APOM and high-density lipoprotein cholesterol are associated with lung function and per cent emphysema

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ABSTRACT Chronic obstructive pulmonary disease (COPD) is linked to cardiovascular disease; however, there are few studies on the associations of cardiovascular genes with COPD.

We assessed the association of lung function with 2100 genes selected for cardiovascular diseases among 20 077 European-Americans and 6900 African-Americans. We performed replication of significant loci in the other racial group and an independent consortium of Europeans, tested the associations of significant loci with per cent emphysema and examined gene expression in an independent sample. We then tested the association of a related lipid biomarker with forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ratio and per cent emphysema.

We identified one new polymorphism for FEV1/FVC (rs805301) in European-Americans ($p=1.3\times10^{-6}$) and a second (rs707974) in the combined European-American and African-American analysis ($p=1.38\times10^{-7}$). Both single-nucleotide polymorphisms (SNPs) flank the gene for apolipoprotein M (*APOM*), a component of high-density lipoprotein (HDL) cholesterol. Both were replicated in an independent cohort. SNPs in a second gene related to apolipoprotein M and HDL, *PCSK9*, were associated with FEV1/FVC ratio among African-Americans. rs707974 was associated with per cent emphysema among European-Americans and African-Americans and *APOM* expression was related to FEV1/FVC ratio and per cent emphysema. Higher HDL levels were associated with lower FEV1/FVC ratio and greater per cent emphysema.

These findings suggest a novel role for the apolipoprotein M/HDL pathway in the pathogenesis of COPD and emphysema.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of death globally [1] and is characterised by persistent airflow obstruction [2, 3]. Emphysema is defined anatomically by permanent enlargement of airspaces distal to terminal bronchioles with destruction of alveolar walls [4].

Familial studies suggest a genetic influence on COPD [5–8]. Recent genome-wide association studies (GWAS) have identified loci associated with the ratio of forced expiratory volume in 1 s (FEV_1) to forced vital capacity (FVC) and COPD among participants of European ancestry [9–14]. Many of these genes have been shown to influence susceptibility to COPD [15–17]; however, they explained little more than 3% of the variance in lung function.

Emphysema also has a familial predisposition [18]. However, understanding of the genetic basis for emphysema, beyond α_1-antitrypsin deficiency, is more limited. One GWAS identified one genetic locus for radiologist-defined emphysema on computed tomography (CT) [19] and a second identified genetic loci in α-mannosidase-related genes for quantitatively assessed emphysema [20]. Candidate gene association studies have identified additional genes for emphysema [21–26].

Complimentary genotyping strategies to better delineate the genetic basis of COPD and emphysema are therefore warranted. One such strategy is a "gene-centric" genotyping chip, which includes a large panel of candidate genes and often better gene coverage than GWAS chips. No such chips have been designed specifically for lung disease; however, the Institute of Translational Medicine and Therapeutics/Broad/Candidate-gene Association Resource (CARe) Consortium (IBC) chip [27] includes 2100 candidate genes primarily selected for cardiovascular disease.

Respiratory and cardiac function are tightly linked at cellular [28, 29], physiological [30], structural [31] and anatomical levels. For example, endothelial dysfunction is implicated in the pathogenesis of atherosclerosis [32] and emphysema in animal models [33–36] and humans [37], the latter *via* ceramide-mediated endothelial cell apoptosis [38–40]. High-density lipoprotein (HDL) may also be relevant to COPD and emphysema, as HDL increases *in vitro* ceramide levels [41]. HDL levels and function are affected by apolipoprotein M [42–44].

We examined associations of FEV_1/FVC ratio on the IBC chip in European-American and African-American participants in the CARe consortium [45]. Findings were replicated in the SpiroMeta consortium [12]. We performed additional analyses of identified genes with the percentage of emphysema-like lung (per cent emphysema), gene expression and HDL with lung function and per cent emphysema in the Multi-Ethnic Study of Atherosclerosis (MESA) Single Nucleotide Polymorphism (SNP) Health Association Resource (SHARe) and MESA COPD studies.

Methods

Study samples

The appropriate institutional review boards approved the study protocols and written informed consent was obtained from all participants.

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Analyses of lung function

The association of genes and lung function were assessed in the seven CARe cohorts that measured spirometry: Atherosclerosis Risk in Communities (ARIC), Coronary Artery Risk Development in Young Adults (CARDIA), Cleveland Family Study (CFS), Cardiovascular Health Study (CHS), Framingham Heart Study (FHS), Jackson Heart Study (JHS) and the subset of MESA with spirometry. These cohorts have been described previously [46–55] and are summarised in the online supplementary material. Exclusion criteria were lack of valid spirometric or genetic data, age <23 years and a restrictive pattern of spirometry, defined as FVC less than the lower limit of normal [56] and FEV1/FVC ratio >0.70.

Replication of lung function SNPs

Replication of FEV1/FVC ratio was performed in the SpiroMeta consortium, a large independent sample of 14 GWAS studies [12]. Replication for airflow obstruction was performed using publically available data from the SpiroMeta and Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortia [10], which partly overlap with European-American participants in the CARe consortium. Details are provided in the online supplementary material.

Analyses of per cent emphysema

Per cent emphysema was examined in all participants in MESA SHARe, which comprises all participants who consented to genetic analyses in the MESA [46], MESA Family [57] and MESA Air Pollution [58] studies. Spirometry was not required.

Gene expression analyses

mRNA expression was examined in peripheral blood mononuclear cells in the MESA COPD Study, an independent sample described in the online supplementary material.

