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Relation between circulating CC16 concentrations, lung function, and development of chronic obstructive pulmonary disease across the lifespan: a prospective study

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SUMMARY

Background Low concentrations of the anti-infl ammatory protein CC16 (approved symbol SCGB1A1) in serum have been associated with accelerated decline in forced expiratory volume in 1 s (FEV1) in patients with chronic obstructive pulmonary disease (COPD). We investigated whether low circulating CC16 concentrations precede lung function defi cits and incidence of COPD in the general population.

Methods: We assessed longitudinal data on CC16 concentrations in serum and associations with decline in FEV1 and incidence of airflow limitation for adults who were free from COPD at baseline in the population-based Tucson Epidemiological Study of Airway Obstructive Disease ([TESAOD] n=960, mean follow-up 14 years), European Community Respiratory Health Survey ([ECRHS-Sp] n=514, 11 years), and Swiss Cohort Study on Air Pollution and Lung Diseases in Adults ([SAPALDIA] n=167, 8 years) studies. Additionally, we measured circulating CC16

concentrations in samples from children aged 4–6 years in the Tucson Children's Respiratory Study (n=427), UK Manchester Asthma and Allergy Study (n=481), and the Swedish Barn/children, Allergy, Milieu, Stockholm, Epidemiological survey (n=231) birth cohorts to assess whether low CC16 concentrations in childhood were predictive for subsequent lung function

Interpretation: Low concentrations of CC16 in serum are associated with

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reduced lung function in childhood, accelerated lung function decline in adulthood, and development of moderate airflow limitation in the general adult population.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is among the chronic diseases with the highest morbidity and mortality burdens worldwide.[1] Development of this disease might be related to an accelerated decline in lung function during adult life, impaired lung function development during childhood, or a combination of these features.[2] Although smoking is the main risk factor for an accelerated decline in lung function and the inception of COPD,[3] not all smokers develop the disease and a notable proportion of cases are seen in never smokers. Apart from α 1- antitrypsindeficiency, which is only seen in about 1% of all cases of COPD,[4] there are no established biomarkers to identify adults at risk before disease onset or children who might be predisposed to lung function deficits in adult life. CC16 (approved symbol SCGB1A1) is a homodimeric pneumoprotein that is mainly produced by club cells in the distal airways, but is measurable in circulation.[5-7] Recurrent exposure to noxious environmental factors, such as cigarette smoke, results in chronically decreased numbers of club cells and concentrations of CC16 in serum.[8,9] Growing evidence suggests that CC16 has antiinflammatory and antioxidative properties in the lungs,[5-7] and might have a protective role against obstructive lung diseases.[9] In most clinical studies,[10-15] reduced concentrations of CC16 in blood and airways have been associated with increased prevalence and severity of COPD. Additionally, low concentrations of CC16 in serum were associated with decline in forced expiratory volume in 1 s (FEV^1) in patients with COPD in the ECLIPSE study[16] and Lung Health Study.[17] However, whether serum CC16 is a predictor of lung function trajectories and development of COPD in the general population is unknown.
We did a prospective study of participants in six longitudinal studies to investigate whether baseline concentrations of circulating CC16 are associated with lung function decline and incidence of airfl ow limitation in adults. Additionally, we assessed the relation between low CC16 concentrations and lung function in childhood, before exposure to any effects from active smoking.

METHODS

Participants

To assess the effects of CC16 concentration in adults, we used data from the Tucson Epidemiological Study of Airway Obstructive Disease (TESAOD)[18] as the main sample. We tested for replication of results with data from three Spanish centres in the European Community Respiratory Health Survey (ECRHS-Sp)[19] and from the Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA).[20] For studies in children, we used data from the birth cohorts of the Tucson Children' s Respiratory Study (CRS),[21] the UK Manchester Asthma and Allergy Study (MAAS),[22] and the Swedish Barn/ children, Allergy, Milieu, Stockholm,

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Epidemiological survey (BAMSE).[23] For each cohort study protocols were approved by the institutional ethics committee and written informed consent was obtained from study participants (or their parents for the birth cohorts).

