The protective effect of club cell secretory protein (CC-16) on COPD risk and progression: a Mendelian randomisation study

SUPPLEMENTARY RESULTS

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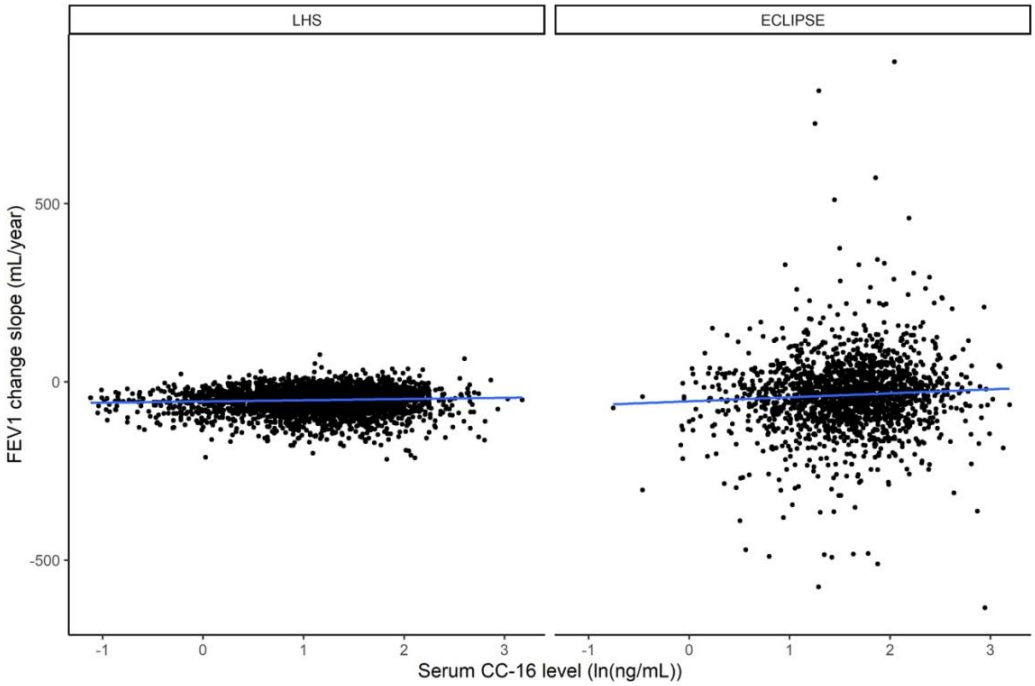
1. TABLE S1: ASSOCIATION BETWEEN SERUM CC-16 LEVEL AND CHANGE IN FEV_1 IN THE BIOMARKER COHORTS

	n	CC-16 effect on annual change in FEV1 (β)†	SE	p
LHS	3444	2.43	0.93	0.01*
ECLIPSE	1821	6.25	3.94	0.11
Meta-analysis	5265	2.64	0.91	0.004**

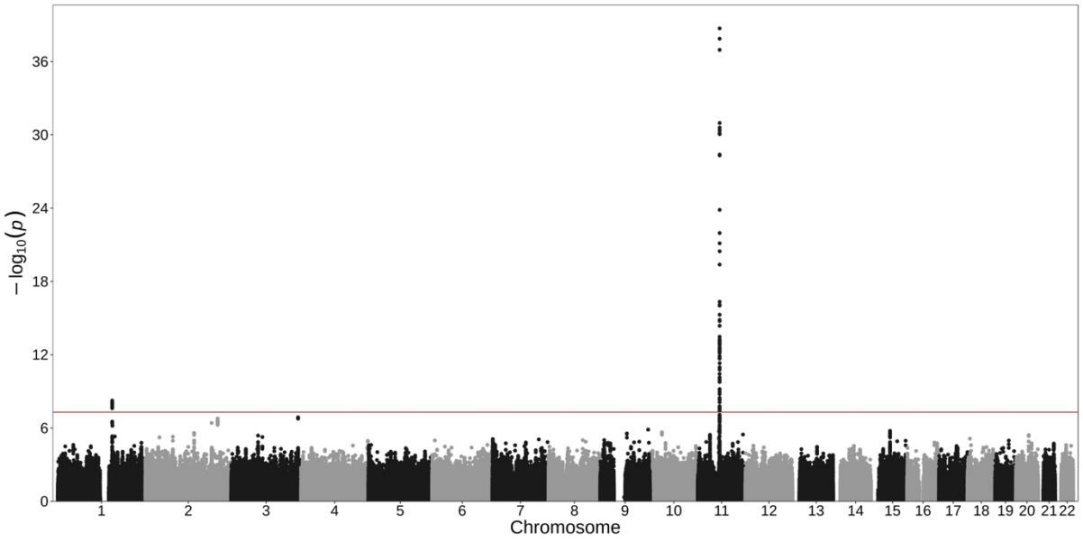
Multiple linear mixed effects model for change in FEV_1 with ln(CC-16), adjusted for age, sex, smoking status, baseline forced expiratory volume in 1 s (FEV1), body mass index, and the interactions of each factor with time. †change in annual rate of FEV1 decline (mL/year) per unit increase in ln(ng/mL) CC-16. SE: standard error. LHS: Lung Health Study. *p<0.05 **p<0.01.

