SUPPLEMENTARY APPENDIX

The relation of circulating CC16 to lung function growth, decline, and development of COPD across the lifespan

Stefano Guerra[1,2], MD, PhD; Marilyn Halonen[1], PhD; Monica M Vasquez[1,3], MPH; Amber Spangenberg[1], BS; Debra A. Stern[1], MS; Wayne J. Morgan[1], MD; Anne L. Wright[1], PhD; Iris Lavi[2], PhD; Lluïsa Tarès[2], BS; Anne-Elie Carsin[2,4], MS; Carlota Dobaño[5], PhD; Esther Barreiro[6], MD; Jan-Paul Zock[2], PhD; Jesús Martínez-Moratalla[7], MD; Isabel Urrutia[8], MD, PhD; Jordi Sunyer[2,4], MD, PhD; Dirk Keidel[9], MA; Medea Imboden[9], PhD; Nicole Probst-Hensch[9], PhD; Jenny Hallberg[10], PhD; Erik Melén[10], MD, PhD; Magnus Wickman[10], MD, PhD; Jean Bousquet[11], MD, PhD; Danielle C. M. Belgrave[12], PhD; Angela Simpson[12], MD, PhD; Adnan Custovic[12], MD, PhD; Josep M Antó[2,4], MD, PhD; Fernando D Martinez[1], MD

1. Arizona Respiratory Center, University of Arizona, Tucson, AZ, USA
2. Centre for Research in Environmental Epidemiology (CREAL), Universitat Pompeu Fabra, and CIBER Epidemiología y Salud Pública (CIBERESP), Barcelona, Spain
3. Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, AZ
4. IMIM Hospital del Mar Medical Research Institute, Barcelona
5. ISGlobal, Barcelona Ctr. Int. Health Res. (CRESIB), Hospital Clínic, Universitat de Barcelona, Barcelona
6. Pulmonology Department-Muscle and Respiratory System Research Unit (URMAR), IMIM-Hospital del Mar, and CIBER de Enfermedades Respiratorias (CIBERES), Barcelona
7. Servicio de Neumología del Complejo Hospitalario Universitario de Albacete, and Servicio de Salud de Castilla - La Mancha, Albacete, Spain
8. Pneumology Service, Galdakao-Usánsolo Hospital, Bizkaia, Spain
9. Swiss Tropical and Public Health Institute, and University of Basel, Switzerland
10. Institute of Environmental Medicine, Karolinska Institutet, and Sachs' Children and Youth Hospital, Stockholm, Sweden
11. University Hospital Montpellier; and Respiratory and Environmental Epidemiology Team, INSERM 1018, CESP Centre, Villejuif, France
12. Centre for Respiratory Medicine and Allergy, Institute of Inflammation and Repair, University of Manchester & University Hospital of South Manchester, UK

Table of contents

Supplementary methods and results	page 3
Supplementary Tables E1 – E12	page 5
Supplementary Figures E1 – E5	page 17
Results from prospective CC16 measurements	page 22
Supplementary references	page 28

Supplementary methods and results

Lung function tests

In TESAOD, protocols for lung function testing were consistent across surveys and American Thoracic Society (ATS) guidelines were integrated into the protocols after 1979[1]. ECRHS-Sp and SAPALDIA shared the same lung function testing protocol[2], which also complied with ATS guidelines[3]. They also used the same type of spirometer (EasyOne, NDD Medical Technologies, Andover, MA) in survey 3, although different instruments were used in the two studies for survey 2. In CRS, spirometry was performed at YR11 using a custom-built pneumotach-based sytem and at YR16 using a portable Schiller Spirovit SP-1 (Schiller AG, Baar, Switzerland). In MAAS, spirometry was performed at YR5, YR8, YR11 and YR16 according to ATS guidelines[3] using a Lilly pneumotachograph system (Jaeger, Germany). The test was repeated at intervals of 30 seconds until 3 technically acceptable traces were obtained. All children were asymptomatic at time of testing. β2-agonists were withheld for at least 4 hours prior to testing. In BAMSE, lung function testing was performed at 8 years of age using the 2200 Pulmonary Function Laboratory (Sensormedics, Anaheim, CA, USA)[4] and at 16 years using the Jaeger MasterScreen-IOS system (Carefusion Technologies, San Diego, California). The same spirometry test protocols were used on both occasions. The highest values of FEV1 were extracted and used for analysis, provided that the subject's effort was coded as being maximal by the test leader, the flow-volume curve passed visual quality inspection, and that the two highest FEV1 readings were reproducible according to ATS/ERS criteria.

For analyses on adult cohorts, in TESAOD percent predicted values for lung function indices were computed using reference equations generated from the same population by Knudson and colleagues[5]. In ECRHS-Sp and SAPALDIA, reference equations by Hankinson et al[6] were used. Equations from Hankinson et al[6] were also used to compute lower limit of normal (LLN) values for all cohorts.

CC16 measurements and adjustment

All samples were cryopreserved at -80°C, with the exception of ECRHS-Sp samples which were stored at -20°C. Circulating CC16 was measured in stored samples from "baseline" (survey 1 in TESAOD, survey 2 in ECRHS-Sp and SAPALDIA, YR6 in CRS, YR5 in MAAS, and YR4 in BAMSE) using a commercially available enzyme-linked immunosorbent assay kit (BioVendor with branches in Asheville NC and Modrice, Czech Republic). In addition, in TESAOD CC16 levels were also measured in serum samples from follow-up surveys in the subset of 601 participants for whom they were available (see pages 25-31 in this appendix). Samples from TESAOD, SAPALDIA, and CRS were analyzed in Dr Halonen's laboratory at the University of Arizona. Samples from ECRHS-Sp were analyzed in Dr Barreiro's laboratory at IMIM-Hospital del Mar, Barcelona. Samples from BAMSE were analyzed in Dr Dobaño's laboratory at the Centre for International Health Research (CRESIB), Barcelona. Samples from MAAS were analyzed by Dr Jiakai Wu at the Centre for Respiratory Medicine and Allergy University Hospital of South Manchester laboratory.

Serum samples were used for all cohorts, with the exception of BAMSE (plasma) and 78 plasma samples from CRS. CC16 levels for these 78 plasma samples were adjusted for comparability with serum levels based on a correction factor that was derived from analyses on 80 CRS participants who had both serum and plasma samples available at the YR26 survey. Levels of CC16 were measured in serum and plasma samples from these 80 subjects using the same ELISA kit from BioVendor (kit range: 1.57-50.0 ng/ml). All samples had detectable levels of CC16 and raw values were log-transformed (base 10) before being used in statistical analyses. Serum and plasma CC16 levels were strongly correlated (Pearson $r=0.90$, $p<0.001$, Figure E3), but geometric mean levels of CC16 were significantly higher in serum compared to plasma (7.9 ng/ml [95%CI: 7.2-8.8] vs. 7.2 ng/ml [95%CI: 6.5-7.9], respectively, $p<0.001$ by Paired T-Test). After removing two observations with low serum-plasma concordance (Figure 1, open circles), serum CC16 levels were on average 0.0354 log[ng/ml] higher than plasma CC16 levels. This difference was applied as a correction factor to the log plasma values at age 6 years and the serum and plasma values at age 6 years were subsequently combined in CRS analyses.