TESAOD[18] is a population-based prospective cohort study of non-Hispanic white households in Tucson, AZ, USA. At baseline and in up to 11 subsequent surveys done over 24 years, participants completed standardized question naires on respiratory health and did lung function tests. For this study, we used data from 960 participants who at enrolment were aged 21–70 years, had a ratio of FEV^1 to forced vital capacity (FEV^1/FVC) of 70% or greater, had serum samples available for measurement of CC16 concentrations, and had lung function test results from at least one follow-up survey. ECRHS-Sp[19] included a random sample of individuals aged 20–44 years and an enrichment sample of people who reported taking asthma medication or having had asthma attacks or shortness of breath at night in the previous year. Participants completed a detailed questionnaire and did lung function tests at the time of the first survey and two follow-up surveys around 9 and 20 years later. We used data for 514 participants who had FEV^1/FVC ratios of 70% or greater and sufficient serum samples for measurement of CC16 concentrations taken at the year 9 survey (none was available from the first survey) and lung function test results from the year 20 survey. SAPALDIA[20] enrolled a random sample of adults aged 18–62 years recruited through population registries from eight areas in Switzerland. Standardised questionnaires and spirometry were completed at the time of the first survey and follow-up surveys around 11 and 19 years later. We used data from 167 participants who were selected as controls for a nested study of COPD because they had normal lung function (FEV^1/FVC ratio 70% or greater and FVC % predicted 80% or more), sufficient serum samples for measurement of CC16 concentrations taken at the year 11 survey (no serum samples were available from the first survey), and lung function test results from the year 19 survey.

CRS[21] is a longitudinal study that recruited 1246 healthy infants at birth. Baseline and multiple follow-up standardised questionnaires were completed up to adulthood (age 26 years), including specific questions on active smoking from age 16 years. Blood samples were taken at 6 years of follow-up (mean age 6·1 [SD 0·8] years) and lung function was assessed with spirometry at 11 years (mean age 10·9 [0·7] years) and 16 years of follow-up (16·7 [0·6]). We used data from 438 participants who had serum (n=360) or plasma (n=78) samples available for measurement of CC16 concentrations at the 6 years survey and who had lung function test results from at least one of the surveys at years 11 and 16.

MAAS[22] is a population-based birth cohort. Participants were recruited prenatally and followed up prospectively until age 16 years, with visits at years 5, 8, 11, and 16. Standardised questionnaires were completed and lung function tests were done at all visits. We used data from 481 participants who had serum samples available for measurement of CC16 concentrations from the year 5 visit and lung function results from year 5 and at least one of years 8, 11, or 16. BAMSE[23] is a population-based birth cohort that completed standardised questionnaires at baseline and multiple follow-up visits to age 16 years. Lung function tests were done at years 8 and 16. We used data from 231 participants who were selected for nested molecular studies by a

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random and asthma-enriched sampling strategy, had available plasma samples from year 4 for measurement of CC16 concentrations, and had lung function test results at year 8, 16, or both.

LUNG FUNCTION

Complete information on lung function tests is provided in the appendix. We used guidelines of the Global Initiative for Chronic Obstructive Lung Disease for definitions:[1] airflow limitation was defined as FEV^1/FVC ratio of less than 70% and stage 2 airflow limitation as FEV^1/FVC ratio of less than 70% and FEV^1 % predicted less than 80%. Sensitivity analyses were done with lower limit of normal cutoff values (appendix). Because bronchodilator response was not tested in most studies, we have assessed prebronchodilator results and we used the term incident airflow limitation throughout this report.