2. FIGURE S1: ANNUAL CHANGE IN FEV_1 VERSUS SERUM CC-16 CONCENTRATION



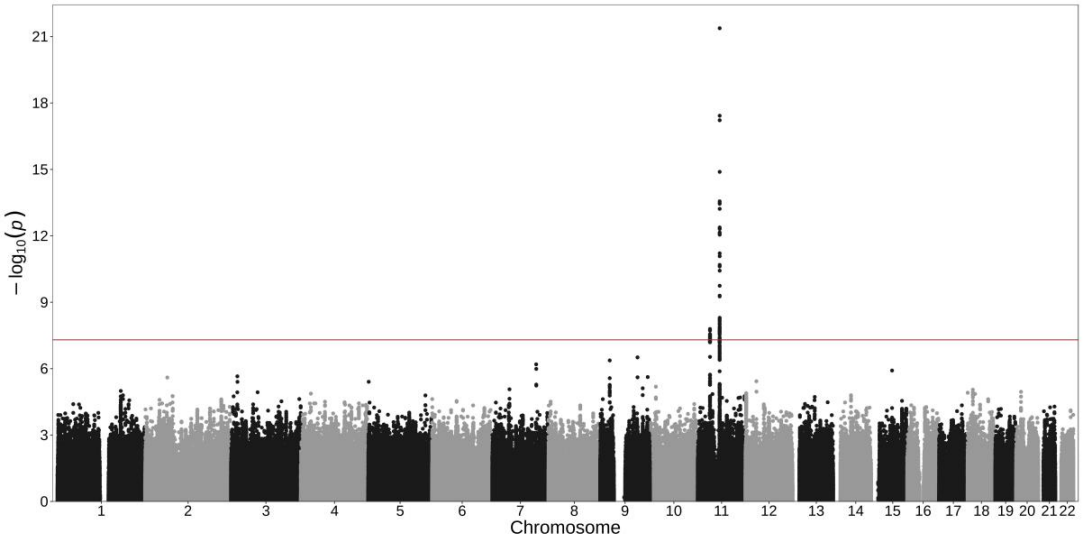
Annual change in FEV1 (calculated as the slope of FEV_1 versus time) versus CC-16 concentration in ln(ng/mL). Blue line = robust linear regression (unadjusted).

