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Adhesion molecules, endothelin-1 and lung function in seven population-based cohorts

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Abstract

Context—Endothelial function is abnormal in chronic obstructive pulmonary disease (COPD); whether endothelial dysfunction causes COPD is unknown.

Objective—Test associations of endothelial biomarkers with FEV_1 using instrumental variables.

Methods—Among 26 907 participants with spirometry, ICAM-1, P-selectin, E-selectin and endothelin-1 were measured in subsets.

Results—ICAM-1 and P-selectin were inversely associated with FEV_1 among European-Americans (−29 mL and −34 mL per standard deviation of log-transformed biomarker, $p<0.001$), as was endothelin-1 among African-Americans (−22 mL, $p=0.008$). Genetically-estimated ICAM-1 and P-selectin were not significantly associated with FEV_1. The instrumental variable for endothelin-1 was non-informative.

Conclusion—Although ICAM-1, P-selectin and endothelin-1 were inversely associated with FEV_1, associations for ICAM-1 and P-selectin do not appear causal.

Keywords

Genetic polymorphisms; growth factors/cytokines/inflammatory mediators; respiratory disease

Introduction

Chronic obstructive pulmonary disease (COPD), defined as an obstructive pattern of spirometry that is not reversible with bronchodilators, is the fourth leading cause of death worldwide (Decramer et al., 2012). The major risk factor for COPD in developed countries is cigarette smoking; however, smoking is neither a necessary nor a sufficient cause of COPD. Endothelial function is abnormal in severe COPD (Dinh-Xuan et al., 1991), but the extent to which endothelial dysfunction contributes to COPD pathogenesis remains unclear given the current, cross-sectional literature on the topic. Such cross-sectional studies cannot traditionally define the direction of an association and may be subjected to bias and confounding.

There are strong biological hypotheses to support a causal role for endothelial dysfunction in COPD. Mechanisms by which abnormalities in endothelial cell function may lead to the development or progression of COPD include adhesion molecule-mediated monocyte, neutrophil, and T-cell adhesion and infiltration (Hogg et al., 2004); vaso- and bronchoconstriction promoted by endothelin-1 (ET-1) (Redington et al., 1995; Yanagisawa et al., 1988); vascular endothelial growth factor (VEGF)-dependent, ceramide-mediated apoptosis of endothelial cells (Petrache et al., 2005); and micro-thrombosis in the pulmonary vasculature (Voelkel & Cool, 2003).

Biomarkers for endothelial dysfunction include adhesion molecules and ET-1, which are also appealing therapeutic targets in COPD given their extant pharmacologic inhibitors. Cross-sectional associations between these biomarkers and lung function have been demonstrated in several studies. The adhesion molecules, Intracellular Adhesion Molecule 1 (ICAM-1), P-selectin and E-selectin are elevated in COPD compared to controls and are associated with lung function in the general population (Cella et al., 2001; Rusznak et al., 2000; Thyagarajan et al., 2009; Walter et al., 2008). ET-1 is elevated in the bronchial epithelial cells and airways of asthmatic and COPD patients (Spiropoulos et al., 2003;

Trakada et al., 2000; Vittori et al., 1992), and plasma ET-1 is elevated in COPD exacerbations (Roland et al., 2001). Nonetheless, prior studies have not clarified whether elevated levels of these circulating biomarkers bear a causal relation to COPD development, or if they simply reflect the cascading results of hypoxic, infectious and cigarette-related endothelial damage.

Instrumental variable analysis may provide an approach to circumvent problems of reverse causation and uncontrolled confounding in the non-experimental setting. Genotype can be used to estimate biomarker levels, which serve as instrumental variables that may be analyzed with respect to the disease of interest. Since genotype is fixed at conception and is free of environmental or behavioral confounding, associations between genetically-estimated biomarker levels and disease support a causal relationship (Ebrahim & Davey Smith, 2008; Lawlor et al., 2008). This technique, also known as Mendelian Randomization, has been used to demonstrate that low density lipoprotein (LDL) cholesterol and lipoprotein(a), but not C-reactive protein or high-density lipoprotein, may be causally related to cardiovascular disease (Elliott et al., 2009; Kamstrup et al., 2009; Linsel-Nitschke et al., 2008; Voight et al., 2012). It has not been applied, to our knowledge, to ICAM-1, the selectins or ET-1, with respect to lung function.