MEASUREMENT OF CC16 CONCENTRATIONS

Circulating CC16 was measured in stored samples taken at baseline in TESAOD, year 9 in ECRHS-Sp, year 11 in SAPALDIA, year 6 in CRS, year 5 in MAAS, and year 4 in BAMSE (all termed baseline in this study) with a commercially available ELISA kit (BioVendor, Asheville, NC, USA, and Modrice, Czech Republic). Additionally, 601 (63%) participants in TESAOD had follow-up serum samples available for prospective measurement of CC16 concentrations (mean duration of follow-up 6・5 years, range 1・0–11・0). Serum samples were used for all cohorts except the BAMSE cohort and 78 participants in CRS, where measurements were made in plasma. In CRS, these were adjusted to enable comparison with serum concentrations (appendix). The distribution of CC16 concentrations was skewed (long tail to the right) and, therefore, we used log-transformation-generated inverse standardised concentrations[24] to allow testing of effects related to 1 SD decrease from baseline. We separated CC16 concentrations into tertiles to investigate the risks associated with low CC16 concentrations for FEV^1 decline and incident airflow limitation in adults and for deficits in FEV^1 growth in children.

STATISTICAL ANALYSES

In the adult cohorts, we used multivariate linear regression models to test the effects of CC16 concentration at baseline on FEV^1 decline. Rate of FEV^1 decline (change per year) was included as the dependent variable and various known potential risk factors (including CC16 concentration and FEV^1 at baseline) as the independent variables. To avoid effects of observations with short follow-up periods in TESAOD, we included only participants who had follow up data for 5 years or longer (all ECRHS-Sp and SAPALDIA participants were followed up for at least 5 years). Results were confirmed in an analysis of all TESAOD participants. Additionally, because multiple observations were available for each participant in TESAOD, we applied random coefficients models[25] that adjusted for the Within household cluster correlation and within-participant serial correlation of repeated observations. These models included covariates and an interaction term between CC16 tertiles and years of follow-up to test whether decline in FEV^1

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differed across CC16 tertiles.
Incident airflow limitation was studied in participants from TESAOD and ECRHS-Sp. In TESAOD, the relation between baseline CC16 concentration and the risk of incident airflow limitation was tested with Cox`s proportional hazards models. Because there was only one follow-up survey after baseline in ECRHS-Sp, associations between CC16 concentration and incident airflow limitation were tested with logistic regression. Model discrimination was assessed with the Harrell's C statistic in Cox's models and by the area under the curve in logistic regression models. Because in TESAOD a household-based recruitment strategy was used, we used household-clustered sandwich estimators of SEs in all models for this cohort. Prospective CC16 data for participants in TESAOD were analysed by generating two variables: one based on the quartiles of the rate of change of CC16 concentrations in serum between baseline and follow-up,[24] and one based on the combination of CC16 concentrations at baseline and follow-up. For the latter, CC16 concentrations were classified in three categories of persistently low (being in the lowest CC16 tertile at the baseline and follow-up survey), inconsistently low (being in the lowest tertile in only one survey), and persistently high (not being in the lowest tertile at either survey). These categories were used as independent variables in the Cox's and logistic regression models. Follow-up for the Cox's proportional hazards models started at the time of prospective measurement. In analyses of participants in the birth cohorts, we used random eff ects models[25] to assess the eff ects of low CC16 concentrations on lung function growth in childhood. To remove potential effects of active smoking, we did sensitivity analyses based on the same models but with data for children who smoked by age 16 years in CRS and BAMSE removed. In MAAS, information on active smoking was not available at year 16 and, therefore, we have included only data from years 8 and 11 in this sensitivity analysis. All analyses were done with Stata SE version 12.0 and SPSS version 22.

[TABLE 1]

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. SG, MMV, DAS, A-EC, DK, JH, and DCMB had access to data from the cohorts in the study. All authors had final responsibility for the decision to submit the paper for publication.

RESULTS

The TESAOD study population included 570 (59%) women, 553 (58%) ever smokers, and 89 (9%) participants with asthma. Mean age at baseline was 45 years and a mean FEV^1 % predicted was 98%; baseline characteristics are presented in the appendix. Distributions of demo graphic and clinical characteristics were similar in the replication cohorts, although ECRHS-Sp had fewer women and SAPALDIA fewer smokers and participants with asthma (appendix). In a

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comparison of TESAOD participants included and excluded from this study, those included were slightly more likely to be women and older and less likely to be underweight than those excluded, but the two groups were otherwise similar (appendix).