Phenotypic measures

Spirometry

Pre-bronchodilator spirometry was performed by trained and certified spirometry technicians in accordance with American Thoracic Society guidelines. Spirometry methods and equipment were highly standardised and in some cases identical across cohorts, as described in the online supplementary material.

Per cent emphysema

Per cent emphysema was assessed in MESA SHARe on lung fields of cardiac CT scans, which image ~70% of lung volume from the carina to the lung bases, at a single centre by trained readers, as previously described, and validated compared to full-lung scans [59]. Per cent emphysema was defined as percentage of total voxels in the lung < -950 HU. The MESA COPD study used the same approach on full-lung scans using Apollo software (Vida Diagnostics, Coralville, IA, USA).

HDL

HDL was measured in EDTA plasma using the cholesterol oxidase method (Roche Diagnostics Corporation, Indianapolis, IN, USA) after precipitation of non-HDL cholesterol with magnesium/dextran [60].

Genotyping

All CARe participants were genotyped using the IBC Illumina iSELECT array, a 50 000 gene-centric SNP array [27]. All genotyping was performed at a single centre. Quality control methods are described in the online supplementary material.

MESA SHARe participants were genotyped using the Affymetrix Genome-Wide Human SNP Array 6.0 platform (Affymetrix, Santa Clara, CA, USA) at a single centre.

Statistical analyses

Analyses of candidate genes with FEV1/FVC ratio employed linear regression, stratified by race and adjusted for age, age^2, height, height^2, sex, smoking status, pack-years, pack-years^2, site (if applicable) and the first 10 principal components for ancestry. Association testing of rank-normalised residuals was performed under an additive genetic model [61–63].

Cohort-specific association results were meta-analysed, again stratified by race, using inverse variance weighting in METAL [64] with cohort-specific and overall genomic control. *A priori*, we planned to replicate our top loci identified among European-Americans in the African-American cohorts and *vice versa* as distinct cohorts. Race-specific results were then meta-analysed in METAL [64] for the combined European-American and African-American analyses. The Bonferroni-adjusted thresholds for statistical

significance in CARe were 1.31×10^{-6} in European-Americans and 1.13×10^{-6} in African-Americans and combined analyses, which are exceedingly conservative for the IBC chip.

Analyses for log-transformed per cent emphysema used the same analytical approach supplemented with a linear mixed effects model for family-based-data [62], and adjustment for age, sex, site, scanner, height, weight, tube current, cigarettes per day, pack-years, asthma and principal components for ancestry.

Analysis details for gene expression and HDL association studies with lung function and per cent emphysema are provided in the online supplementary material.

To address multiple comparisons, we considered analysis of per cent emphysema to be analogous to a modified Holm's procedure [65] on the pathway of apolipoprotein M. We hypothesised that SNPs rs805301 and rs707974 are in linkage disequilibrium with the causative *APOM* (apolipoprotein M) variant that affects per cent emphysema, which affects FEV1/FVC ratio, thus the Holm–Bonferonni corrected threshold for statistical significance for subsequent analyses was set at 0.025.

Results

The mean ± SD age of the 26 977 CARe participants with spirometry was 54 ± 13 years, 52% were ever-smokers, with a median of 20 pack-years. Additional characteristics of the 20 077 European-American and 6900 African-American participants are shown by cohort and race in table 1.

Association study of 2100 candidate genes with lung function in CARe

Among European-Americans, we identified one new SNP (rs805301) for FEV1/FVC ratio (fig. 1a). Among African-Americans, no SNPs were significantly associated with FEV1/FVC ratio using the Bonferroni cut-off; however, three SNPs were significant with the less conservative cut-off ($p<1 \times 10^{-5}$) (fig. 1b). In the combined European-American and African-American analysis, we identified a second new SNP (rs707974) for FEV1/FVC ratio (fig. 1c and table 2).

The new SNP (rs805301) identified in European-Americans was selected for the IBC chip as a variant in *APOM* based upon Genome Build 36 and is annotated in *BAG6* (B-cell lymphoma-2-associated athanogene 6), which is the upstream flanking gene of *APOM*, on Genome Build 37.3. It replicated among African-Americans ($p=0.036$) and remained significant in the combined meta-analysis (table 2). The risk allele (C) was associated with a decrease in FEV1/FVC ratio in both racial groups.

The new SNP identified in the combined European-American and African-American analysis, rs707974, was the second most significant SNP in African-Americans (table 2) and would have been significant with a less stringent Bonferroni cut-off. It was also selected as an *APOM* variant and is now annotated in *GPANK1* (G patch domain and ankyrin repeats 1), the downstream flanking gene of APOM separated by an open-reading frame, C6orf47. It was not significant for FEV1/FVC ratio in European-Americans ($p=2.84 \times 10^{-5}$).

The SNPs rs805301 and rs707974 were not in high linkage disequilibrium in European-Americans or African-Americans ($r^2=0.07$ and $r^2=0.03$, respectively), suggesting that they are separate loci (fig. 2). They were also not in high linkage disequilibrium with the previously described *AGER* (advanced glycosylation end product receptor) SNP rs2070600 in European-Americans ($r^2=0.035$ and $r^2=0.37$, respectively) [12, 13]. In addition, rs805301 remained associated with FEV1/FVC after adjustment for rs2070600 ($p=6.82 \times 10^{-4}$) and rs2070600 was only nominally associated with FEV1/FVC among African-Americans ($p=0.009$). These findings suggest that associations of rs805301and rs707974 with lung function are unrelated to *AGER*.