3. FIGURE S2: MANHATTAN PLOT FOR SERUM CC-16 GENOME-WIDE ASSOCIATION STUDY (GWAS) IN LUNG HEALTH STUDY



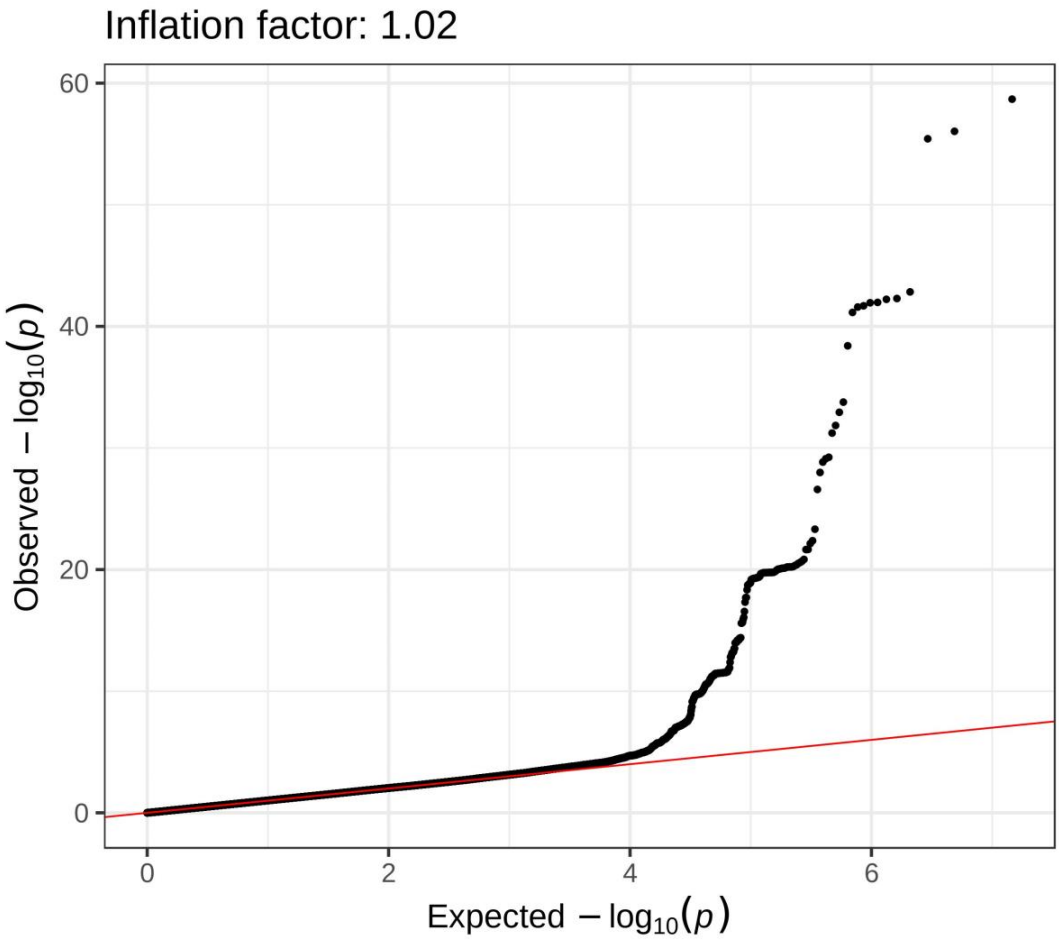
GWAS p values (–$\log_{10}$ scale) (Y axis) versus single nucleotide polymorphism positions across 22 chromosomes (X axis). Horizontal red line represents the genome-wide significance cut-off of $5x10^{-8}$. CC-16, club cell secretory protein-16.

4. FIGURE S3: MANHATTAN PLOT FOR SERUM CC-16 GENOME-WIDE ASSOCIATION STUDY (GWAS) IN ECLIPSE STUDY



GWAS p values (–$\log_{10}$ scale) (Y axis) versus single nucleotide polymorphism positions across 22 chromosomes (X axis). Horizontal red line represents the genome-wide significance cut-off of $5x10^{-8}$. CC-16, club cell secretory protein-16.

5. FIGURE S4: QUANTILE-QUANTILE PLOT FOR SERUM CC-16 GENOME-WIDE ASSOCIATION STUDY (GWAS)



Meta-analysis of Lung Health Study and ECLIPSE study GWAS. CC-16, club cell secretory protein-16. Observed p-values ($-\log_{10}$ scale) (Y axis) versus expected p-values ($-\log_{10}$ scale) (X axis). Red line represents where observed p values are equal to the expected.

6. TABLE S2: JOINT AND CONDITIONAL ANALYSES ON CHROMOSOME 11 CC-16 PROTEIN QUANTITATIVE TRAIT LOCI (pQTLS)

				Joint analysis			Conditional analysis		
SNP rsID	**Chr**	**Position§**	**Effect/Alt allele**	**Allele effect β†**	**SE**	**p**	**Allele effect β†**	**SE**	**p**
rs11032840	11	34779464	G/T	0.076	0.011	1.79×10^{-12}	0.076	0.011	1.79×10^{-12}
rs3741240	11	62186542	G/A	0.117	0.013	9.91×10^{-19}	-	-	-
rs11231085	11	62190448	G/C	0.110	0.013	3.37×10^{-16}	0.077	0.011	8.59×10^{-12}

Joint analysis: estimated joint effects of the three pQTLs on chromosome 11. Conditional analysis: estimated effect of the pQTLs on serum CC16 levels conditional on the top pQTL (rs3741240). §hg19 build of human reference genome. †change in ln(ng/mL) CC-16 per effect allele. SNP: single nucleotide polymorphism. rsID: reference SNP cluster identifier. Chr: chromosome. SE: standard error.