Statistical analyses

Comparisons across groups were completed with analysis of variance and X^2 tests for continuous and categorical variables, respectively. Non-parametric and exact tests were also used as appropriate.

In the adult cohorts, we tested the effects of baseline CC16 on subsequent FEV1 decline using multivariate linear regression models. These models included the rate of FEV1 decline as the dependent variable and a list of potential predictors as the independent variables, which included sex, age, height, smoking status-intensity, pack-years, asthma, baseline FEV1, and baseline CC16. Smoking intensity was assessed by the reported usual number of cigarettes smoked per day and used as a continuous variable in statistical models. Because multiple observations for each participant were available in TESAOD, the rate of FEV1 decline was first computed by regressing FEV1 levels against age for each subject. In contrast, since only two observations were available in ECRHS-Sp and SAPALDIA, for these two cohorts the rate of FEV1 decline was computed as the difference in FEV1 between survey 3 and 2 divided by the follow-up time for each subject. To avoid effects of observations with short follow-up periods, only participants with ≥ 5 years follow-up were included in these analyses in TESAOD (all ECRHS-Sp and SAPALDIA participants had ≥ 5 years follow-up). However, results were confirmed in TESAOD in analyses that included all participants.

Because multiple observations for each participant were available in TESAOD, in this cohort we also used random coefficients models[7], which adjusted for the intra-household cluster correlation and intra-subject serial correlation of repeated observations, to assess the effects of CC16 at baseline on FEV1 decline during the study follow-up. These models included covariates and an interaction term between CC16 tertiles and years of follow-up to test whether FEV1 decline differed across CC16 tertiles.

Incident airflow limitation was studied in the adult cohorts. In TESAOD, the relation of baseline CC16 to the risk of incident airflow limitation was studied in Cox proportional hazards models. The time to event was defined as the first survey in which a participant met the criteria for incident airflow limitation (or stage 2 airflow limitation) for cases and as the last completed survey for controls (i.e., participants who did not meet criteria in any of the completed surveys). Analyses on incident airflow limitation were replicated only in ECRHS-Sp because there were only 7 incident cases in SAPALDIA. Because in ECRHS-Sp only one follow-up survey was available after the baseline CC16 measurements, CC16 associations with incident airflow limitation were tested in logistic regression models. Model discrimination was assessed by the Harrell's C statistics in Cox models and by the area under the curve (AUC) in logistic regression models. Because a household-based recruitment strategy was used in TESAOD, household-clustered sandwich estimators of standard errors were used in regression and Cox models for this cohort.

In analyses on birth cohorts, random effects models[7] were used to assess the effects of low CC16 on lung function growth in childhood. In order to remove potential effects by active smoking, in sensitivity analyses the same models were also tested after removing observations of children who smoked by age 16 in CRS and BAMSE. Because in MAAS information on active smoking was not available from YR16, in these analyses only YR8 and YR11 observations were included for this cohort.

Association between baseline serum CC16 and subsequent decline of FEV1 among all TESAOD participants

When multivariate regression models predicting decline of FEV1 were run using information from all TESAOD participants (rather than only participants with ≥ 5 yrs follow-up as done for Table 2), the following beta coefficient (95% CI) was obtained for 1-SD decrease in baseline CC16 levels: -7.4 (-12.7, - 2.1) ml/yr; p=0.007.

Supplementary tables

Table E1. Baseline characteristics of the 960 TESAOD study participants.

Characteristics at baseline survey	
Females: N (%)	570 (59.4%)
Age: mean (range) in years	45 (21 – 70)
Body Mass Index (N=927): N (%)	
Under-weight (< 18.5 Kg/m^2)	14 (1.5%)
Normal-weight (≥18.5, < 25 Kg/m^2)	538 (58.0%)
Over-weight (≥ 25, < 30 Kg/m^2)	308 (33.2%)
Obese (≥ 30 Kg/m^2)	67 (7.2%)
Smoking status (N=959): N (%)	
Never	406 (42.3%)
Former	220 (22.9%)
Current	333 (34.7%)
Pack-years: median; IQR^ (N=553)	17.3; 7.0 – 34.7
Ever physician-confirmed asthma* (N=959): N (%)	89 (9.3%)
FEV1 % predicted: mean (SD)	97.6% (16)
FEV1/FVC ratio: mean (SD)	82.7% (7)

* Defined as a positive report that a doctor told the participant that he/she had asthma
^ Among ever smokers

Table E2. Characteristics of the 514 ECRHS-Sp participants at survey 2.

Characteristics at baseline survey	
Females: N (%)	249 (48.4%)
Age: mean (range) in years	41 (28 – 55)
Body Mass Index: N (%)	
Under-weight (< 18.5 Kg/m^2)	1 (0.2%)
Normal-weight (≥18.5, < 25 Kg/m^2)	201 (39.1%)
Over-weight (≥ 25, < 30 Kg/m^2)	217 (42.2%)
Obese (≥ 30 Kg/m^2)	95 (18.5%)
Smoking status (N=503): N (%)	
Never	179 (35.6%)
Former	117 (23.3%)
Current	207 (41.1%)
Pack-years: median; IQR**	17.1; 8.8 – 32.0
Ever physician-confirmed asthma*: N (%)	54 (10.5%)
FEV1 % predicted: mean (SD)	103.2% (12)
FEV1/FVC ratio: mean (SD)	81.0% (5)

* Defined as a positive report that a doctor told the participant that he/she had asthma
** Among ever smokers

Table E3. Characteristics of the 167 SAPALDIA participants at survey 2.

Characteristics at baseline survey	
Females: N (%)	88 (52.7%)
Age: mean (range) in years	48 (30 – 69)
Body Mass Index: N (%)	
Under-weight (< 18.5 Kg/m^2)	1 (0.6%)
Normal-weight (≥18.5, < 25 Kg/m^2)	87 (52.1%)
Over-weight (≥ 25, < 30 Kg/m^2)	56 (33.5%)
Obese (≥ 30 Kg/m^2)	23 (13.8%)
Smoking status: N (%)	
Never	89 (53.3%)
Former	52 (31.1%)
Current	26 (15.6%)
Pack-years: median; IQR**	12.9; 4.0 – 27.2
Ever physician-confirmed asthma*: N (%)	9 (5.4%)
FEV1 % predicted: mean (SD)	104.1% (11)
FEV1/FVC ratio: mean (SD)	78.3% (5)

* Defined as a positive report that a doctor told the participant that he/she had asthma
** Among ever smokers

Table E4. Comparison of baseline characteristics of TESAOD participants included and excluded from the present study.