Sensitivity analyses restricted to participants free of clinical cardiovascular disease, aged ⩽55 years and free of asthma yielded similar results, as did analyses additionally adjusted for diabetes, hypertension and asthma (online supplementary material). Analyses stratified by smoking status yielded similar results (online supplementary material).

The other top loci in African-Americans were in *NFKBIA* (nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor-α) and *PCSK9* (proprotein convertase subtilisin/kexin type 9) (table 2). *PCSK9* is related to apolipoprotein M [67] and six of the top 30 SNPs for FEV1/FVC in African-Americans were in *PCSK9* (fig. 2). SNPs in neither gene replicated in European-Americans. Regional association plots for additional loci are displayed in online supplementary figure S3.

Results for the FEV1 are displayed in online supplementary figures S4, S5 and S6. Top SNPs associated with FEV1/FVC ratio and FEV1 are presented in online supplementary tables S1 and S2, respectively.

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TABLE 1 Characteristics of European-American and African-American participants with spirometry in the Candidate-gene Association Resource cohorts

	European-American participants							African-American participants							Combined
	ARIC	FHS	CHS	CARDIA	MESA	CFS	All	ARIC	JHS	CHS	CARDIA	MESA	CFS	All	
Subjects n	8825	5316	3479	1228	1049	180	20 077	2740	1597	503	1052	799	209	6900	26 977
Age years	54±6	48±13	73±6	36±3	65±10	51±15	55±13	53±6	50±12	73±6	34±4	65±10	48±14	52±13	54±13
Male %	47	46	44	47	50	47	46	37	38	35	40	47	41	39	44
Height cm	169±9	170±9	165±9	172±9	169±10	170±10	169±10	168±9	169±9	164±9	170±10	168±10	169±10	168±9	169±10
BMI kg·m^{-2}	27±5	27±5	26±4	26±5	28±5	32±8	27±5	30±6	32±7	28±5	29±7	30±6	34±8	30±7	28±6
Cigarette smoking %															
Never	41	52	47	61	40	50	46	49	69	51	62	40	40	55	48
Former	36	35	43	20	51	21	36	23	17	35	11	45	35	23	33
Current	23	13	10	19	9	29	18	28	14	14	27	15	25	22	19
Pack-years#	26 (12–40)	16 (7–30)	30 (13–50)	10 (4–19)	19 (8–35)	14 (5–32)	23 (10–38)	17 (8–30)	14 (7–26)	21 (10–38)	6 (3–11)	15 (6–28)	11 (5–24)	14 (6–27)	20 (9–36)
FEV_1 % pred	94±16	98±14	89±22	99±11	92±16	96±18	94±17	97±17	95±15	92±24	100±14	94±19	92±19	96±17	95±17
FEV_1/FVC	0.74±0.08	0.76±0.07	0.69±0.10	0.79±0.06	0.73±0.09	0.77±0.07	0.74±0.09	0.76±0.08	0.80±0.09	0.70±0.11	0.81±0.06	0.75±0.10	0.78±0.08	0.77±0.09	0.75±0.09

Data are presented as mean±SD or median (interquartile range), unless otherwise stated. ARIC: Atherosclerosis Risk in Communities; FHS: Framingham Heart Study; CHS: Cardiovascular Health Study; CARDIA: Coronary Artery Risk Development in Young Adults; MESA: Multi-Ethnic Study of Atherosclerosis; CFS: Cleveland Family Study; JSH: Jackson Heart Study; BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity. #: in ever-smokers.

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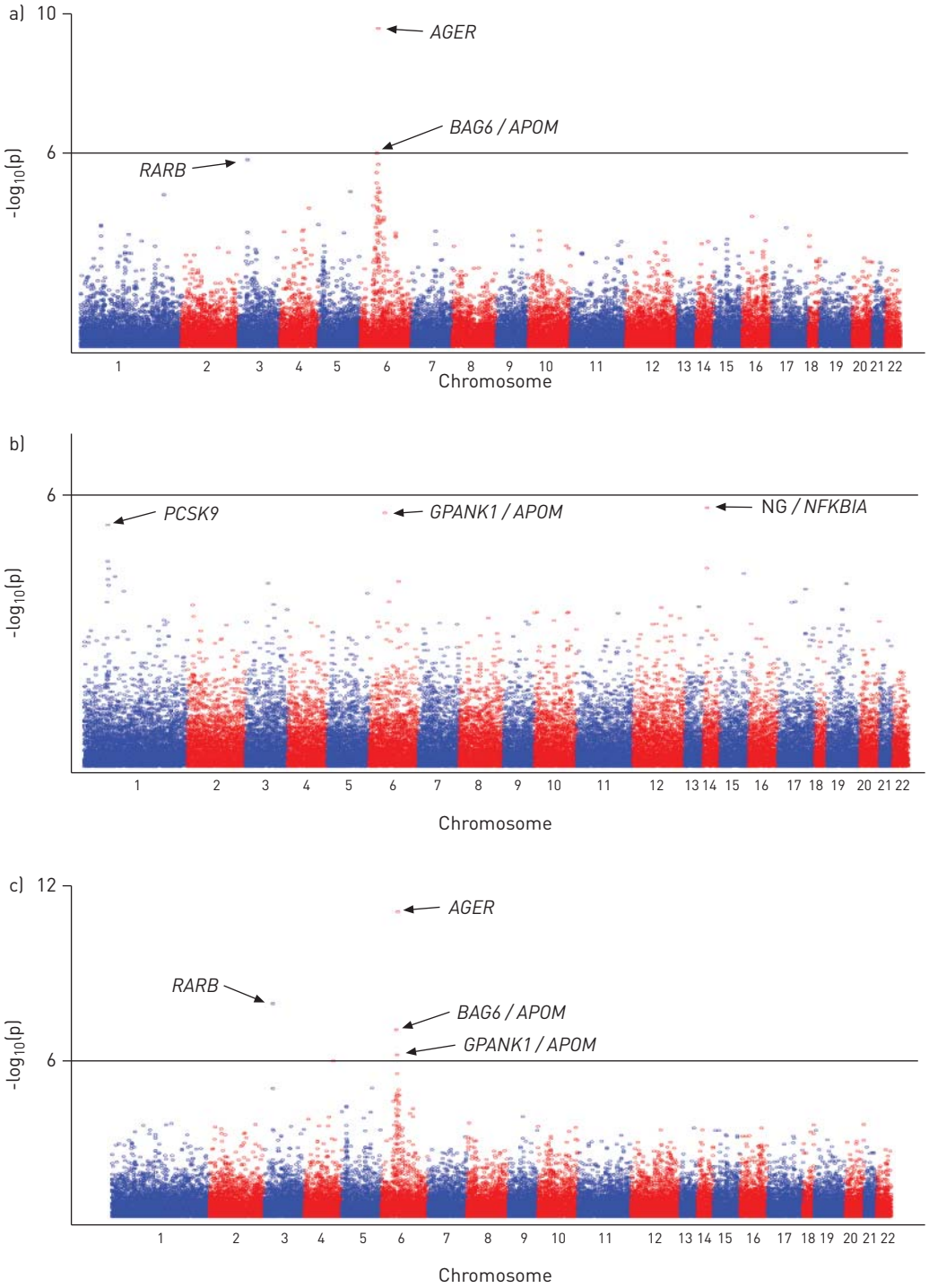