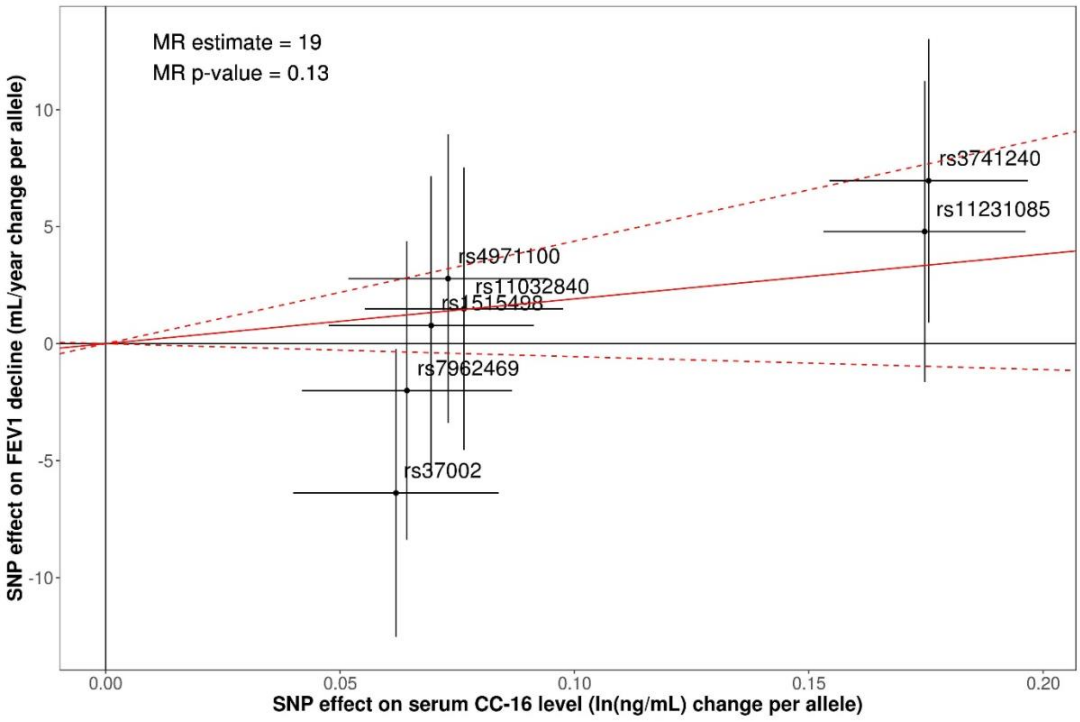
7. TABLE S3: ASSOCIATIONS BETWEEN CC-16 PROTEIN QUANTITATIVE TRAIT LOCI (pQTLS) AND COPD OUTCOMES

				COPD risk			COPD progression (Change in FEV_1 in mL/year)					
				ICGC			LHS			ECLIPSE		
SNP rsID	Chr	Position§	Effect/Alt allele	Allele effect β†	SE	p	Allele effect β‡	SE	p	Allele effect β	SE	p
rs4971100	1	155155731	A/G	-3.9×10^{-3}	0.01	0.7	-1.06	0.81	0.19	2.78	3.15	0.38
rs1515498	3	189508302	A/G	-2.2×10^{-3}	0.01	0.83	1.43	0.83	0.08	0.78	3.26	0.81
rs37002	5	1356944	C/T	-0.02	0.01	0.13	-0.57	0.85	0.50	-6.38	3.14	0.04*
rs11032840	11	34779464	G/T	3.8×10^{-3}	0.01	0.71	0.10	0.79	0.90	1.49	3.08	0.63
rs3741240	11	62186542	G/A	-0.02	0.01	0.14	1.63	0.82	0.05*	6.96	3.09	0.02*
rs11231085	11	62190448	G/C	-0.02	0.01	0.11	2.89	0.83	5.4×10^{-4}*	4.79	3.28	0.14
rs7962469	12	52348259	A/G	-0.03	0.01	1.2×10^{-3}*	-0.51	0.87	0.56	-2.00	3.25	0.54

§hg19 build of human reference genome. †ln(odds ratio) for COPD per effect allele CC-16 per effect allele. ‡change in annual rate of FEV_1 decline (mL/year) per effect allele *p<0.05. ICGC, International COPD Genetics Consortium; COPD, chronic obstructive pulmonary disease; LHS, Lung Health Study; ECLIPSE, Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; SNP, single nucleotide polymorphism; rsID, reference SNP cluster identifier; Chr, chromosome; Alt, alternate; SE, standard error.

8. FIGURE S5: IVW MENDELIAN RANDOMIZATION (MR) PLOT FOR COPD PROGRESSION IN ECLIPSE STUDY

Inverse variance weighted regression model, adjusted for linkage disequilibrium between single-nucleotide polymorphisms (SNPs), intercept constrained to zero. The model relates the per-allele effects of the SNPs on serum CC-16 level to their per-allele effects on lung function decline. The red line represents the estimated effect. Error bars represent 95% confidence intervals. SNPs are annotated by their rs identifier. CC-16, club cell secretory protein-16; FEV_1, forced expiratory volume in 1 second.