In TESAOD, the median concentration of CC16 in serum was 8·0 ng/mL (IQR 5·7–10·9). Baseline CC16 concentrations were higher in men than women in TESAOD, had a U-shaped distribution across age groups, and were strongly and inversely associated with current smoking and pack-years (table 1, appendix). No association was found with asthma, but CC16 concentration in serum was directly associated with FEV^1 % predicted, FVC % predicted, and FEV^1/FVC ratio (table 1).

The TESAOD participants included in this study completed a mean of 6·8 lung function tests (SD 3·0, range 2·0–12·0) over a mean follow-up of 13·7 years (7·0, 1·0–23·0), yielding 6549 lung function test results. Younger age, normal weight, never smoking, and slightly better lung function at baseline were related to having longer follow-up, but no relation was found between baseline CC16 concentration in serum and length of follow-up (appendix). After adjustment for sex, age, height, smoking status and intensity, pack-years, asthma, and FEV^1 at baseline, CC16 concentration in serum at baseline of TESAOD predicted decline in FEV^1 during follow-up, with a 1 sD decrease in baseline CC16 concentration being associated with 4·4 mL/year additional decline in FEV^1 (table 2).

A similar association was seen between baseline CC16 concentration and subsequent FEV^1 decline in SAPALDIA. In ECRHS-Sp, a similar association was seen, but to a lesser degree (table 2). These results were confirmed by reanalysis after participants with FEV^1/FVC ratios below the lower limit of normal at baseline were removed (TESAOD, p=0·0033; ECRHS-Sp, p=0·023; SAPALDIA, p=0·052) and after further adjustment for body-mass index (TESAOD, p=0·0008; ECRHS-Sp, p=0·012; SAPALDIA, p=0·057).

The mean rate of decline in FEV^1 in TESAOD was faster in men and women with low CC16 concentrations at baseline than that in men and women with high CC16 concentrations (mean difference 9·7 mL/year, 95% CI 3·0–16·3 in men, p=0·0043; 6·3 mL/year, 2·1–10·6 in women, p=0·0034; figure 1).

We found a stronger association between CC16 concentration and FEV^1 decline in smokers than in never smokers in TESAOD, but this interaction was not replicated in ECRHS-Sp or SAPALDIA (appendix). We found no interaction with age, even though associations with CC16 concentration were generally stronger in subjects aged 45 years and older than in those younger than 45 years in TESAOD (appendix).

Consistent with results on FEV^1 decline, TESAOD participants with CC16 concentrations in the lowest tertile were at increased risk of incident airflow limitation and incident stage 2 airflow limitation during follow-up, although only the latter association remained significant after adjustment for covariates (table 3). In fully adjusted models, 1 SD decrease in baseline CC16 concentration was associated with a 27% increase in risk of incident stage 2 airflow limitation (table 3). This

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inverse association was replicated in the ECRHS-Sp study (table 4), although only when CC16 concentration was used on a continuous scale. In the 601 TESAOD participants with CC16 concentrations measured in follow-up samples, after full adjustment we found that steeper decrease from baseline CC16 concentration to follow-up levels was associated with increasing risk of incident stage 2 airflow limitation (appendix). Additionally, compared with participants who never had low CC16 concentrations, those with persistently low concentrations had an additional 9·0 mL/year (4·3–13·7) decline in FEV^1 (p=0·0004) during follow up.
The baseline characteristics of the children in CRS, MAAS, and BAMSE are summarised in the appendix. The CRS cohort included slightly more boys than girls, 19% of children had a mother who smoked and 21% a father who smoked, and 9% had confirmed asthma. Fewer children in BAMSE had parents who smoked and higher proportions of children in MAAS and BAMSE (20% and 37%, respectively) had confirmed asthma. Univariate and multivariate analyses of CRS participants showed significant associations with reduced concentrations of CC16 for male sex and wheezing in the previous year (appendix). In adjusted random effects models on the three birth cohorts, low concentrations of CC16 at age 4–6 years predicted a deficit in FEV^1 in later childhood (fi gure 2). The lowest CC16 tertile was associated overall with a 68 mL deficit in FEV^1 up to age 16 years (p=0·0001), which was equivalent to 2–3% of expected FEV^1. Estimates were not substantially reduced by further adjustment for asthma, and associations were confirmed after restricting analyses to children who had never smoked up to age 16 years (–71 mL, p<0·0001; appendix).