We therefore first tested for cross-sectional associations between soluble ICAM-1, P-selectin, E-selectin, and ET-1 and lung function among 20 021 European-Americans and 6886 African-Americans from the National Institutes of Health/National Heart, Lung, and Blood Institute (NIH/NHLBI) Candidate-gene Association Resource (CARe) (Musunuru et al., 2010). We then tested whether the observed associations may be causal by examining the association of genetically-estimated biomarker levels with lung function.

Methods

Study sample

We included the seven NIH/NHLBI cohorts in CARe that measured lung function: Atherosclerosis Risk in Communities (ARIC) Study, offspring and generation three cohorts from Framingham Heart Study (FHS), Cardiovascular Health Study (CHS), Jackson Heart Study (JHS), Coronary Artery Risk Development in Young Adults (CARDIA), Multi-Ethnic Study of Atherosclerosis (MESA) Lung Study and Cleveland Family Study (CFS). The recruitment and designs of the cohorts have been previously described and are summarized in the Online Supplement. The cohorts included in this analysis recruited unrelated participants except for FHS and CFS, which included families. All cohorts enrolled European- and African-American participants except for FHS and JHS, which enrolled only European-Americans and African-Americans, respectively.

Exclusion criteria were lack of valid spirometric or genetic data, age less than 23 years, and a restrictive pattern of spirometry, defined as forced vital capacity (FVC) less than the lower limit of normal (Hankinson et al., 1999) with a forced expiratory volume in 1 s (FEV_1)/FVC ratio of greater than 0.70 (see Online Supplement). In addition, carriers of T-alleles at SNP rs5491 were excluded from analyses of ICAM-1 as such participants demonstrate factitiously low levels of ICAM-1 on the R&D Systems Assay used by the majority of cohorts (see Online Supplement) (Bielinski et al., 2008).

Appropriate Institutional Review Boards approved study protocols and written informed consent was obtained from all participants.

Quantitative phenotypes

Spirometry—Pre-bronchodilator spirometric measurements were performed by trained and certified spirometry technicians using highly standardized methods and spirometry systems in accordance with American Thoracic Society guidelines (see Online Supplement). Airflow limitation was defined as FEV_1/FVC ratio less than the lower limit of normal (Hankinson et al., 1999).

Biomarkers—Fasting blood samples were drawn, processed and stored using standardized procedures in central laboratories. Soluble biomarker concentrations were determined as described in the Online Supplement. Biomarker measurements were performed at the same visit as spirometry and were measured on the whole cohort or a random subset except as noted in the Online Supplement.

Covariates—Age, gender and race were self-reported. Participants who reported smoking fewer than 100 cigarettes in their lifetime were classified as never smokers, and those who reported smoking during the last 30 d were classified as current smokers. Pack-years of cigarette smoking were calculated as years smoked × cigarettes per day/20. Height and weight were measured using standardized protocols with a stadiometer and balance scales.

Genotyping

The CARe consortium genotyping platform was the ITMAT-Broad-CARe (IBC) Illumina-iSELECT array, a 50 000 gene-centric SNP genotyping array. The SNPs were selected to densely tag approximately 2100 candidate genes primarily related to cardiovascular disease, including the genes for adhesion molecules and ET-1. Additional details regarding procedures and quality control are provided in the Online Supplement.

Statistical analysis

Our primary endpoint for this analysis was the FEV_1, since previous studies have shown associations between this measurement and adhesion molecules. The FEV_1/FVC ratio served as a secondary endpoint. Soluble biomarker concentrations were log-transformed to obtain approximately normal distributions. Race-stratified Pearson correlations between log-biomarker levels were computed.