FIGURE 1 Manhattan plots of association results for forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ratio stratified by race and combined. Manhattan plots are ordered by the chromosome position of the association results for FEV1/FVC ratio. The top loci are labelled with arrows. a) Meta-analysis of 38 294 single nucleotide polymorphisms (SNPs) among 20 077 European-American participants; b) meta-analysis of 44 416 SNPs among 6900 African-American participants; c) meta-analysis of SNPs among combined European-American and African-American participants. The solid black lines represent 1×10^{-6}. *AGER*: advanced glycosylation end product receptor; *RARB*: retinoic acid receptor-β; *BAG6*: B-cell lymphoma-2-associated athanogene 6; *APOM*: apolipoprotein M; *PCSK9*: proprotein convertase subtilisin/kexin type 9; *GPANK1*: G patch domain and ankyrin repeats 1; NG: near gene; *NFKBIA*: nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor-α.

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TABLE 2 Top five single nucleotide polymorphisms (SNPs) associated with the forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio among European-American and African-American participants in the Candidate-gene Association Resource (CARe) cohorts

SNP (function)[#]	Subjects	Chromosome	Gene[¶]	Coded allele	Allele frequency[+]	β ± SE[§]	p-value
European-Americans	20 077						
rs2070600 (ns)		6	*AGER/RNF5*	T	0.05	0.162 ± 0.026	**2.19×10^{-10}**
rs805301 (intron)		6	*BAG6/APOM*	C	0.37	-0.054 ± 0.011	**1.32×10^{-6}**
rs1286664 (intron)		3	*RARB*	T	0.17	0.067 ± 0.014	2.07×10^{-6}
rs6941112 (intron)		6	*STK19/C4B*	A	0.33	0.053 ± 0.011	2.90×10^{-6}
rs3117582 (upstream)		6	*BAG6*	T	0.89	0.081 ± 0.018	5.23×10^{-6}
African-Americans	6900						
rs1951269 (unknown)		14	*NG/NFKBIA*	A	0.78	-0.097 ± 0.021	4.08×10^{-6}
rs707974 (3'UTR)		6	*GPANK1/APOM*	G	0.02	0.291 ± 0.064	5.17×10^{-6}
rs565436 (intron)		1	*PCSK9*	A	0.60	0.08 ± 0.018	9.32×10^{-6}
rs533375 (intron)		1	*PCSK9*	A	0.25	-0.083 ± 0.021	5.29×10^{-5}
rs7156874 (intron)		14	*PSMA6*	A	0.02	0.271 ± 0.068	7.33×10^{-5}
Combined European-American and African-American participants	26 977						
rs2070600 (ns)		6	*AGER/RNF5*	T	0.04	0.168 ± 0.025	**8.37×10^{-12}**
rs1286664 (intron)		3	*RARB*	T	0.18	0.067 ± 0.014	**1.60×10^{-8}**
rs805301 (intron)		6	*BAG6/APOM*	C	0.43	-0.050 ± 0.009	**1.26×10^{-7}**
rs707974 (3'UTR)		6	*GPANK1/APOM*	G	0.10	0.088 ± 0.016	**1.38×10^{-7}**
rs6941112 (intron)		6	*STK19/C4B*	A	0.30	0.052 ± 0.011	1.22×10^{-6}

