0.0064	0.0021	2.09×10^{-5}	+++	0.9314
		chr5:147819730	rs10075211	t	c	0.5643	−0.0063	0.0021	2.09×10^{-5}	−−−	0.9415
		chr5:147831463	rs6887366	a	t	0.5677	−0.0067	0.0021	2.41×10^{-5}	−−−	0.8996
		chr5:147829724	rs1988819	t	c	0.4346	0.0064	0.0021	2.73×10^{-5}	+++	0.8897
		chr5:147827981	rs1985524	c	g	0.4328	0.0065	0.0021	3.02×10^{-5}	+++	0.8684
		chr5:147827466	rs4597955	a	g	0.5653	0.0042	0.0021	3.36×10^{-5}	+++	0.2972
		chr5:147836715	rs7733410	a	g	0.4254	0.0069	0.0021	6.42×10^{-5}	+++	0.9967
		chr5:147820305	rs10041517	t	c	0.3303	0.0065	0.0022	7.00×10^{-5}	−++	0.7765
		chr5:147816802	rs13156542	t	c	0.5538	−0.0059	0.0020	7.73×10^{-5}	−−−	0.7984
		chr5:147835163	rs10037493	t	c	0.4234	0.0056	0.0021	1.25×10^{-4}	+++	0.9289
	ADAM19	chr5:156849167	rs13155908	a	g	0.8769	−0.0104	0.0029	6.56×10^{-6}	−−−	1.56×10^{-4}
		chr5:156849189	rs10058865	a	c	0.1108	0.0109	0.0031	1.41×10^{-5}	+++	3.84×10^{-4}
		chr5:156866145	rs13190258	a	c	0.8802	−0.0097	0.0030	4.36×10^{-5}	−−−	7.62×10^{-4}
		chr5:156877473	rs59327154	a	g	0.8308	−0.0093	0.0027	4.55×10^{-5}	−−−	9.26×10^{-4}
		chr5:156851428	rs2287749	t	c	0.1220	0.0097	0.0030	5.20×10^{-5}	+++	7.37×10^{-4}
		chr5:156909971	rs11748149	a	g	0.0980	0.0121	0.0031	5.55×10^{-5}	+++	7.79×10^{-4}
		chr5:156889456	rs72811319	a	g	0.1030	0.0123	0.0031	7.36×10^{-5}	+++	8.96×10^{-4}
		chr5:156903456	rs11739504	a	c	0.9039	−0.0122	0.0032	8.71×10^{-5}	−−−	1.03×10^{-3}
		chr5:156846458	rs34458213	a	t	0.8831	−0.0091	0.0030	8.93×10^{-5}	−−−	1.18×10^{-3}
		chr5:156880634	rs72811309	a	g	0.1020	0.0124	0.0031	9.21×10^{-5}	+++	1.08×10^{-3}
		chr5:156880625	rs72811307	t	c	0.8980	−0.0122	0.0031	1.03×10^{-4}	−−−	1.14×10^{-3}
		chr5:156890868	rs11134799	t	c	0.8969	−0.0121	0.0031	1.07×10^{-4}	−−−	1.24×10^{-3}
		chr5:156880896	rs72811310	t	g	0.8974	−0.0118	0.0031	1.29×10^{-4}	−−−	1.33×10^{-3}
		chr5:156839688	rs11466816	t	g	0.1126	0.0090	0.0031	1.36×10^{-4}	+++	1.48×10^{-3}

* 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.

ADAM19 rs1422795 gave P = 0.02 in the unconditional analysis and is thus not listed in this table.

** Direction refers to the sign of the beta coefficient by cohort in the following order: FHS (Framingham Health Study), ARIC (Atherosclerosis Risk in Communities), CHS (Cardiovascular Health Study). A "+" indicates a positive beta coefficient and a "−" indicates a negative beta coefficient