[TABLE 2] [FIGURE 1]

DISCUSSION

In this study we found consistent associations between decreased circulating CC16 concentrations and reduced lung function in childhood, accelerated lung function decline in adulthood, and development of moderate airflow limitation in the general adult population. These findings are consistent with those from previous studies of patients with COPD, in whom lower baseline CC16 concentrations have been associated with steeper decline in FEV^1.[16,17] That we found this association in people without COPD suggests that the relation between low CC16 concentration and decline in FEV^1 is established before disease inception. This theory is supported by our finding that low circulating CC16 concentrations early in life predicted lung function deficits during childhood. Thus, low CC16 concentrations are a risk factor for lung function deficits across the lifespan. Among the factors that might affect CC16 concentrations in early childhood, we found that sex, age, body-mass index, maternal smoking, and active wheezing were independent predictors of low circulating CC16 concentrations. However, all these factors explained only a small proportion (around 6%) of the variability in CC16 concentrations. This finding supports the involvement of other genetic, physiological, environmental, and develop mental factors, as has been suggested previously.[26-29] Whether the relations between CC16 concentrations, lung function growth and decline, and development of

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COPD are causal or are confounded by other unmeasured factors cannot be conclusively determined from our data. Nevertheless, our results from the birth cohorts support the conclusion that CC16 concentrations have effects on lung function that are at least partly independent of cigarette smoking. Multidetector CT has shown narrowing and disappearance of small airways in the early stages of COPD,[30] which might be relevant to the CC16-related deficits seen in this disease. The molecular mechanisms by which CC16 could protect against development of COPD are unknown. Consistent with possible anti-inflammatory and anti-oxidative activities of this molecule in the lung,[5-7] CC16-deficient mice have increased lung epithelial injury, airway inflammation, and susceptibility to oxidative stress and infectious agents.[31-33] Some animal studies also indicate increased susceptibility of knockout mice to COPD-related phenotypes in response to cigarette smoking,[34,35] although one study failed to find an increased risk of emphysema.[17] Results from in-vitro studies suggest that CC16 affects inflammatory cell function[36,37] and that these effects might be linked to inhibition of PLA2 activity,[38] expression of prostaglandins,[39] chemotaxis,[36,40] and cytokine production.[37,41.] Although this study supports the temporal relation of CC16 concentrations and subsequent lung health outcomes, the possible relevance of this biomarker in the clinical or public health setting needs to be determined. In most of the cohorts we analysed, we assessed data from one CC16 measurement per participant, although we did assess two per person in a subset of TESAOD participants, which supported the potential added value of temporal trajectories of CC16 in risk prediction. No information was available on other physiological predictors of systemic CC16 concentrations, such as glomerular filtration rate (which is inversely related to CC16 concentrations in serum)[42] or diurnal variation.[43] Long-term studies that can control for such physiological factors and model serial CC16 measurements are needed to establish conclusively the potential of this molecule as a biomarker for prevention or treatment of COPD, whether in the general population or targeted subgroups. In TESAOD, the effects of CC16 concentrations seemed to be stronger in smokers than in never smokers, but this finding was not replicated in the other two adult cohorts. Thus, the interaction with

smoking might not be real or could be due to differences between populations or methods. This study has some limitations. No bronchodilator response was tested in TESAOD and, therefore, we were unable to assess how CC16 effects relate to the risk of COPD defined by post-bronchodilator lung function.[1] Also, differences in the numbers of available tests, length of follow-up, and instruments might have weakened comparability of cohorts for lung function. This could also be the case for CC16 measurements, owing to differences between cohorts in blood collection protocols and serum storage conditions, although CC16 concentrations are generally stable in serum.[44] However, we standardised CC16 concentrations and separated them into tertiles within each cohort and adopted a prospective study approach in participants with similar follow-up periods, which should have reduced the effects of such differences. Additionally, although these differences may affect the comparability of cohorts, they do not alter the internal validity of the results.
Among the strengths of our study are the long-term and population-based nature of the cohorts assessed, the extensive longitudinal characterisation of lung function, and

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the replication of findings in multiple independent cohorts that covered similar parts of the lifespan. Thus, we found low circulating concentrations of CC16 to be an independent predictor for subsequent deficits in lung function growth in childhood and for accelerated lung function decline and incident COPD in adulthood.