Data are presented as n, unless otherwise stated. The European-American SNPs represent five loci (r^2 range 0.005–0.20). Bold indicates statistical significance. β: effect estimate; ns: nonsynonymous coding SNP; UTR: untranslated region; *AGER*: advanced glycosylation end product-specific receptor (also known as *RAGE*); *RNF5*: ring finger protein 5; *BAG6*: B-cell lymphoma-2-associated athanogene 6 (also known as *BAT3*); *APOM*: apolipoprotein M; *RARB*: retinoic acid receptor β; *STK19*: serine/threonine kinase 19; *C4B*: complement component 4B (Chido blood group); NG: near gene; *NFKBIA*: nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor-α; *GPANK1*: G patch domain and ankyrin repeats 1; *PCSK9*: proprotein convertase subtilisin/kexin type 9; *PSMA6*: proteasome (prosome, macropain) subunit-α 6. [#]: Function is reported dbSNP Genome Build 37.3 (www.ncbi.nlm.nih.gov/projects/SNP/). [¶]: If two genes are listed for the SNP, the first gene listed was annotated using dbSNP (Genome Build 37.3) and the second gene was annotated using the Institute of Translational Medicine and Therapeutics/Broad/CARe Consortium chip (annotation from Genome Build 36); if there is only one gene listed, there was no discrepancy between Genome Build 37.3 and Genome Build 36. [+]: Frequency of the allele labelled in the table as the coded allele. [§]: Rank-normalised residuals of FEV_1/FVC ratio adjusted for age, age^2, height, height^2, sex, smoking status, pack-years, pack-years^2, the first 10 principal components for ancestry, genomic control at the cohort and meta-analysis level; a cohort-specific site covariate was included in the regression model for cohorts with multiple sites (Atherosclerosis Risk in Communities, Coronary Artery Risk Development in Young Adults, Cardiovascular Health Study and Multi-Ethnic Study of Atherosclerosis).

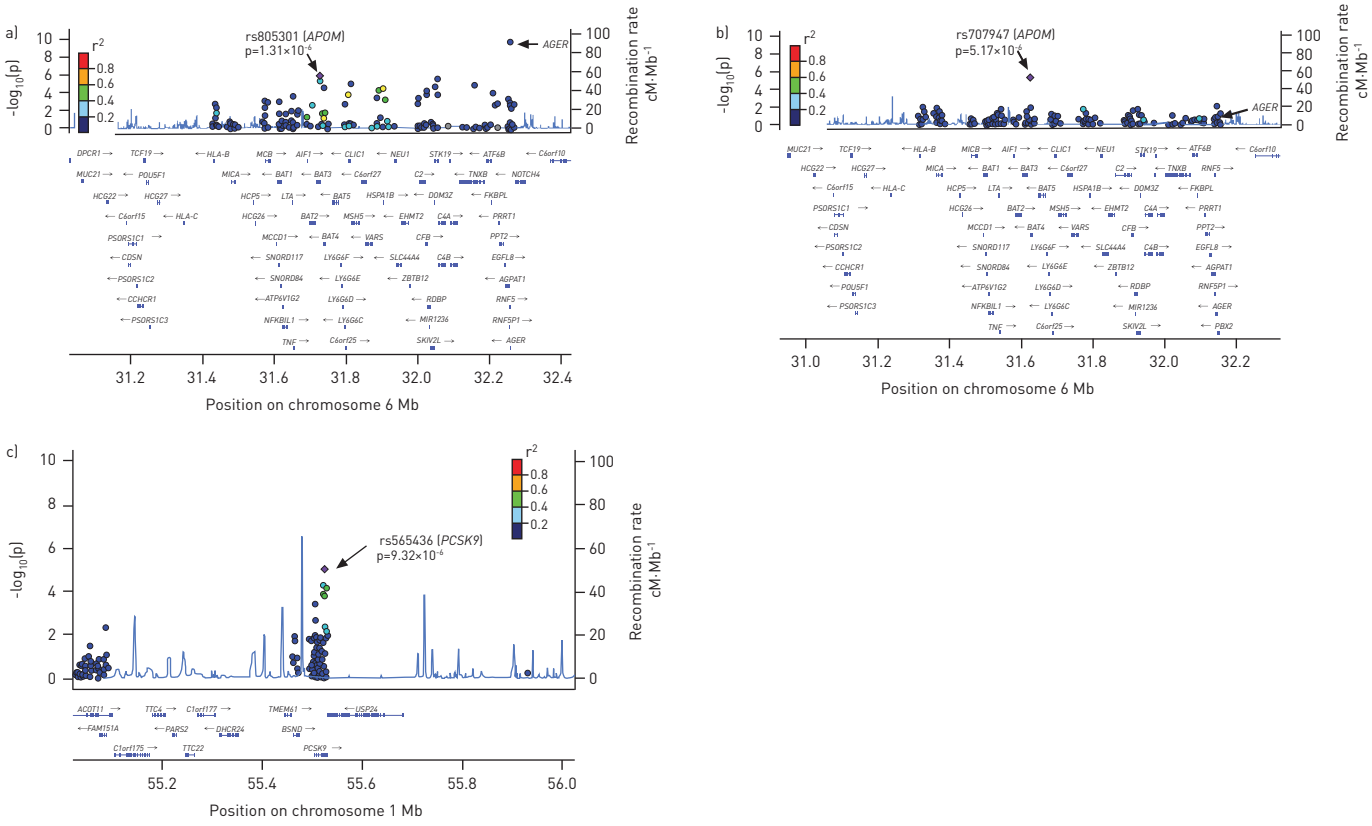

FIGURE 2 Regional association plots of top single nucleotide polymorphisms (SNPs) flanking the *APOM* (apolipoprotein M) (rs805301 and rs707974) and *PCSK9* (proprotein convertase subtilisin/kexin type 9) SNPs for forced expiratory volume in 1 s/forced vital capacity ratio in a) European-American participants, b) African-American participants (chromosome 6), and c) African-American participants (chromosome 1). The selected SNPs with the lowest p-value are illustrated by the purple diamond. The correlations (r^2) of surrounding SNPs in the region are indicated by the colours shown on the graph. For the SNPs flanking *APOM* (rs805301 and rs707974), a 600-kb flanking size was selected to include the *AGER* (advanced glycosylation end product-specific receptor) SNP on the plot, whereas a flanking size of 500 kb was selected for the *PCSK9* SNP. Plots were generated using LocusZoom [66]. The genome builds/linkage disequilibrium populations implemented were hg18/HapMap Phase II CEU and hg/19 1000 Genomes Nov 2010 AFR for European-American and for African-American participants, respectively.