9. TABLE S4: GWAS CATALOGUE LOOK-UP RESULTS

rsID	Chr	Effect allele	Trait	P value	Sample	Reference
rs4971100	1	A	Magnesium levels	$1x10^{-07}$	2,317 European, 1,283 African American ancestry children	Chang et al[1]
rs4971100	1	A	Magnesium levels	$4x10^{-07}$	2,317 European, 1,283 African American ancestry children	Chang et al[1]
rs4971100	1	A	Estimated glomerular filtration rate	$9x10^{-17}$	567,460 European, 165,726 East Asian, 13,842 African American, 13,359 South Asian, 4,961 Hispanic ancestry individuals	Wuttke et al[2]
rs4971100	1	A	Estimated glomerular filtration rate	$2x10^{-07}$	165,726 East Asian ancestry individuals	Wuttke et al[2]
rs4971100	1	A	Serum uric acid levels	$8x10^{-19}$	121,745 Japanese ancestry individuals, at least 88,461 European ancestry individuals	Nakatochi et al[3]
rs4971100	1	A	Serum uric acid levels	$5x10^{-16}$	121,745 Japanese ancestry individuals, at least 88,461 European ancestry individuals	Nakatochi et al[3]
rs3741240	11	A	Chronic obstructive pulmonary disease-related biomarkers	$1x10^{-26}$	Up to 1,951 European ancestry smokers	Kim et al[4]
rs7962469	12	?	Lung function (FEV1/FVC)	$8x10^{-19}$	Approximately 370,000 European ancestry individuals	Kichaev et al[5]

Taken from the NHGRI-EBI Catalog of Human Genome-Wide Association Studies (https://www.ebi.ac.uk/gwas/) which lists known SNP-trait associations with $p<1x10^{-5}$.