Lung function was regressed on each log-transformed biomarker using generalized linear models stratified by race and adjusted for sex, age, height, weight, cohort, site, smoking status, pack-years and the first three genetic principal components. These latter terms were generated using EIGENSTRAT (Harvard University, Boston, MA) (Price et al., 2006). Generalized estimating equations were used to account for familial clustering (Zeger & Liang, 1986). Covariates were specified *a priori* and were chosen based upon known determinants of lung function (sex, age, smoking, principal components) as well as precision variables (height, weight, cohort, site). Interactions with site and cohort were tested using interaction terms.

To characterize gene-biomarker associations, each log-transformed biomarker was regressed on its respective candidate SNPs within *ICAM1*, *SELP*, *SELE* or *EDN1* using multiple linear regression under an additive genetic model. The threshold for statistical significance was set at a Bonferroni-corrected $p<0.05$. Significantly-associated SNPs were retained and their association with lung function was examined in similar models to examine gene-lung function associations.

Next, each log-transformed biomarker was regressed on the respective retained SNPs using unadjusted linear regression to derive a prediction equation. This prediction equation was

used to determine a genetically-estimated predicted value for each biomarker for each participant based upon genotype at the retained SNPs. The proportion of variance in soluble biomarker levels explained by the genetic prediction equations was computed (R^2), as well as the proportion of variance explained by the prediction equations plus the covariates (adjusted R^2).

Instrumental variable analysis proceeded as follows. The association of the genetically-estimated biomarker values, or instrumental variables, with lung function was tested using generalized linear models. The delta method of deriving approximate probability distributions was used to compute standard errors that reflect the variance in the subject-specific predicted values. All models were stratified by race and adjusted for the same covariates.

The threshold for statistical significance for these analyses was set at $p<0.05$ since there was only one primary outcome (FEV_1) and all performed analyses are reported (Rothman, 1990). Instrumental variable analyses were also repeated using all SNPs found to be significantly associated with a given biomarker among the 50 000 gene-centric SNPs in CARe.

Cross-sectional and instrumental variable analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC). Additive genetic models were executed in PLINK version 1.07 (Harvard University, Boston, MA) (Purcell et al., 2007).

Results

Characteristics of the 20 021 European- and 6886 African-American participants included from the seven cohorts are shown in Table 1. The mean age of the included participants was 54 years, 44% were men, 52% had smoked cigarettes with a median of 20 pack-years and 17% had airflow limitation.

In this sample, ICAM-1 was measured for 10 289 participants, P-selectin for 4243, E-selectin for 418 and ET-1 for 2432. Median biomarker levels are shown in Table 1. Compared to participants without biomarker measurements, participants with biomarker measurements were slightly younger, and less likely to smoke or to have airflow limitation (Online Supplementary Table 1).

ICAM-1 was correlated with P-selectin ($r=0.15$ and $r=0.28$ among European- and African-Americans, respectively [both $p<0.001$]) and E-selectin ($r=0.31$ [$p<0.001$] and $r=0.26$ [$p=0.005$], respectively). In contrast, ET-1 levels were negatively correlated with P-selectin ($r=-0.38$, $p=0.04$) and not correlated with ICAM-1 ($p=0.76$).

Endothelial biomarkers and lung function

Higher levels of ICAM-1 were significantly associated with a lower FEV_1 among European-Americans (Table 2). An association of similar magnitude was observed among African-Americans, and the combined analysis showed that a one standard deviation (SD) increase in ICAM-1 was associated with a 34 mL decrement in the FEV_1. In European-Americans, the relationship between ICAM-1 and the FEV_1/FVC ratio was consistent with that for the FEV_1 but fell short of statistical significance. A similar pattern of association with lung function was observed for P-selectin. In the small sample with E-selectin levels, there were no significant, inverse relationships between the biomarker and lung function.

Higher levels of ET-1 were significantly associated with lower FEV_1 among African-Americans, with a one SD increase in ET-1 associated with a 22 mL decrement in FEV_1.