Replication of SNPs flanking APOM in SpiroMeta

Both rs805301 and rs707974 replicated for FEV1/FVC ratio in 20 288 European participants in the SpiroMeta consortium in a consistent direction (β= -0.03, p=0.02 and β=0.05, p=0.02, respectively).

We reviewed publically available results from the SpiroMeta-CHARGE GWAS meta-analysis of airflow obstruction [10]. SNP rs805301 was associated with airflow obstruction (p=0.004) and rs707974 was nominally associated with airflow obstruction in individuals without asthma (p=0.026) (online supplementary material).

Association of SNPs flanking APOM with per cent emphysema in MESA

SNP rs707974 was significantly associated with per cent emphysema among 2552 European-Americans and 2483 African-Americans ($p=4.74\times10^{-4}$ and p=0.009, respectively) and in combined analyses ($p=1.67\times10^{-5}$) (table 3) in MESA. The characteristics of these participants are shown in online supplementary table S3. The direction of the association of rs707974 with per cent emphysema and lung function was consistent: risk allele (A) was associated with greater per cent emphysema and a lower FEV1/FVC ratio.

The association with per cent emphysema persisted in an independent sample of 1138 European-American and 1563 African-American MESA participants who did not have spirometry measures and who were therefore excluded from the lung function analysis (p=0.02 and p=0.003, respectively). Additional adjustment for socioeconomic status yielded similar results, whereas restriction to 418 European-Americans and 209 African-Americans with FEV1/FVC ratio <0.70 yielded nonsignificant results; however, the effect size was greater in African-Americans and similar in European-Americans in these groups compared to the

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overall MESA sample (online supplementary material). SNP rs805301 was not significantly associated with per cent emphysema.

PCSK9 was nominally associated with per cent emphysema in European-Americans (p=0.04) but not African-Americans. *AGER* SNP rs2070600 was significantly associated with per cent emphysema among European-Americans and African-Americans ($p=2.54\times10^{-4}$ and p=0.001, respectively).

Gene expression of SNPs flanking APOM in MESA COPD

APOM expression was significantly inversely associated with FEV1/FVC ratio (table 4) in an independent sample of 101 participants in the MESA COPD Study, the characteristics of which are described in the online supplementary material. We secondarily examined expression of *GPANK1*, *BAG6* and *PCSK9*. *GPANK1* expression was associated with FEV1/FVC ratio (β= -0.096, 95% CI -0.175- -0.017; p=0.02), whereas *BAG6* and *PCSK9* expression were not associated with FEV1/FVC ratio.

APOM expression was positively associated with per cent emphysema in minimally adjusted models and after adjustment for *BAG6* (table 4). *BAG6* expression was not associated with per cent emphysema, except after adjustment for *APOM* (p=0.01). *PCSK9* was significantly associated with per cent emphysema (β=1.150, 95% CI 1.0–1.32; p=0.016).

Association of HDL with lung function and per cent emphysema in MESA

Among 3044 participants with spirometry, higher HDL levels were independently associated with a lower FEV1/FVC ratio (-0.24% per 10 $mg\cdot dL^{-1}$ HDL, 95% CI -0.45- -0.03; p=0.027).

Among 8367 participants with per cent emphysema data, higher HDL levels were independently associated with greater per cent emphysema (0.53% increase in per cent emphysema per 10 $mg\cdot dL^{-1}$ HDL, 95% CI 0.34–0.73; p<0.001). Figure 3 shows the multivariate relationship of HDL to per cent emphysema, which was nonlinear (p<0.001) with a plateau at HDL levels >60 $mg\cdot dL^{-1}$.

To assess for potential survival bias among older participants, we repeated the HDL-emphysema analysis among 5241 participants aged 45–65 years and found consistent results. Findings were also consistent within strata of sex, race and smoking history (online supplementary material).

Discussion

This large biracial study identified two new SNPs for FEV1/FVC ratio, one in European-Americans (rs805301) and one in the combined European-American and African-American analysis (rs707974). Both SNPs were originally selected as *APOM* polymorphisms and are now annotated in genes flanking *APOM*. Both replicated in an independent sample. In addition, rs707974 was significantly associated with per cent emphysema in both European-Americans and African-Americans, *APOM* gene expression was associated with FEV1/FVC and per cent emphysema and HDL was associated with FEV1/FVC and per cent emphysema.