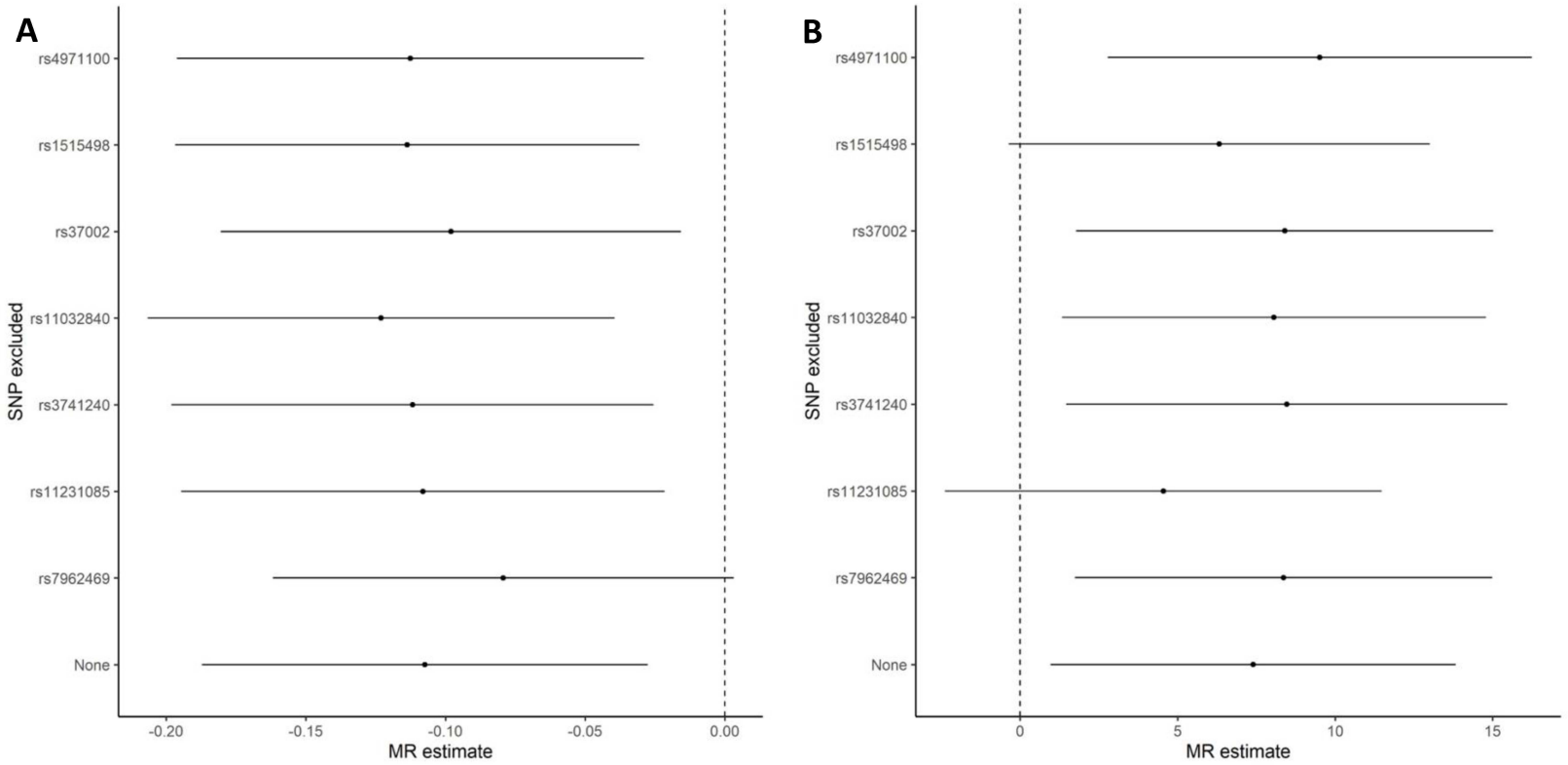
10.TABLE S5: PARTIAL F STATISTICS FOR INDIVIDUAL INTRUMENTAL VARIABLES

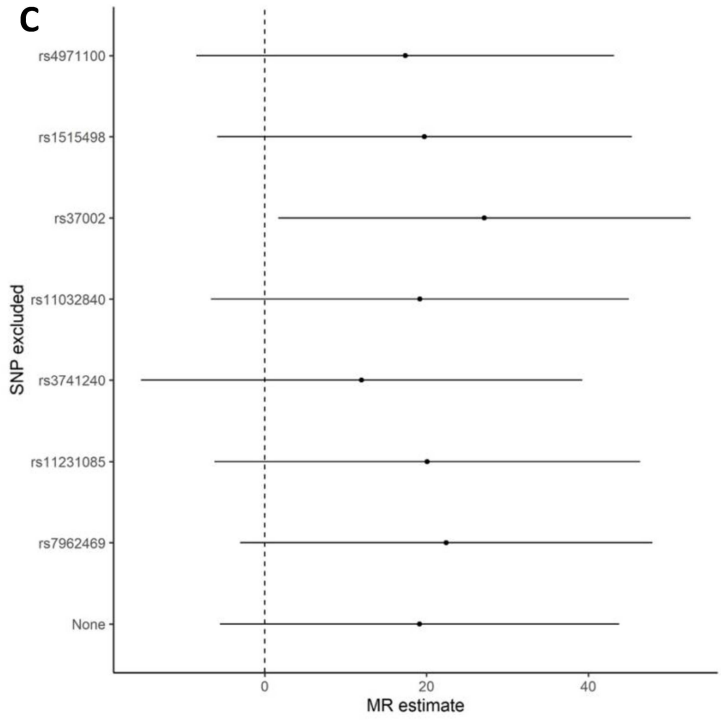
		Partial F	
rsID	**Chr**	**LHS**	**ECLIPSE**
rs4971100	1	34.09	11.93
rs1515498	3	27.86	11.14
rs37002	5	11.52	21.45
rs11032840	11	21.47	31.17
rs3741240	11	168.23	96.09
rs11231085	11	176.63	76.2
rs7962469	12	16.97	14.78

F statistics derived from ANOVA comparing linear models for ln(CC-16) concentration with and without the SNP as covariate. rsID, reference SNP cluster identifier; Chr, chromosome.