The association of ET-1 with FEV_1/FVC ratio was in a consistent direction, but was not statistically significant.

There were no significant interactions by cohort or site.

Genetically-estimated biomarker levels and lung function

Among European-Americans, the proportion of variance in biomarker levels explained by the retained candidate SNPs (R^2) was 1.3% for ICAM-1 and 5.9% for P-selectin. Among African-Americans, the R^2 was 1.0% for ICAM-1 and 1.6% for P-selectin (Supplementary Table 2). No SNPs within *SELE* or *EDN1* were significantly associated with E-selectin or ET-1, respectively, and therefore instrumental variables were not created for these two biomarkers.

There were no significant associations found between genetically-estimated levels of ICAM-1 or P-selectin and the FEV_1 among European- or African-Americans (Table 3). Effect estimates were close to the null and 95% confidence intervals excluded clinically meaningful differences. Similarly null relationships were observed for the FEV_1/FVC ratio. Findings were similar in sensitivity analyses among non-smokers, heavy smokers and participants with airflow limitation (Online Supplementary Tables 6–8). No significant associations were found when restricting to participants less than 55 years of age, participants with at least one biomarker measurement, or, for ICAM-1, all participants including T-allele carriers at rs5491.

Discussion

In this large, bi-racial study of seven pooled cohorts, soluble levels of ICAM-1 and P-selectin were inversely associated with the FEV_1 in a trend consistent with an obstructive pattern of spirometry. However, there was no evidence to suggest a causal relationship of these biomarkers to lung function given the negative findings for genetically-estimated levels of ICAM-1 or P-selectin and lung function. We also demonstrated, for the first time in a large sample, that serum ET-1 levels were inversely related to lung function, although we were unable to genetically estimate ET-1 levels due to inadequate power.

These associational results are consistent with biologic and epidemiologic data supporting alterations in adhesion molecules in COPD. ICAM-1, P-selectin and E-selectin are known to recruit neutrophils, monocytes and T-cells, which are central to COPD histopathology and pathogenesis (Hogg et al., 2004). Cell cultures from patients with COPD increase ICAM-1 gene expression and release more ICAM-1 versus cells from controls (Rusznak et al., 2000). Studies of “bronchitics” and patients with COPD have found mixed evidence for increased expression of ICAM-1, P- and E-selectin among the subset of patients with an obstructive component to their disease (Cella et al., 2001; Di Stefano et al., 1994; Ferroni et al., 2000; Noguera et al., 1998; Riise et al., 1994). Prior reports from the CARDIA and Framingham cohorts have shown ICAM-1 and P-selectin to be inversely associated with the FEV_1 (Thyagarajan et al., 2009; Walter et al., 2008), data from which were included in the current study.

No prior large studies, to our knowledge, have demonstrated an association between serum ET-1 and the FEV_1. In one small case-control study, sputum ET-1 levels were shown to be elevated in nine COPD patients at baseline, but not plasma levels (Chalmers et al., 1999). In a sample of 67 COPD patients, plasma ET-1 levels were inversely associated with baseline FEV_1 and FVC, and during exacerbations, both sputum and plasma levels of ET-1 were shown to increase (Roland et al., 2001). There is mixed evidence from small studies regarding whether baseline peripheral plasma ET-1 levels are elevated in COPD and

emphysema compared to controls, and whether elevations are limited to subjects with pulmonary hypertension (Carratu et al., 2008; Celik & Karabiyikoglu, 1998; Yamakami et al., 1997). ET-1 classically exerts potent intravascular effects such as vasoconstriction and resultant pulmonary hypertension, up-regulation of inflammatory cytokines and leukocyte accumulation, but also causes airway effects including mucous secretion, airway edema and bronchial hyper-responsiveness (Helset et al., 1996; Redington et al., 1995; Roland et al., 2001; Yanagisawa et al., 1988).