	Group 1: Included subjects (N=960)	**Group 2: Excluded subjects** (N=620)	**p-value: Groups** 1 & 2	**Group 3: All TESAOD subjects*** (N=1580)
	N (%)	N (%)		N (%)
Sex	N=960	N=620	0.019	N=1580
Females	570 (59.4%)	331 (53.4%)		901 (57.0%)
Males	390 (40.6%)	289 (46.6%)		679 (43.0%)
Age categories	N=960	N=620	0.047	N=1580
21 ≤ years < 30	245 (25.5%)	194 (31.3%)		439 (27.8%)
30 ≤ years < 45	212 (22.1%)	141 (22.7%)		353 (22.3%)
45 ≤ years < 60	278 (29.0%)	152 (24.5%)		430 (27.2%)
60 ≤ years ≤ 70	225 (23.4%)	133 (21.5%)		358 (22.7%)
BMI Categories	N=927	N=598	0.039	N=1525
Underweight	14 (1.5%)	23 (3.9%)		37 (2.4%)
Normal Weight	538 (58.0%)	331 (55.4%)		869 (57.0%)
Overweight	308 (33.2%)	191 (31.9%)		499 (32.7%)
Obese	67 (7.2%)	53 (8.9%)		120 (7.9%)
Smoking Status	N=959	N=620	0.155	N=1579
Never	406 (42.3%)	244 (39.4%)		650 (41.2%)
Former	220 (22.9%)	131 (21.1%)		351 (22.2%)
Current	333 (34.7%)	245 (39.5%)		578 (36.6%)
Ever physician-confirmed asthma	N=959	N=619	0.626	N=1578
No	870 (90.7%)	566 (91.4%)		1436 (91.0%)
Yes	89 (9.3%)	53 (8.6%)		142 (9.0%)
	Mean (SD)	Mean (SD)		Mean (SD)
Pack-years (only smokers)	N=553 23.3 (21.4)	N=375 21.4 (20.4)	0.166	N=928 22.5 (21.0)
FEV1 % Predicted	N=960 97.6 (15.8)	N=620 97.6 (15.6)	0.974	N=1580 97.6 (15.7)
FVC % Predicted	N=960 97.7 (15.7)	N=620 97.8 (15.6)	0.906	N=1580 97.7 (15.7)
FEV1/FVC ratio	N=960 82.7 (6.5)	N=620 83.0 (6.6)	0.396	N=1580 82.9 (6.6)

Table E5. Results of a multivariate linear regression model predicting log-transformed serum CC16 levels [in log10(ng/ml)] in TESAOD.

N=926	**Coefficient (SE)**	**p-value**	**95% Confidence Interval**
Sex			
Males	Reference		
Females	-0.078 (0.017)	<0.001	(-0.111, -0.045)
Age categories			
21 ≤ years < 30	Reference		
30 ≤ years < 45	-0.070 (0.022)	0.002	(-0.114, -0.027)
45 ≤ years < 60	-0.015 (0.022)	0.506	(-0.059, 0.029)
60 ≤ years ≤ 70	0.042 (0.024)	0.083	(0.006, 0.089)
BMI categories			
Normal weight	Reference		
Underweight	-0.114 (0.061)	0.065	(-0.234, 0.007)
Overweight	-0.043 (0.017)	0.014	(-0.077, -0.009)
Obese	-0.071 (0.030)	0.017	(-0.130, -0.013)
Smoking status			
Never	Reference		
Former	-0.014 (0.022)	0.520	(-0.060, 0.029)
Current	-0.121 (0.021)	<0.001	(-0.163, -0.079)
Pack-years	-0.001 (0.001)	0.006	(-0.002, -0.001)
Intercept	1.02 (0.02)	<0.001	(0.98, 1.06)

Table E6. Baseline characteristics of TESAOD participants with < and ≥ 13.7 years of follow-up.

Characteristics at baseline survey	Participants with < 13.7 yrs follow-up N = 432	Participants with ≥ 13.7 yrs follow-up N = 528	P value
Females: N (%)	257 (59.5%)	313 (59.3%)	0.95
Age: mean (SD)	48 (16)	43 (15)	<0.0001
Body Mass Index*: N (%)			
Under-weight (< 18.5 Kg/m^2)	8 (1.9%)	6 (1.2%)	0.004
Normal-weight (≥18.5, < 25 Kg/m^2)	219 (52.6%)	319 (62.4%)	
Over-weight (≥ 25, < 30 Kg/m^2)	148 (35.6%)	160 (31.3%)	
Obese (≥ 30 Kg/m^2)	41 (9.9%)	26 (5.1%)	
Smoking status: N (%)**			
Never	166 (38.4%)	240 (45.5%)	0.051
Former	100 (23.2%)	120 (22.8%)	
Current	166 (38.4%)	167 (31.7%)	
FEV1 % predicted: mean (SD)	96.2% (17)	98.7% (15)	0.014
FEV1/FVC ratio: mean (SD)	82.3% (7)	83.1% (6)	0.064
Serum CC16 in ng/ml: geometric mean	7.66	7.66	0.98

* Total N = 927 because of 33 missing cases
** Total N = 959 because of one missing case

Table E7. Association between baseline serum CC16 and subsequent decline of FEV1 in never and ever smokers from TESAOD, ECRHS-Sp, and SAPALDIA.

	Increase in FEV1 decline associated with 1-SD decrease in baseline CC16	
	NEVER SMOKERS ***Beta coefficient* (95% CI)*** ***p value*** ***N subjects / N observations***	***SMOKERS*** ***Beta coefficient** (95% CI)*** ***p value*** ***N subjects / N observations***
TESAOD	-1.8 (-4.3, 0.7) ml/yr p = 0.16 N subjects = 350 / N observations = 2713	-6.1 (-10.0, -2.2) ml/yr p = 0.002 N subjects = 450 / N observations = 3401
ECRHS-Sp	-3.5 (-8.0, 1.0) ml/yr p = 0.13 N subjects = 179 / N observations = 358	-1.9 (-4.2, 0.4) ml/yr p = 0.10 N subjects = 316 / N observations = 632
SAPALDIA	-6.5 (-13.3, 0.2) ml/yr p = 0.06 N subjects = 89 / N observations = 178	-4.6 (-11.9, 3.0) ml/yr p = 0.24 N subjects = 75 / N observations = 150

* Adjusted for sex, age, height, asthma, initial FEV1 levels. ECRHS-Sp models also included center and sample type (random versus enriched) and SAPALDIA models study area.
** Adjusted for sex, age, height, smoking status and intensity, pack-years, asthma, initial FEV1 levels. ECRHS-Sp models also included center and sample type (random versus enriched) and SAPALDIA models study area.

Table E8. Association between baseline serum CC16 and subsequent decline of FEV1 in participants who were <45 and ≥45 years old at baseline in TESAOD, ECRHS-Sp, and SAPALDIA.

	Increase in FEV1 decline associated with 1-SD decrease in baseline CC16	
	Participants <45 years old at baseline ***Beta coefficient* (95% CI)*** ***p value*** ***N subjects / N observations***	***Participants ≥45 years old at baseline*** ***Beta coefficient* (95% CI)*** ***p value*** ***N subjects / N observations***
TESAOD	-2.2 (-6.2, 1.7) ml/yr p = 0.26 N subjects = 394 / N observations = 2945	-5.6 (-9.2, -1.9) ml/yr p = 0.003 N subjects = 406 / N observations = 3169
ECRHS-Sp	-1.4 (-3.8, 0.9) ml/yr p = 0.24 N subjects = 341 / N observations = 682	-4.9 (-9.4, -0.4) ml/yr p = 0.03 N subjects = 154 / N observations = 308
SAPALDIA	-5.6 (-13.5, 2.3) ml/yr p = 0.16 N subjects = 69 / N observations = 138	-2.9 (-9.2, 3.3) ml/yr p = 0.35 N subjects = 95 / N observations = 190

* Adjusted for sex, age, height, smoking status and intensity, pack-years, asthma, initial FEV1 levels. ECRHS-Sp models also included center and sample type (random versus enriched) and SAPALDIA models study area.