TABLE 3 Association of single nucleotide polymorphisms (SNPs) flanking *APOM* (apolipoprotein M) with per cent emphysema among European-American and African-American participants in the Multi-Ethnic Study of Atherosclerosis

SNP	Subjects n	Reference allele[#]	Allele frequency[¶]	β±SE[+]	p-value
European-Americans	2552				
rs805301		C	0.37	+0.019±0.018	0.29
rs707974		G	0.10	-0.098±0.028	**4.74×10^{-4}**
African-Americans	2483				
rs805301		C	0.58	+0.013±0.018	0.46
rs707974		G	0.02	-0.160±0.061	**0.009**
Combined European-Americans and African-Americans	5035				
rs805301		C	NR[+]	+0.015±0.001	0.14
rs707974		G	NR[+]	-0.094±0.022	**1.67×10^{-5}**

Bold indicates statistical significance. β: effect estimate; NR: not reported. [#]: reference allele frequency; [¶]: log-transformed per cent emphysema < -950 HU adjusted for age, sex, site, computed tomography scanner, height, weight, weight >220 lb, cigarettes per day, pack-years, asthma and principal components for ancestry; [+]: association testing was performed under an additive genetic model stratified by race and subsequently in the combined population controlling for race/ethnicity for each quantitative phenotype. Statistical significance set at 0.025 using a Holm–Bonferonni corrected threshold for significance. The SNPs are not in tight linkage disequilibrium ($r^2=0.059$).

TABLE 4 Association of *APOM* (apolipoprotein M) gene expression with forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ratio and computed tomography (CT) per cent emphysema in 101 participants in the MESA COPD study

	Difference in FEV1/FVC per increase in *APOM* gene expression#	p-value	Difference in per cent emphysema¶ per increase in *APOM* gene expression#	p-value
Model 1	-0.091 (-0.178– -0.003)	**0.04**	1.05 (0.10–2.0)	**0.03**
Model 2	-0.092 (-0.178– -0.003)	**0.04**	1.04 (0.13–1.95)	0.03
Model 3			0.76 (-0.14–1.67)	0.10
Model 4			1.14 (0.20–2.09)	**0.02**

Data are presented as mean (95% CI), unless otherwise stated. We tested the association of gene expression with FEV1/FVC and with per cent emphysema in the Multi-Ethnic Study of Atherosclerosis (MESA) chronic obstructive pulmonary disease (COPD) study implementing linear regression models weighted to account for the sampling schema, as described in the online supplementary material. Bold indicates statistical significance. Model 1: adjusted for age, sex, cohort and race/ethnicity; model 2: model 1 additionally adjusted for smoking status and pack-years; model 3: model 2 additionally adjusted for height and weight; model 4: model 3 additionally adjusted for *BAG6* (B-cell lymphoma-2-associated athanogene 6) (probe-set 210208). #: *APOM* probe-set 214910_s_at; ¶: log-transformed CT per cent emphysema $<$ -950 HU.

The identified SNPs flanking *APOM* are unlikely to be causative variants, but might be linked with a functional *APOM* variant. Consistent with this thinking, the *APOM* promoter SNP rs805297 alters *APOM* expression [68] and is in weak linkage disequilibrium with rs707974 among African-Americans and European-Americans (r^2=0.36 and r^2=0.32, respectively) and rs805301 among European-Americans (r^2=0.23) (online supplementary fig S7).

A*POM* encodes apolipoprotein M, a lipoprotein-associated plasma protein [69]. The majority of apolipoprotein M is found in HDL [70]. In murine models, modifying *APOM* gene expression changes apolipoprotein M plasma concentration, which affects HDL levels, pre-β-HDL formation, reverse cholesterol transport and remodels plasma HDL [42, 70]. Hence, *APOM* gene expression alters the function and quality of HDL.

Apolipoprotein M and HDL are relevant to the pathogenesis of COPD, particularly emphysema, *via* three related pathways. First, HDL inhibits tumour necrosis factor-stimulated sphingosine kinase activity in human endothelial cells, thereby increasing ceramide and decreasing sphingosine-1-phosphate (S1P) cellular levels [41, 71]. Ceramide, a second-messenger molecule, modulates endothelial cell apoptosis and is implicated in emphysema pathogenesis [39, 40]. Secondly, HDL-associated apolipoprotein M is the plasma carrier for S1P and this HDL–apolipoprotein M subclass presents S1P to the S1P1 endothelial cell receptor, which is endothelium-protective [44, 72]. S1P has an essential role in maintaining endothelial barrier integrity in the lung and is implicated in emphysema pathogenesis [44, 73]. Thirdly, HDL binds and incorporates α_1-antitrypsin. HDL-bound α_1-antitrypsin inhibits extracellular matrix degradation and apoptosis in vascular smooth muscle [74, 75].

The relevance of *APOM* and HDL to COPD pathogenesis is further reinforced by our findings that *PCSK9* polymorphisms were associated with FEV1/FVC ratio and, nominally, per cent emphysema, and that *PCSK9* gene expression was associated with per cent emphysema. *PCSK9* augments the degradation of low density lipoprotein receptors [76] and gain-of-function mutations in *PCSK9* cause familial hypercholesterolaemia [77]. HDL levels in patients with *PCSK9* mutations are generally increased [78–80] and most placebo-controlled trials of PCSK9 inhibitors have shown modest increases in HDL levels [81–85]. Furthermore, plasma levels of PCSK9 are associated with plasma apolipoprotein M levels [67].

The association of apolipoprotein M and HDL with FEV1/FVC and emphysema are probably distinct from their relationships to cardiovascular disease and we speculate that the roles of HDL and apolipoprotein M in the lungs are different from their roles in atherosclerosis. Although HDL has long been thought to be atheroprotective, the definitive epidemiological study on HDL and cardiovascular disease suggested no benefit [86] and large-scale randomised clinical trials of cholesterol ester transfer proteins, which raise HDL levels, have yet to show a benefit on clinical cardiovascular events [87, 88]. The literature on apolipoprotein M in cardiovascular disease is relatively small and mixed, with animal studies suggesting atheroprotective effects [42, 43]; however, in humans, plasma apolipoprotein M levels were not associated with atherosclerotic disease [89].