Although the magnitude of the biomarker-lung function associations shown in our analysis was modest, this is expected in a general population sample. These results, however, are also much less likely to be biased by factors that affect clinical cases versus controls (e.g. medication and treatment effects, comorbidities, selection bias) and hence may provide biological insights that are likely to be magnified in clinical populations, especially if the biomarkers play a causal role. To investigate this possibility, we performed instrumental variable analysis.

We examined the relationships of genetically-estimated levels of adhesion molecules with lung function, since these associations are theoretically free of reverse causation, as well as environmental or behavioral confounding (Ebrahim & Davey Smith, 2008). There was no convincing evidence to suggest that genetically-determined variability in ICAM-1 or P-selectin is causally related to lung function. In fact, with respect to the association between genetically-estimated ICAM-1 and P-selection and the FEV_1, effect estimates approached zero, and narrow 95% confidence intervals effectively ruled out any clinically meaningful relationships (i.e. all upper and lower limits were absolutely less than 200 mL). The negative findings were consistent in the large, total sample, as well as in race-stratified and sensitivity analyses.

We are not aware of any prior studies using this technique to examine whether these biomarkers are causally associated with lung function. Two recent instrumental variable analyses yielded non-significant results with respect to COPD and the inflammatory biomarkers CRP (Dahl et al., 2011) and IL6 (van Durme et al., 2011). A recent study also suggested that genetic variants associated with plasma E-selectin levels may be causally associated with diabetes risk (Qi et al., 2010).

In addition to the use of novel techniques, this study's strengths include a large pooled sample from well-characterized population-based cohorts, a strong biological hypothesis, and consistent methods for spirometry, biomarker assay and genotyping. Nonetheless, there are a number of limitations. While pooling data from heterogeneous cohorts is inferior to performing one extremely large study, pooling allowed an adequate sample size of participants broadly recruited from the general US population who were phenotyped and genotyped using highly standardized and often identical protocols. We furthermore found no evidence for confounding or interaction by cohort. With regards to biomarker measurements, this study analyzed soluble levels, whereas membrane-bound adhesion molecules play a more direct role in neutrophil diapedesis and parenchymal infiltration (DiStasi & Ley, 2009). However, soluble ICAM-1 and the selectins are correlated with their membrane-bound expression and provide an index for endothelial activation (Fijnheer et al., 1997; Pigott et al., 1992). ET-1 is also produced in large part by endothelium and secreted into the bloodstream (Yanagisawa et al., 1988), where it exerts potent vasoconstrictive and inflammatory effects.

The inherent limitations of instrumental variable analysis include the following underlying assumptions (Glymour et al., 2012). There must be no unmeasured common determinants of genotype and lung function, such as population heterogeneity, which we have addressed by

race stratification and adjustment for principal components of ancestry. Valid instrumental variables should only be associated with the outcome via the biomarker. To address this, we only used SNPs within each biomarker's respective candidate gene; however, we cannot completely exclude the possibility of genetic pleiotropy given the current state of knowledge. Weak instruments, which are only weakly correlated with the biomarker of interest, may also introduce bias. While our genotype-biomarker associations had only modest predictive power, probably due to the relative importance of non-genetic sources of variation for these biomarkers, our instrument strengths for ICAM-1 and P-selectin were similar to prior publications (Elliott et al., 2009; Kamstrup et al., 2009; Linsel-Nitschke et al., 2008). Using additional SNPs outside of the candidate genes did not substantially improve our instrument strength and introduced the potential problem of genetic confounding via pleiotropic effects of the ABO blood group gene (*ABO*) on glycosylation and transport of adhesion molecules. Since we were unable to generate reliable instrumental variables for E-selectin or ET-1, we did not analyze these biomarkers in this fashion.

Another potential limitation was power, despite the large sample. The derivation of instrumental variables relied upon the subsets with biomarker measurements, and the number with E-selectin and ET-1 was too small to generate reliable estimates. The same was broadly true for African-American subjects. For ICAM-1 and P-selectin, among European-Americans, we maximized our power by using the genotype-biomarker associations demonstrated in subsets to impute instrumental variables for the entire sample.