Table E9. Characteristics of the 438 CRS participants at the YR6 survey.

Characteristics at baseline survey	
Females: N (%)	213 (48.6%)
Age: mean (range) in years	6.0 (4 – 10)
Ethnicity: N (%)	
Both parents non-Hispanic White	265 (60.5%)
One Hispanic and one non-Hispanic White parent	63 (14.4%)
Both Hispanic white parents	57 (13.0%)
Other ethnicity / missing	53 (12.1%)
Body Mass Index (N=399): mean (SD) in Kg/m^2	16.0 (1.9)
Current maternal smoking (N=427): N (%)	80 (18.7%)
Current paternal smoking (N=394): N (%)	81 (20.6%)
Active physician-confirmed asthma (N=435)*: N (%)	40 (9.2%)

* Defined as a positive report of physician diagnosis of asthma plus active symptoms during the previous year.

Table E10. Characteristics of the 481 MAAS participants at the YR5 survey.

Characteristics at baseline survey	
Females: N (%)	222 (46.2%)
Age at YR5: mean (range) in years	5.04 (4.78 - 5.70)
Ethnicity: N (%)**	
Caucasian	459 (95.4%)
Asian	8 (1.7%)
Oriental	2 (0.4%)
African	2 (0.4%)
Other	10 (2.1%)
Body Mass Index: mean (SD) in Kg/m^2	16.37 (1.50)
Current maternal smoking at YR5: N (%)	76 (15.8%)
Current paternal smoking at YR5: N (%)	97 (20.2%)
Active physician-confirmed asthma at YR5*: N (%)	96 (20.1%)

* Defined as at least two of the following three conditions: current wheeze, current use of asthma medication, doctor diagnosis of asthma.

** Because non-Caucasians ethnicity groups had very small numbers, for analysis this variable was recoded as 1=Caucasian, 0=all other ethnic groups.

Table E11. Characteristics of the 231 BAMSE participants at the YR4 survey.

Characteristics at baseline survey	
Females: N (%)	103 (44.6%)
Age: mean (range) in years	4.0 (4 – 5)
Ethnicity: N (%)	
Caucasian	231 (100%)
Body Mass Index: mean (SD) in Kg/m^2	16.2 (1.3)
Current maternal smoking: N (%)	29 (12.6%)
Current paternal smoking (N=230): N (%)	26 (11.3%)
Active physician-confirmed asthma*: N (%)	86 (37.2%)

* Defined as a positive report to at least two of the following: Physician-diagnosed asthma ever; asthma medication in the last 12 months; and wheezing/breathing difficulties in the last 12 months.

Table E12. Association of baseline characteristics with CC16 values at age 6 years in CRS. N=438 for univariate analyses (unless otherwise specified); N=388 for multivariate regression analyses.

	Serum CC16 levels in ng/ml **Results from univariate analyses**	**Results from multivariate regression predicting log serum CC16 in log10(ng/ml)**
	Geometric Mean (95%CI) *P value*	*Beta coefficients (95% CI)* *P value*
Sex		
Females (N=213)	8.47 (8.0-8.9)	reference
Males (N=225)	7.47 (7.1-7.9)	-0.044 (-0.079, -0.008)
	P = 0.001	P = 0.017
Ethnicity		
Both parents non-Hispanic whites (N=265)	8.16 (7.8-8.6)	
One Hispanic and one non-Hispanic white parent (N=63)	7.83 (7.0-8.7)	
Both parents Hispanic whites (N=57)	7.24 (6.4-8.2)	
Other ethnicity / missing (N=53)	7.79 (6.9-8.8)	
	P = 0.24	
Maternal smoking at YR6		
No (N=347)	8.05 (7.7-8.4)	reference
Yes (N=80)	7.47 (6.8-8.2)	-0.043 (-0.093, 0.006)
	P = 0.15	P = 0.086
Paternal smoking at YR6		
No (N=313)	7.83 (7.5-8.2)	
Yes (N=81)	8.33 (7.6-9.1)	
	P = 0.24	
Active physician-confirmed asthma at YR6:		
No (N=395)	8.00 (7.7-8.3)	
Yes (N=40)	7.38 (6.2-8.7)	
	P = 0.25	
Active wheezing during past year at YR6:		
No (N=308)	8.24 (7.9-8.6)	reference
Yes (N=126)	7.22 (6.7-7.8)	-0.058 (-0.098, -0.019)
	P = 0.003	P = 0.004
Positive skin prick test at YR6:		
No (N=325)	8.09 (7.7-8.5)	
Yes (N=192)	7.70 (7.2-8.2)	
	P = 0.24	
	Spearman rho with CC16 levels	
Age in years	0.095	0.036 (0.005, 0.067)
	P = 0.05	P = 0.024
BMI in Kg/m^2	-0.085	-0.010 (-0.020, 0.000)
(N=399)	P = 0.09	P = 0.041

Supplementary figures

Figure E1. Flow-chart for the selection of the 960 TESAOD participants included in the current study