11.FIGURE S6: LEAVE-ONE-OUT SENSITIVITY ANALYSES

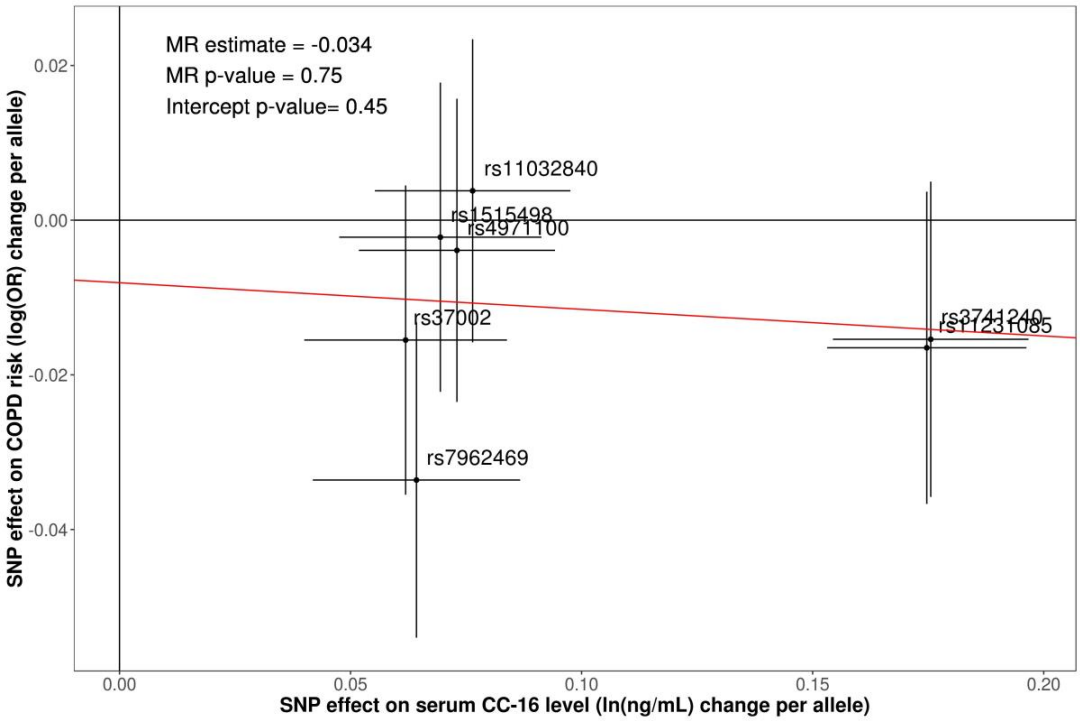
Mendelian randomisation (MR) estimates and 95% confidence intervals from inverse variance weighted models using all 7 SNPs ("None") and after removing a single SNP (as indicated on Y axis). MR outcomes: (A) "COPD risk" in ICGC dataset, (B) "COPD progression" (annual change in FEV_1) in Lung Health Study, (C) "COPD progression" in ECLIPSE study.




C
rs4971100
rs1515498
rs37002
rs11032840
rs3741240
rs11231085
rs7962469
None
SNP excluded
0
20
40
MR estimate