We believe that the uniformity of negative results in this large sample makes type II error unlikely, and strongly suggests that genetically-determined variability in ICAM-1 and P-selectin does not play a major causal role in low lung function. Of note, we did not rule out the possibility that environmentally-determined variability in ICAM-1 and P-selectin could still contribute to decrements in lung function, or that these biomarkers may mediate environmental risk related to smoking and other risk factors.

Conclusions

Soluble ICAM-1 and P-selectin were consistently and inversely associated with lung function; however, there was no evidence for an association between lung function and genetically-estimated levels of ICAM-1 and P-selectin, and hence no support for a causal relationship. Serum ET-1 was inversely associated with the FEV_1 among African-Americans, which is novel, biologically plausible, and potentially therapeutically evocative, however inadequate power prevented the examination of causal inference for ET-1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of European-American and African-American participants in the Candidate-gene Association Resource (CARe) with spirometry and genotypic information.

	European-Americans							African-Americans							Total
	ARIC	FHS**	CHS	CARDIA	MESA	CFS	All	ARIC	JHS	CHS	CARDIA	MESA	CFS	All	
N	8818	5288	3459	1227	1049	180	20021	2737	1593	502	1048	799	207	6886	26907
Age (years) mean±SD	54.3±5.7	47.9±12.8	72.9±5.6	35.7±3.3	64.9±9.9	51.3±15.0	55.2±13.0	53.2±5.8	50.2±11.8	73.2±5.6	34.4±3.8	64.8±9.7	47.6±14.1	52.3±13.0	54.4±13.0
Male sex (%)	46.6	46.1	44.4	47.4	50.3	47.2	46.3	36.5	38.1	35.5	40.5	46.9	40.1	38.7	44.4
BMI (kg/m^2) mean±SD	26.8±4.7	27.2±5.2	26.2±4.4	25.8±5.2	27.6±4.9	31.9±7.5	26.8±4.9	29.6±6.0	31.8±7.5	28.3±5.1	29.0±6.8	29.5±5.6	34.4±8.2	30.1±6.6	27.7±5.6
Smoking (%)															
Never	41.2	51.6	47.1	60.6	39.8	50.0	46.2	49.1	68.5	51.4	61.8	40.6	40.1	54.4	48.3
Former	35.5	35.0	42.8	20.5	50.8	20.6	36.4	22.9	17.1	34.3	10.8	44.6	35.3	23.4	33.1
Current	23.3	13.4	10.1	18.8	9.3	29.4	17.5	28.1	14.3	14.3	27.4	14.9	24.6	22.1	18.7
Pack-years * median (IQR)	26 (12, 40)	16 (7, 30)	30 (13, 50)	10 (4, 19)	16 (4, 33)	14 (5, 32)	22 (9, 38)	17 (7, 30)	14 (7, 26)	21 (9, 38)	6 (3, 10)	13 (4, 25)	11 (4, 23)	14 (6, 27)	20 (8, 36)
ppFEV$_1$ mean±SD	94.0±16.2	97.5±16.2	89.1±21.6	99.0±11.4	91.9±16.2	95.6±17.6	94.3±16.8	97.2±16.9	94.6±15.0	91.5±23.8	100.0±13.9	94.3±18.8	91.9±18.8	96.1±17.1	94.8±16.9
FEV$_1$/FVC (%) mean±SD	74±8	76±7	69±10	79±6	73±9	77±7	74±9	76±8	81±9	70±11	81±6	75±10	78±8	77±9	75±9
FEV$_1$/FVC <LLN† (%)	19.3	15.8	22.4	12.0	16.0	11.1	18.2	15.2	8.7	25.9	10.3	16.5	17.9	13.9	17.1
Biomarker assay dates	1986–89	1998 2002–05	1989–93	2000–01	2000–02	2001–06		1986–89	2000–04	1989–93	2000–01	2000–02	2001–06		
ICAM-1															
N	468	5258	1333	998	536	179	8772	175	-	186	754	198	204	1517	10289
Median level ng/mL (IQR)	273 (239, 323)	239 (207, 283)	317 (278, 363)	137 (121, 162)	271 (239, 304)	281 (237, 313)	247 (203, 299)	238 (179, 295)		290 (198, 362)	157 (134, 186)	249 (187, 308)	249 (168, 319)	184 (146, 263)	240.5 (192, 296)
P-selectin															
N	423	1930	–	1006	–	–	3359	118	–	–	766	–	–	884	4243
Median level ng/mL (IQR)	37.6 (22, 57)	35.7 (28, 45)		35.6 (29, 43)			35.8 (28, 45)	36.4 (23, 63)			36.0 (30, 42)			36.0 (30, 43)	35.9 (28, 44)
E-selectin															
N	–	–	–	–	295	–	295	–	–	–	–	123	–	123	418
Median level ng/mL (IQR)					46.4 (34, 59)		46.4 (34, 59)					51.6 (39, 64)		51.6 (39, 64)	48.0 (35, 60)
Endothelin-1															
N	–	–	–	–	–	–	–	841	1,591	–	–	–	–	2432	2432
Median level pg/mL (IQR)								1.3 (1.0, 1.6)	1.2 (0.9, 1.6)					1.2 (0.9, 1.6)	1.2 (0.9, 1.6)