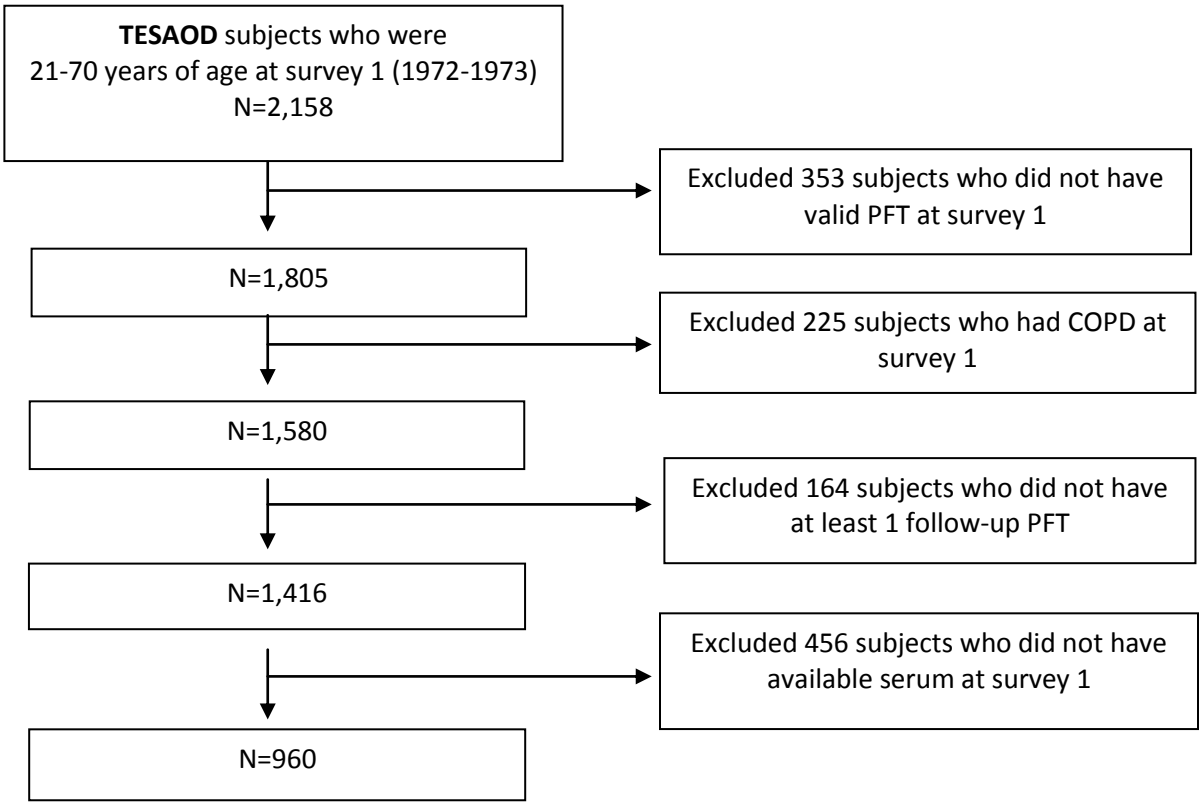


Figure E2. Flow-chart for the selection of the 514 ECRHS-Sp participants included in the current study

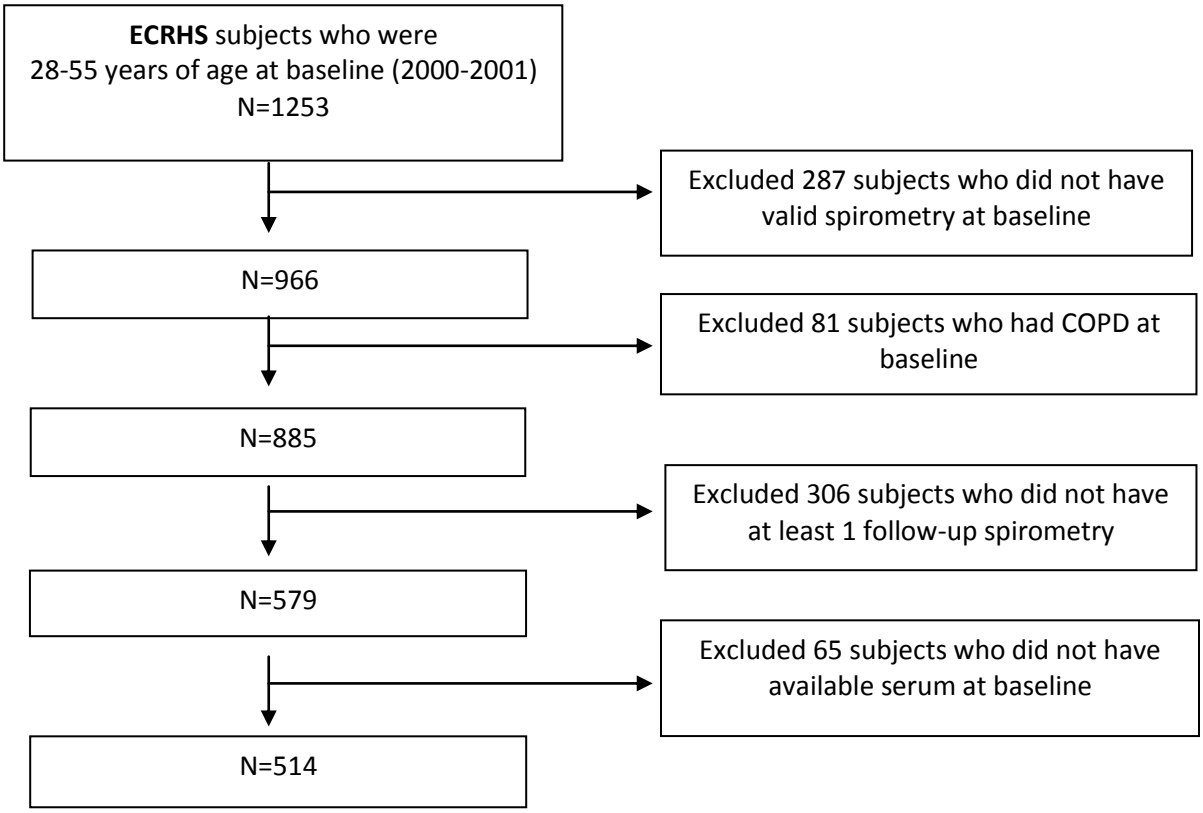


Figure E3. Scatter-plot of serum and plasma levels of CC16 from 80 CRS participants at YR26