[FIGURE 2]

CONTRIBUTORS

SG and FDM designed the study. SG, MMV, DAS, A-EC, DK, JH, and DCMB analysed the data. ASp measured CC16 concentrations in blood samples from the TESAOD, SAPALDIA, and CRS cohorts, with supervision by MH. LT measured CC16 concentrations in blood samples from the ECRHS cohort with supervision by EB. IL measured CC16 in the blood samples from the BAMSE cohort with supervision by CD. Leadership and coordination for the specific cohorts and projects were provided by SG and MMV for TESAOD, J-PZ, JM-M, IU, JS, and JMA for ECRHS-Sp, MI and NP-H for SAPALDIA, SG, WJM, ALW, and FDM for CRS, ASi, and AC for MAAS, and EM and MW for BAMSE, and JB and JMA for MeDALL (for BAMSE). SG and FDM drafted the report with input from all authors. All authors approved the final version of the paper.

DECLARATION OF INTERESTS

WJM has received personal fees from the American Academy of Allergy, Asthma, and Immunology, American College of Chest Physicians, American Thoracic Society, Cystic Fibrosis Foundation and Genentech.
JB has received personal fees from Almirall, AstraZeneca, Chiesi, GlaxoSmithKline, Meda, Merck, Menarini, Merck Sharpe Dohme, Novartis, Sanofi -Aventis, Stallergenes, Takeda, Teva, and Uriach. DCMB is employed by GlaxoSmithKline; she was employed at the University of Manchester when the study was done and the statements made are in no way infl uenced by or express the views of GlaxoSmithKline. AC has received personal fees from AstraZeneca, GlaxoSmithKline, Novartis, and Thermo Fisher. FDM has received honoraria and fees for travel as an invited speaker from Abbott. The other authors declare no competing interests.

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TABLES AND FIGURES

TABLE 1:

	CC16 concentration in serum (ng/mL)*	p value
Sex		
Women (n=570)	7·38 (7·04–7·74)	..
Men (n=390)	8· 08 (7·67–8·51)	0·014
Age group (years)		
≥21–<30 (n=245)	8·29 (7·71–8·92)	..
≥30–<45 (n=212)	6·67 (6·18–7·19)	..
≥45–<60 (n=278)	7·21 (6·77–7·69)	..
≥60–≤70 (n=225)	8·61 (8·06–9·20)	<0·0001†
Body-mass index category		
Underweight (n=14)	5·63 (3·50–9·05)	..
Normal weight (n=538)	7·77 (7·42–8·14)	..
Overweight (n=308)	7·61 (7·15–8·11)	..
Obese (n=67)	7·04 (6·18–8·03)	0·106‡
Smoking status		
Never (n=406)	8·81 (8·41–9·22)	..
Former (n=220)	8·16 (7·65–8·70)	..
Current (n=333)	6·21 (5·80–6·64)	<0·0001
Physician-confirmed asthma		
No (n=870)	7·72 (7·43–8·01)	..
Yes (n=89)	7·15 (6·52–7·85)	0·222
Pack-years, all (n=959)§	−0·249	<0·0001
Pack-years, only smokers (n=553)	−0·178	<0·0001
FEV_1 % predicted (n=960)	0·121	0·0002
FVC % predicted (n=960)	0·066	0·041
FEV_1/FVC ratio (n=960)	0·073	0·023

TESAOD=Tucson Epidemiological Study of Airway Obstructive Disease. FEV_1=forced expiratory volume in 1 s. FVC=forced vital capacity. *Data are geometric means (95% CI) or Spearman's correlation coefficients for correlation with CC16 concentration. †p_{trend}=0·378. ‡p_{trend}=0·536. §Information was missing for one participant.