* Among ever smokers.

** Includes Offspring and Generation 3 cohorts.

† Predicted and lower limit of normal values calculated using Hankinson reference equations (Hankinson et al., 2010).

Abbreviations: ARIC=Atherosclerosis Risk in Communities, CARDIA=Coronary Artery Risk Development in Young Adults, CFS=Cleveland Family Study, CHS=Cardiovascular Health Study, FHS=Framingham Heart Study, MESA=Multi-Ethnic Study of Atherosclerosis, JHS=Jackson Heart Study, SD=Standard Deviation, kg=kilograms, m=meters, BMI=Body Mass Index, IQR=Inter Quartile Range, ppFEV1=percent predicted forced expiratory volume in one second, FEV1/FVC=ratio of forced expiratory volume in one second over forced vital capacity as a percent, LLN=lower limit of normal, ng/mL=nanograms per milliliter, pg/mL=picograms per milliliter.

Table 2

Association of soluble biomarker levels and lung function among European-American and African-American participants in CARe.

	European-Americans		African-Americans		Total	
	Mean difference in lung function per SD unit of log-transformed biomarker (95% CI)	***p* Value**	**Mean difference in lung function per SD unit of log-transformed biomarker (95% CI)**	***p* Value**	**Mean difference in lung function per SD unit of log-transformed biomarker (95% CI)**	***p* Value**
ICAM-1	*N*=8727		*N*=843		*N*=9570	
FEV_1 (mL)	−29 (−43, −15)	<0.001	−45 (−117, 26)	0.22	−34 (−50, −18)	<0.001
FEV_1/FVC (%)	−0.20 (−0.42, 0.02)	0.07	0.15 (−1.00, 1.30)	0.80	−0.20 (−0.45, 0.04)	0.10
P-Selectin	*N*=3359		*N*=884		*N*=4243	
FEV_1 (mL)	−34 (−51, −18)	<0.001	−13 (−41, 15)	0.36	−32 (−47, −18)	<0.001
FEV_1/FVC (%)	−0.23 (−0.47, 0.01)	0.06	0.23 (−0.18, 0.63)	0.27	−0.15 (−0.37, 0.06)	0.16
E-Selectin	*N*=295		*N*=123		*N*=418	
FEV_1 (mL)	−3 (−53, 48)	0.99	51 (−16, 117)	0.13	16 (−24, 57)	0.42
FEV_1/FVC (%)	−0.22 (−1.03, 0.59)	0.59	1.62 (0.31, 2.92)	0.02	0.19 (−0.50, 0.87)	0.59
Endothelin-1			*N*=2432			
FEV_1 (mL)			−22 (−38, −6)	0.008		
FEV_1/FVC (%)			−0.22 (−0.54, 0.10)	0.18		

ICAM-1 models were adjusted for age, sex, height in centimeters, weight in pounds, smoking status, pack-years, cohort, site and principal components 1–3; race was also adjusted for in analyzing the total sample; clustering by family was accounted for by general estimating equations; rs5491 T-allele carriers excluded.