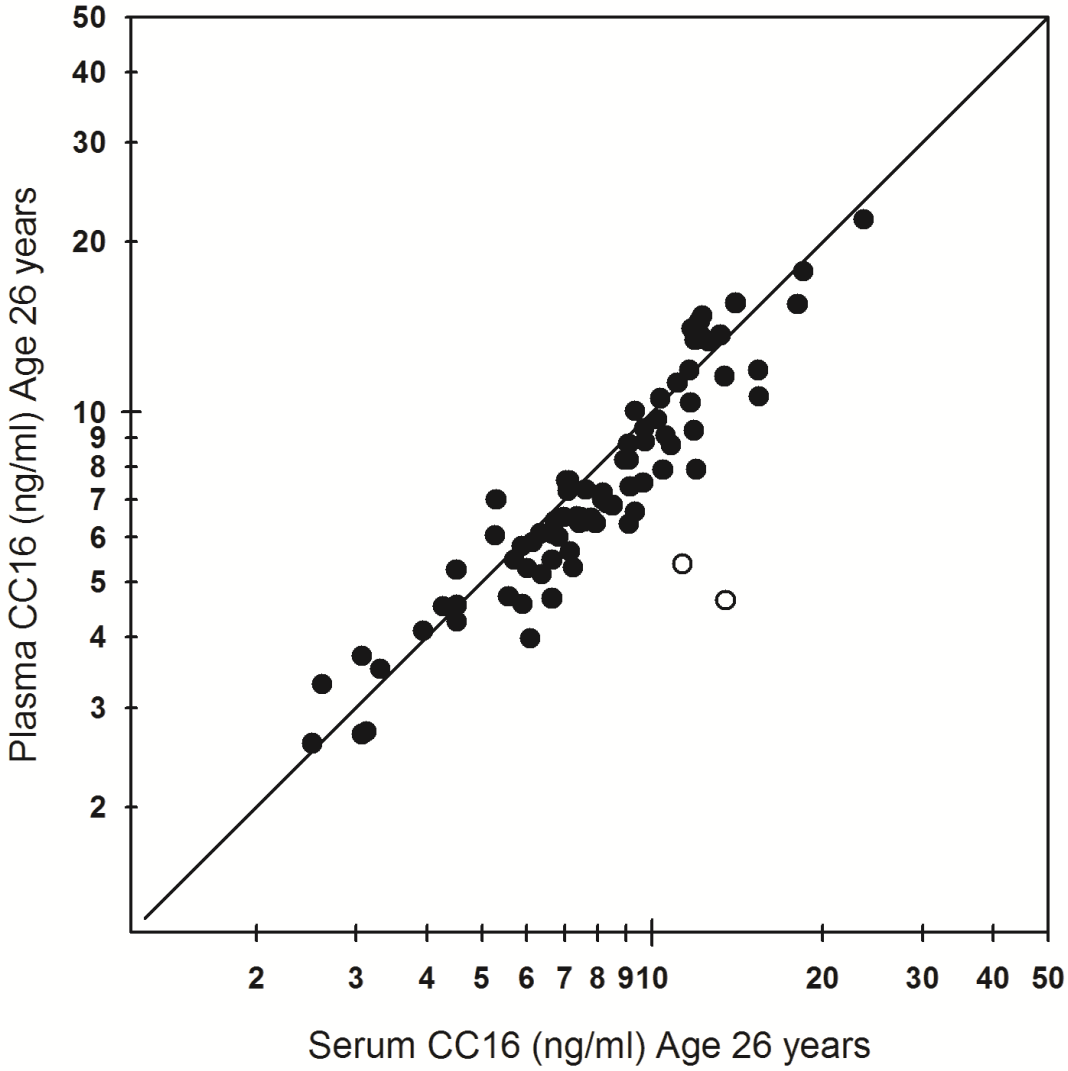
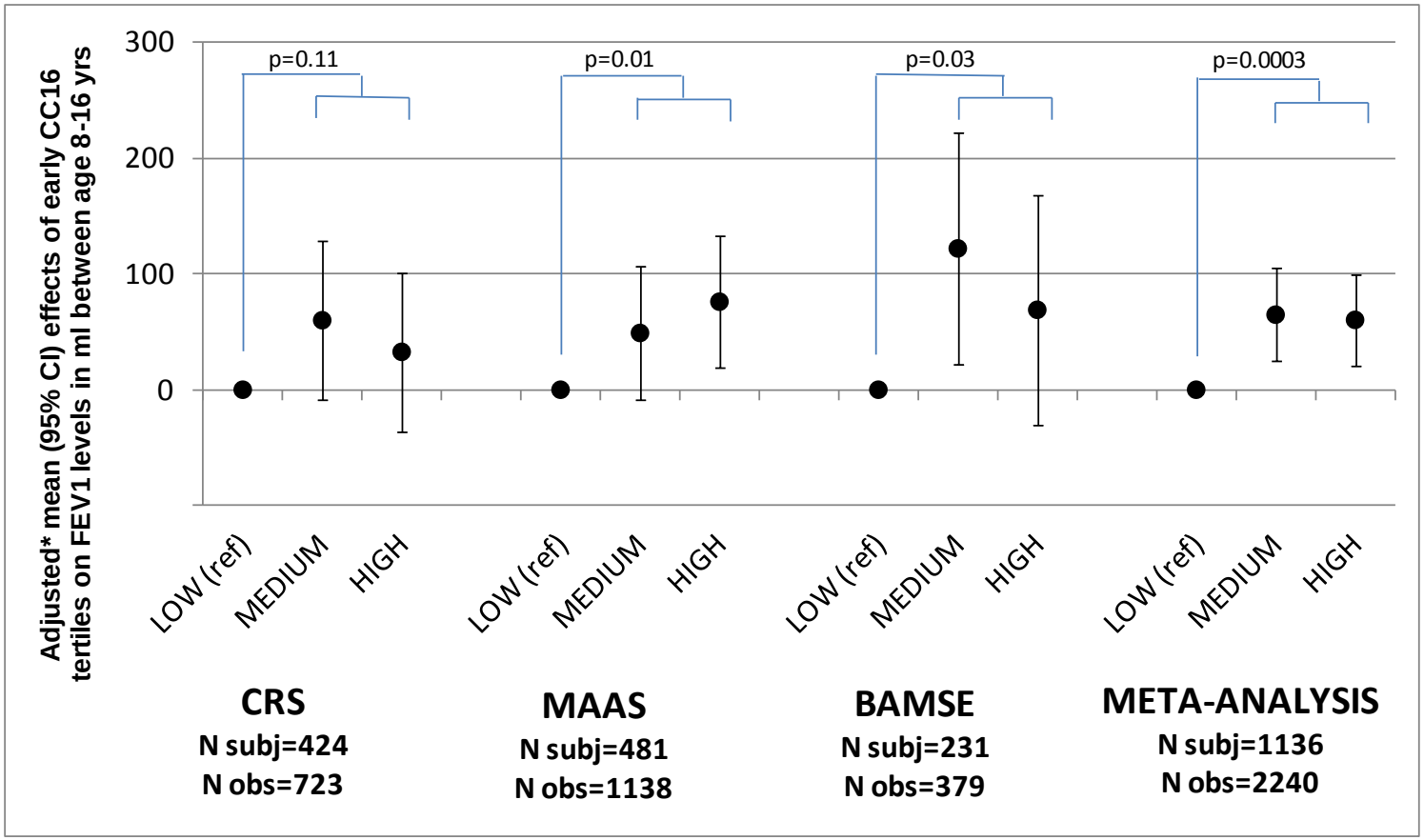


Figure E4. Effects of the medium and high tertile of early circulating CC16 (as compared with the lowest tertile) on subsequent FEV1 levels achieved during childhood up to age 16 years in the CRS, MAAS, and BAMSE birth cohorts **after further adjustment for active asthma at baseline**.