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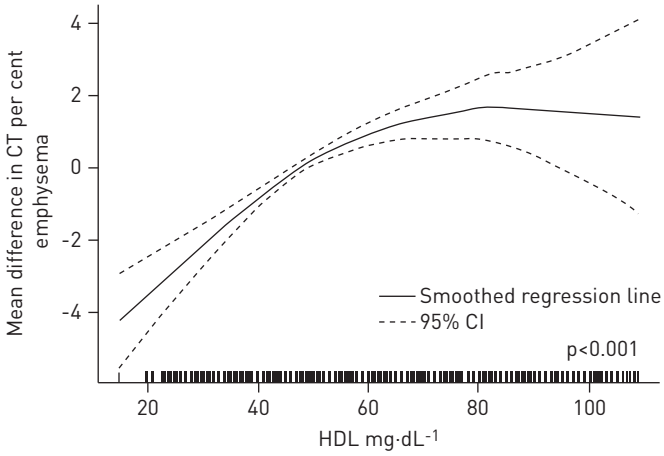

FIGURE 3 Multivariate association between high-density lipoprotein (HDL) and computed tomography (CT) per cent emphysema. Results of multivariate analyses of the relationship between per cent emphysema and plasma HDL among 8367 Multi-Ethnic Study of Atherosclerosis Single Nucleotide Polymorphism Health Association Resource participants are shown. Smoothed regression line adjusted for age, sex, race/ethnicity, height, weight, educational attainment, scanner, tube current, total cholesterol, exercise, pack-years, cigarettes per day, alcohol use, inhaled steroids and use of statins. The figure and p-value was produced using a LOESS smoothing function in a generalised additive model (GAM) in R/GAM (R version 2.13.0; The R Project for Statistical Computing www.r-project.org).

Despite the strong mechanistic support implicating *APOM* in COPD, the latest genome build annotates the new SNPs in genes neighbouring *APOM*, which raises the possibility that they are unrelated to *APOM*. *GPANK1* may be involved in immunity [90] and *BAG6* is implicated in apoptosis [90]; both are associated with lung cancer [91, 92] and neither has been associated with cardiovascular disease. Given that genome builds change over time, the identification of the two new SNPs in the same region in separate racial groups, the gene expression findings and the HDL associations all suggest that *APOM* rather than the other genes are implicated in COPD pathogenesis.

Previous studies of genetic risk for emphysema include two GWAS [19, 20] and candidate gene association studies [21–26]. The GWAS identified *BICD1* (bicaudal D homologue 1) as associated with severe emphysema on radiologist interpretation and α-mannosidase-related genes for per cent emphysema [19]. The candidate gene association studies did not include rs707974 or rs805301.

Two small studies found increased levels of HDL in severe COPD defined by spirometry [93, 94]. Conversely, lower HDL was associated with lower FEV_1 in a population-based study (which did not report the association for FEV_1/FVC ratio) [95] and with advanced COPD and emphysema patients from the Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points Study [96].

The study has several potential limitations. The genomic inflation factor for the meta-analysis in European-Americans was 1.080, suggesting possible population stratification. However, we adjusted our analysis with 10 principal components for ancestry and with cohort-specific and overall genomic control to address population stratification. Furthermore, we replicated the new *APOM* SNP identified in European-Americans (rs805301) in African-Americans, and both SNPs replicated in an independent cohort and with gene expression, all of which makes population stratification less of a concern.

Although we analysed two phenotypes of COPD in general population samples, these traits do not capture the entire phenotypic complexity of clinical COPD. However, results for the two phenotypes were consistent with each other, similar among patients with airflow limitation, and supported by gene expression in a study of clinical disease. Furthermore, multiple prior genes identified for lung function in population-based samples have been replicated in studies of clinical COPD [15–17]. Hence, it is likely that the current findings apply to clinical COPD.

The association between HDL and per cent emphysema may be subject to confounding and reverse causation; a small study suggested that HDL levels decrease in COPD patients undergoing lung transplantation [97]. However, we adjusted for multiple potential confounders in this well-phenotyped cohort and the genetic studies are unlikely to be subject to reverse causation.

Similar to other population-based GWAS, we used pre-bronchodilator spirometry for lung function measurement. However, per cent emphysema was measured on partial-lung CT scans; previously, we validated per cent emphysema on partial-lung scans compared to full-lung scans in this cohort and have

confirmed multiple prior hypotheses using them [30, 59]. Per cent emphysema, like lung function, is related to sex, body size, ancestry and socioeconomic status [98], in addition to current smoking [99]. However, we adjusted for all of these variables in the analyses.

In conclusion, we identified one new SNP related to FEV1/FVC ratio among European-Americans and a second new SNP in the combined European-American and African-American analysis, which was also associated with per cent emphysema. Both new SNPs flank *APOM*, and *APOM* expression was associated with FEV1/FVC ratio and per cent emphysema. *APOM* encodes apolipoprotein M, which is primarily bound to HDL, and higher levels of HDL were associated with lower FEV1/FVC ratio and greater per cent emphysema. Together, these findings suggest a novel effect of the apolipoprotein M–HDL-cholesterol pathway in the pathogenesis of COPD and emphysema. Further examination of this pathway is warranted to determine whether it could be targeted to treat or prevent COPD, and ongoing clinical trials of PCSK9-inhibitors [81–85] and other medications that raise HDL levels [88] may consider monitoring for pulmonary effects.

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