P-selectin models were adjusted for age, sex, height in centimeters, weight in pounds, smoking status, pack-years, cohort, site and principal components 1–3; race was also adjusted for in analyzing the total sample; clustering by family was accounted for among European-Americans and the total sample.

E-selectin models were adjusted for age, sex, height in centimeters, weight in pounds, smoking status, pack-years, site and principal components 1–3; race was also adjusted for in analyzing the total sample.

Endothelin-1 models were adjusted for age, sex, height in centimeters, weight in pounds, smoking status, pack-years, site and principal components 1–3.

SD=Standard Deviation, 95% CI=95% Confidence Interval, ICAM-1=Intercellular Adhesion Molecule 1, FEV_1=forced expiratory volume in one second, mL=milliliters, FEV_1/FVC=ratio of forced expiratory volume in one second over forced vital capacity as a percent.

Table 3

Instrumental variable analysis of the associations of genetically-estimated biomarker levels with lung function using the retained candidate single nucleotide polymorphisms (SNPs).

	European-Americans		African-Americans		Total	
	Mean difference per SD unit of log-transformed genetically-estimated biomarker (95% CI) *N*=20 021	***p* Value**	**Mean difference per SD unit of log-transformed genetically-estimated biomarker (95% CI) *N*=6886**	***p* Value**	**Mean difference per SD unit of log-transformed genetically-estimated biomarker (95% CI) *N*=26 907**	***p* Value**
ICAM-1						
FEV_1 (mL)						
Model 1	25 (−38, 89)	0.44	51 (−100, 201)	0.51	27 (−30, 85)	0.36
Model 2	−4 (−59, 52)	0.90	9 (−147, 166)	0.91	2 (−50, 54)	0.94
FEV_1/FVC (%)						
Model 1	0.18 (−0.8, 1.2)	0.73	−0.57 (−1.7, 0.6)	0.69	−0.054 (−0.9, 1.0)	0.91
Model 2	−0.18 (−1.0, 0.7)	0.68	−1.3 (−4.8, 2.2)	0.52	−0.32 (−1.1, 0.5)	0.37
P-Selectin						
FEV_1 (mL)						
Model 1	2 (−27, 31)	0.89	39 (−69, 147)	0.48	2 (−24, 27)	0.91
Model 2	−7 (−32, 19)	0.61	20 (−77, 117)	0.69	−4 (−60, 53)	0.53
FEV_1/FVC (%)						
Model 1	−0.026 (−0.5, 0.4)	0.90	1.5 (−2.7, 5.6)	0.48	0.072 (−0.4, 0.5)	0.73
Model 2	−0.26 (−0.9, 0.4)	0.31	1.1 (−2.6, 4.7)	0.57	−0.14 (−0.5, 0.2)	0.49

None of the selected candidate SNPs for E-Selectin or Endothelin-1 was significantly associated with biomarker levels, hence instrumental variables were not created.

Multivariate model 1 was adjusted for sex, age, height and cohort. Race was also adjusted for in the total sample. For ICAM-1, T-allele carriers at rs5491 excluded.

Multivariate model 2 was adjusted for sex, age, height, cohort, smoking status, pack-years, weight, waist circumference and principal components. Race was also adjusted for in the total sample. For ICAM-1, T-allele carriers at rs5491 excluded.

SD=Standard Deviation, 95% CI=95% Confidence Interval, ICAM-1=Intercellular Adhesion Molecule 1, FEV_1=forced expiratory volume in one second, mL=milliliters, FEV_1/FVC=ratio of forced expiratory volume in one second over forced vital capacity as a percent.