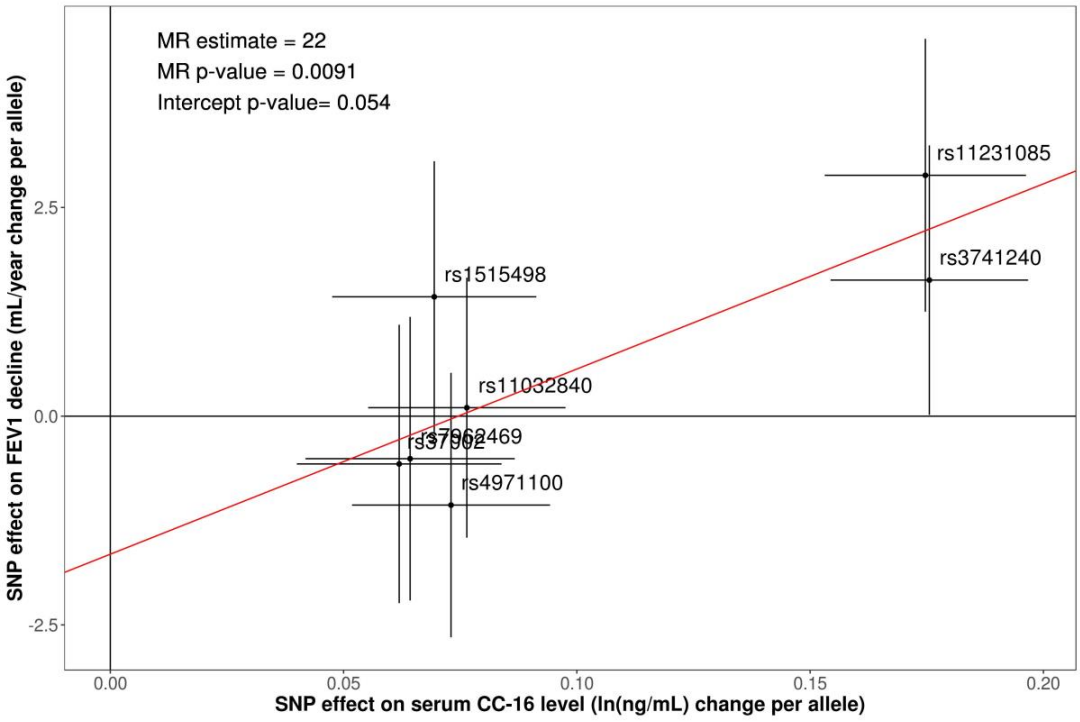
12.FIGURE S7: MENDELIAN RANDOMISATION (MR) EGGER ANALYSIS FOR COPD RISK

Inverse variance weighted regression model, adjusted for linkage disequilibrium between single-nucleotide polymorphisms (SNPs), with unconstrained intercept. The model relates the per-allele effects of the SNPs on serum CC-16 level to their per-allele effects on risk of having chronic obstructive pulmonary disease (COPD) in the International COPD Genetics Consortium (ICGC) dataset. The red line represents the estimated effect. Error bars represent 95% confidence intervals. SNPs are annotated by their rs identifier. OR, odds ratio; CC-16, club cell secretory protein-16.

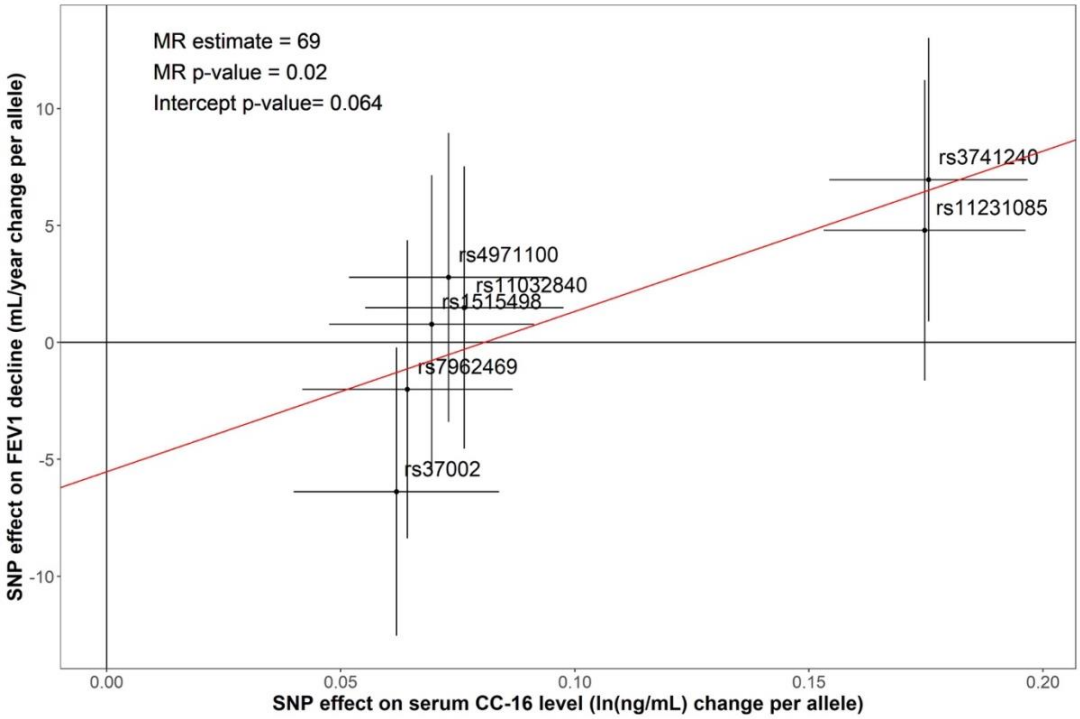


13. FIGURE S8: MENDELIAN RANDOMISATION (MR) EGGER ANALYSIS FOR COPD PROGRESSION IN THE LUNG HEALTH STUDY

Inverse variance weighted regression model, adjusted for linkage disequilibrium between single-nucleotide polymorphisms (SNPs), with unconstrained intercept. The model relates the per-allele effects of the SNPs on serum CC-16 level to their per-allele effects on lung function decline. The red line represents the estimated effect. Error bars represent 95% confidence intervals. SNPs are annotated by their rs identifier. CC-16, club cell secretory protein-16, FEV_1, forced expiratory volume in 1 second.



14. FIGURE S9: MENDELIAN RANDOMISATION (MR) EGGER ANALYSIS FOR COPD PROGRESSION IN THE ECLIPSE STUDY



Inverse variance weighted regression model, adjusted for linkage disequilibrium between single-nucleotide polymorphisms (SNPs), with unconstrained intercept. The model relates the per-allele effects of the SNPs on serum CC-16 level to their per-allele effects on lung function decline. The red line represents the estimated effect. Error bars represent 95% confidence intervals. SNPs are annotated by their rs identifier. CC-16, club cell secretory protein-16, FEV_1, forced expiratory volume in 1 second.

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