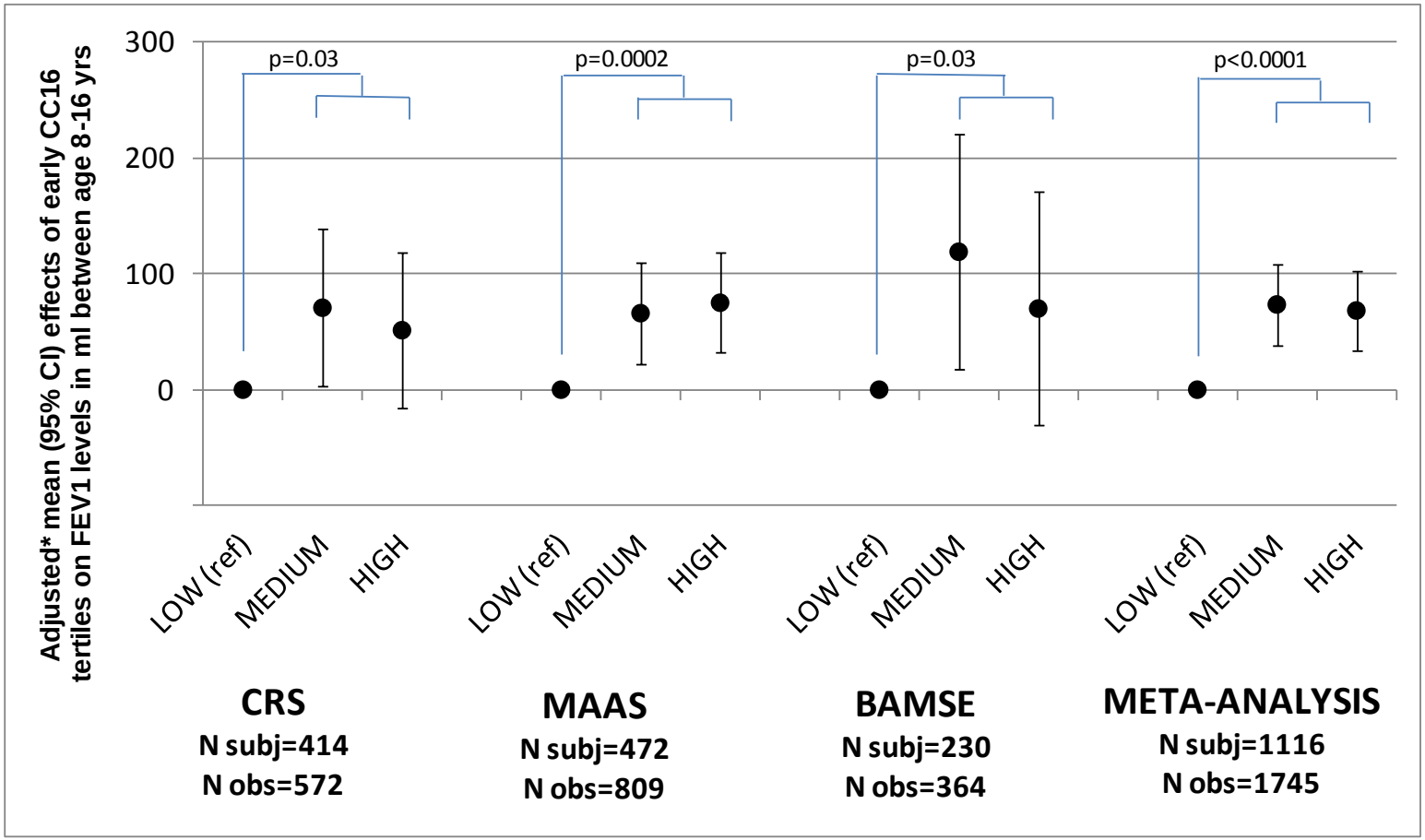


* Results come from random effects models adjusted for sex, age, height, survey, maternal smoking, ethnicity (CRS and MAAS), baseline FEV1 (MAAS), and active asthma at baseline. The dependent variable of the models was FEV1 at ages 11 and 16 yrs in CRS, FEV1 at ages 8, 11, and 16 yrs in MAAS, and FEV1 at ages 8 and 16 yrs in BAMSE.

Early CC16 was measured at age 6 years (mean 6.1 yrs; SD 0.8 yrs) in CRS, 5 years (5.0, 0.1) in MAAS, and 4 years (4.0, 0.1) in BAMSE.

Reported p values refer to the comparison of the lowest early CC16 tertile versus the other two tertiles (medium and high) combined.

Figure E5. Effects of the medium and high tertile of early circulating CC16 (as compared with the lowest tertile) on subsequent FEV1 levels achieved during childhood up to age 16 years in the CRS, MAAS, and BAMSE birth cohorts **after restricting analyses to subjects who never smoked up to age 16 years**. Because in MAAS information on active smoking was not available from YR16, in these analyses only YR8 and YR11 observations were included for this cohort.



* Results come from random effects models adjusted for sex, age, height, survey, maternal smoking, ethnicity (CRS and MAAS), and baseline FEV1 (MAAS). The dependent variable of the models was FEV1 at ages 11 and 16 yrs in CRS, FEV1 at ages 8 and 11 yrs in MAAS, and FEV1 at ages 8 and 16 yrs in BAMSE.

Early CC16 was measured at age 6 years (mean 6.1 yrs; SD 0.8 yrs) in CRS, 5 years (5.0, 0.1) in MAAS, and 4 years (4.0, 0.1) in BAMSE.

Reported p values refer to the comparison of the lowest early CC16 tertile versus the other two tertiles (medium and high) combined.

Summary of results from CC16 measurements on prospective TESAOD samples

Among the 960 TESAOD participants included in this study, 601 (63%) had serum samples available during the follow-up. For prospective CC16 measurements, the sample collected at the earliest follow-up survey after the baseline assessment was used for each participant. Prospective samples came from the follow-up surveys 2 (N=123), 5 (N=51), 6 (N=363), and 7 (N=64). The mean number of years between the baseline and prospective measurement was 6.5 years with a range of 1 to 11 years.

The basic characteristics of the 601 participants with prospective CC16 measurements and the 359 participants with no prospective CC16 measurements are shown in table A. The former were on average older and had better FEV1 and FVC values than the latter.

Table A. Basic characteristics of participants with and without prospective CC16 measurements.

	Participants with prospective measurements (N=601)	**Participants with no prospective measurement** (N=359)	**p-value**
Female sex: N (%)	360 (60%)	210 (59%)	0.67
Age: median (IQR) in years	49 (32-59)	41 (27-58)	0.023
Smoking*: N (%)			0.22
Never	263 (43.83%)	143 (39.83%)	
Former	141 (23.50%)	79 (22.01%)	
Current	196 (32.67%)	137 (38.16%)	
Pack-years (all subjects)*: mean (SD)	13.37 (20.16)	13.58 (19.46)	0.88
Pack-years (only smokers): mean (SD)**	23.80 (21.81)	22.56 (20.67)	0.51
Ever physician-confirmed asthma: N (%)	57 (9.48%)	32 (8.94%)	0.78
Serum CC16: geometric mean (ng/ml)	7.77	7.47	0.29
FEV1% predicted: mean (SD)	98.75 (15.45)	95.68 (16.13)	0.0034
FVC% predicted: mean (SD)	98.86 (15.58)	95.69 (15.83)	0.0025
FEV1/FVC ratio: mean (SD)	82.53 (6.43)	83.12 (6.71)	0.17

*N=600 among participants with prospective measurement because of one subject with missing smoking information
*N=337 smokers with prospective measurement and 216 smokers with no prospective measurement

Prospective CC16 data for the 601 participants with prospective measurements were categorized into two variables:

1 – The rate of change of serum CC16 between baseline and the prospective measurement was computed as the difference between CC16 levels from the prospective measurement and those from the baseline measurement divided by the years between the baseline and prospective measurement. This variable was then divided into quartiles, consistent with previous longitudinal CC16 studies from TESAOD[8].

2 – Participants were categorized into three prospective CC16 groups: "persistently low CC16" (subjects who were in the lowest CC16 tertile both at the baseline and prospective survey), "inconsistently low CC16" (subjects who were in the lowest CC16 tertile in one but not both surveys), and "persistently high CC16" (subjects who at neither survey were in the lowest CC16 tertile).