Table 1: **Associations between characteristics and CC16 concentrations at baseline in 960 TESAOD participants**

.

TABLE 2:

	β coefficient (95% CI) for increase in FEV_1 decline (mL/year) associated with 1 SD decrease in baseline CC16 concentration*	p value
TESAOD (n=800 with 6114 observations, mean follow-up 16·0 years)†	−4·4 (−7·1 to −1·7)	0·0014
ECRHS-Sp (n=495‡ with 990 observations, mean follow-up 11·5 years)	−2·4 (−4·5 to −0·3)	0·023
SAPALDIA (n=164§ with 328 observations, mean follow-up 8·3 years)	−4·5 (−9·0 to 0·0)	0·052
Meta-analysis	−3·3 (−4·8 to −1·8)	<0·0001

FEV_1=forced expiratory volume in 1 s. TESAOD=Tucson Epidemiological Study of Airway Obstructive Disease. ECRHS-Sp=European Community Respiratory Health Survey. SAPALDIA=Swiss Cohort Study on Air Pollution and Lung Diseases in Adults. *β-coefficient adjusted for sex, age, height, smoking status and intensity, pack-years, asthma, and FEV_1 at baseline. ECRHS-Sp models also included centre and sample type (random *vs* enriched) and SAPALDIA models included study area. †Limited to participants with ≥5 years of follow-up. ‡19 of 514 ECRHS-Sp participants had missing information for smoking status, intensity, or both, and were excluded from analyses. §Three of 167 SAPALDIA participants had missing information for smoking intensity and were excluded from analyses.

Table 2: **Association between 1 SD decrease in CC16 concentration in serum at baseline and decline in FEV_1 in adults**

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FIGURE 1:

Figure 1: Decline from baseline FEV1 in men (A) and women (B) across tertiles of CC16 concentrations in serum at baseline Data are estimated from fully adjusted random coefficients models in TESAOD18 participants, models were run separately for men (2546 observations in 389 participants) and women (3864 observations in 569 participants).

Lines represent predicted values for a man and woman aged 45 years at baseline with heights of 175 cm and 170 cm, respectively. Models were adjusted for age, smoking intensity, and physician-confi rmed asthma at baseline and for time-dependent height, smoking status, pack-years, and years of follow-up over time.

TESAOD=Tucson Epidemiological Study of Airway Obstructive Disease.

FEV1=forced expiratory volume in 1 s. High=>9・79 ng/mL. Medium=6・68-9・79 ng/mL. Low=<6・68 ng/mL.