These variables were tested in multivariate regression models predicting FEV1 decline and in Cox models predicting incident airflow limitation, similarly to analyses presented in the main text. These models were adjusted for the same covariates used in main analyses plus a longitudinal smoking category variable (based on combination of smoking status at baseline and prospective measurements) and the change in pack-years between baseline and prospective measurements. Models that included the first variable (i.e., quartiles of CC16 change) were further adjusted for baseline CC16 levels. In Cox models, the follow-up started at the time of the prospective measurement and participants who developed airflow limitation at any time before the prospective measurement were excluded.

Table B shows that, after full adjustment, participants with persistently low CC16 had on average a 9 ml/yr steeper FEV1 decline ($p<0.001$) than participants with persistently high CC16. No significant associations were found using quartiles of CC16 change.

Tables C and D show that similar trends for increased risk of incident stage 2 airflow limitation were found for the groups of participants with CC16 decrease over time and participants with persistently low CC16 levels, although it should be noted that the number of incident cases was quite small in these analyses.

Although these prospective analyses support the potential value of longitudinal measurements of serum CC16, the determinants and clinical relevance of temporal changes of CC16 in adult life remain largely to be determined and further studies are warranted to determine conclusively whether the use of both baseline levels and temporal trajectories of serum CC16 may improve the predictive value of this biomarker in lung health.

Table B. Association between temporal changes of serum CC16 and decline of FEV1 in TESAOD.

	Increase in FEV1 decline ***Beta coefficient* (95% CI)*** ***p value***
MODEL 1	
N subjects = 566	
Coefficient associated with:	
1-SD decrease in baseline CC16	-3.9 (-5.9, -2.0) ml/yr
P value	p<0.001
Coefficients associated with	
4th quartile of CC16 change – steep increase	ref
3rd quartile of CC16 change – slow increase	-0.7 (-6.0, 4.9) ml/yr
2nd quartile of CC16 change – slow decrease	-1.3 (-6.3, 3.8) ml/yr
1st quartile of CC16 change – steep decrease	-4.5 (-10.0, 1.0) ml/yr
P value for trend across quartiles	p=0.11
MODEL 2	
N subjects = 566	
Coefficients associated with	
Persistently high CC16	ref
Inconsistently low CC16	-2.5 (-6.8, 1.8) ml/yr
Persistently low CC16	-9.0 (-13.7, -4.3) ml/yr
P value for trend across groups	p<0.001

* Adjusted for sex, age, asthma, longitudinal smoking categories (based on combination of smoking status at baseline and prospective measurements), baseline pack-years, change in pack-years between baseline and prospective measurements, baseline smoking intensity, and initial FEV1 levels.

Table C. Adjusted HRs associated with baseline levels and temporal changes of serum CC16 for incident airflow limitation in TESAOD.

	HR for incident airflow limitation N subjects who developed airflow limitation / total N subjects (89/456)^		HR for incident stage 2 airflow limitation N subjects who developed stage 2 airflow limitation / total N subjects (26/456)^	
	Adj* HR (95% CI) P value	**Adj** HR (95% CI) P value**	**Adj* HR (95% CI) P value**	**Adj** HR (95% CI) P value**
AdjHR associated with:				
1-SD decrease in baseline CC16	0.91 (0.70, 1.19)	0.81 (0.62, 1.06)	1.37 (0.84, 2.22)	1.21 (0.74, 1.96)
P value	p=0.50	p=0.12	p=0.21	p=0.45
AdjHR associated with				
4th quartile of CC16 change – steep increase (Ref)	1	1	1	1
3rd quartile of CC16 change – slow increase	0.73 (0.34, 1.56)	0.68 (0.32, 1.42)	1.98 (0.20, 19.49)	1.83 (0.18, 18.70)
2nd quartile of CC16 change – slow decrease	1.85 (0.94, 3.63)	1.80 (0.92, 3.53)	7.09 (0.88, 57.04)	6.79 (0.80, 57.50)
1st quartile of CC16 change – steep decrease	1.54 (0.79, 2.99)	1.37 (0.71, 2.64)	6.88 (0.87, 54.12)	6.25 (0.78, 50.32)
P value for trend across quartiles	p=0.04	p=0.08	p=0.01	p=0.01

* Adjusted for sex, age, asthma, longitudinal smoking categories (based on combination of smoking status at baseline and prospective measurements), baseline pack-years, change in pack-years between baseline and prospective measurements, and baseline smoking intensity.

** Adjusted for sex, age, asthma, longitudinal smoking categories (based on combination of smoking status at baseline and prospective measurements), baseline pack-years, change in pack-years between baseline and prospective measurements, baseline smoking intensity, and baseline FEV1/FVC.

Table D. Adjusted HRs associated with prospective CC16 groups for incident airflow limitation in TESAOD.

	HR for incident airflow limitation N subjects who developed airflow limitation / total N subjects (89/456)^		HR for incident stage 2 airflow limitation N subjects who developed stage 2 airflow limitation / total N subjects (26/456)^	
	Adj* HR (95% CI) P value	**Adj** HR (95% CI) P value**	**Adj* HR (95% CI) P value**	**Adj** HR (95% CI) P value**
AdjHR associated with				
Persistently high CC16 (Ref)	1	1	1	1
Inconsistently low CC16	0.74 (0.41, 1.34)	0.75 (0.42, 1.36)	1.65 (0.47 5.84)	1.70 (0.48, 5.98)
Persistently low CC16	0.95 (0.54, 1.66)	0.89 (0.50, 1.59)	3.51 (1.11, 11.09)	3.31 (1.07, 10.21)
P value for trend across groups	p=0.78	p=0.64	p=0.02	p=0.03

* Adjusted for sex, age, asthma, longitudinal smoking categories (based on combination of smoking status at baseline and prospective measurements), baseline pack-years, change in pack-years between baseline and prospective measurements, and baseline smoking intensity.

** Adjusted for sex, age, asthma, longitudinal smoking categories (based on combination of smoking status at baseline and prospective measurements), baseline pack-years, change in pack-years between baseline and prospective measurements, baseline smoking intensity, and baseline FEV1/FVC.

Supplementary references

1. ATS statement--Snowbird workshop on standardization of spirometry. *Am Rev Respir Dis* 1979; **119**(5): 831-8.
2. Downs SH, Schindler C, Liu LJ, et al. Reduced exposure to PM10 and attenuated age-related decline in lung function. *The New England journal of medicine* 2007; **357**(23): 2338-47.
3. Standardization of Spirometry, 1994 Update. American Thoracic Society. *Am J Respir Crit Care Med* 1995; **152**(3): 1107-36.
4. Schultz ES, Gruzieva O, Bellander T, et al. Traffic-related air pollution and lung function in children at 8 years of age: a birth cohort study. *Am J Respir Crit Care Med* 2012; **186**(12): 1286-91.
5. Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am Rev Respir Dis* 1983; **127**(6): 725-34.
6. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med* 1999; **159**(1): 179-87.
7. Brown H, Prescott R. Applied mixed models in medicine. Chichester, UK: John Wiley & Sons, LTD; 2001.
8. Guerra S, Vasquez MM, Spangenberg A, Halonen M, Martinez FD. Serum concentrations of club cell secretory protein (Clara) and cancer mortality in adults: a population-based, prospective cohort study. *Lancet Respir Med* 2013; **1**(10): 779-85.