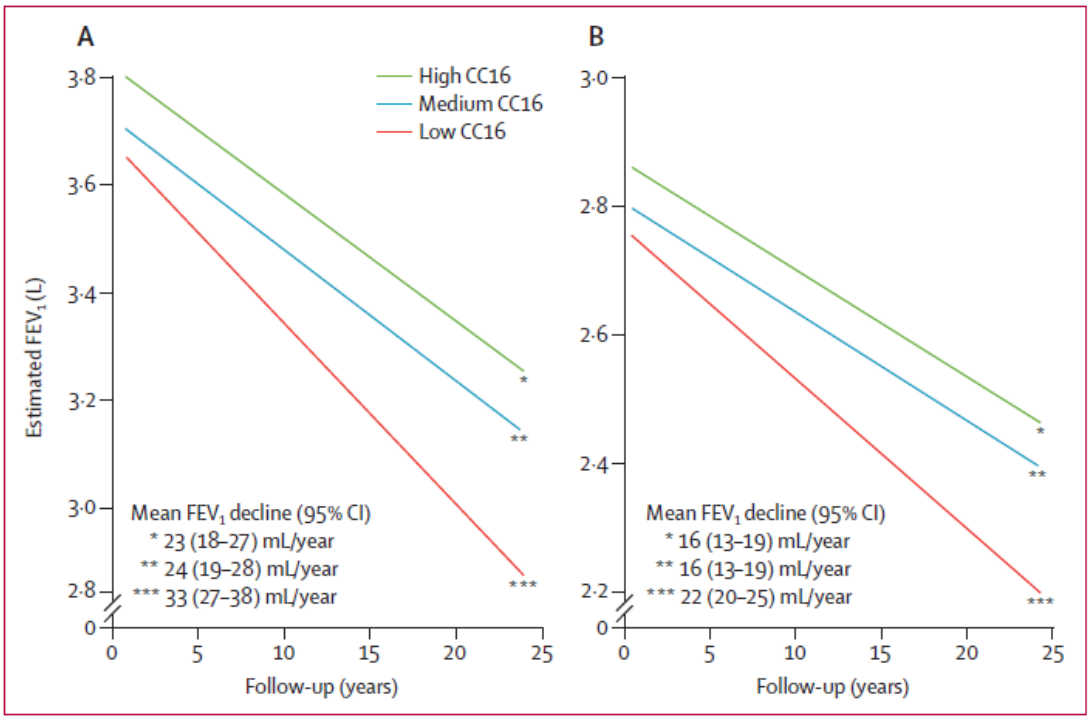

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TABLE 3 AND 4:

	Incident airflow limitation (251 cases)*			Incident stage 2 airflow limitation (106 cases)*		
	Crude HR (95% CI)	Adjusted HR (95% CI)†	Fully adjusted HR (95% CI)‡	Crude HR (95% CI)	Adjusted HR (95% CI)†	Fully adjusted HR (95% CI)‡
Effect associated with standardised baseline CC16 tertiles						
High	1·00	1·00	1·00	1·00	1·00	1·00
Medium	1·09 (0·79–1·49), p=0·613	1·17 (0·86–1·60), p=0·325	1·15 (0·84–1·57), p=0·376	1·53 (0·89–2·60), p=0·121	1·65 (0·97–2·82), p=0·067	1·71 (1·00–2·93), p=0·051
Low	1·45 (1·07–1·96), p=0·017	1·15 (0·84–1·58), p=0·387	1·10 (0·79–1·51), p=0·573	2·36 (1·44–3·88), p=0·0007	1·82 (1·08–3·07), p=0·024	1·81 (1·06–3·09), p=0·029
p_{trend}	0·017	0·403	0·596	0·0005	0·026	0·032
Effect associated with 1 SD decrease in CC16 concentration	1·17 (1·02–1·33), p=0·022	1·09 (0·93–1·27), p=0·284	1·06 (0·91–1·23), p=0·458	1·37 (1·16–1·61), p=0·0002	1·29 (1·04–1·59), p=0·019	1·27 (1·03–1·56), p=0·026
Harrell's C statistics (for models including CC16 tertiles)	0·55	0·73	0·81	0·60	0·79	0·83

TESAOD=Tucson Epidemiological Study of Airway Obstructive Disease. FEV_1=forced expiratory volume in 1 s. *Total population n=960, but two had missing information for smoking status or asthma and were excluded from analyses. †Adjusted for sex, age, smoking status and intensity, pack-years, and asthma at baseline. ‡Adjusted for sex, age, smoking status and intensity, pack-years, asthma, and FEV_1/FVC ratio at baseline.

Table 3: **Risk of incident airflow limitation associated with baseline CC16 concentration in serum in TESAOD**

	Incident airflow limitation (70 cases)*			Incident stage 2 airflow limitation (28 cases)*		
	Crude OR (95% CI)	Adjusted OR (95% CI)†	Fully adjusted OR (95% CI)‡	Crude OR (95% CI)	Adjusted OR (95% CI)†	Fully adjusted OR (95% CI)‡
Effect associated with standardised baseline CC16 tertiles						
High	1·00	1·00	1·00	1·00	1·00	1·00
Medium	1·53 (0·76–3·06), p=0·232	1·47 (0·72–3·02), p=0·289	1·37 (0·56–3·36), p=0·488	2·04 (0·60–6·91), p=0·253	1·89 (0·53–6·66), p=0·324	1·80 (0·46–7·02), p=0·397
Low	2·41 (1·25–4·64), p=0·0083	2·14 (1·06–4·30), p=0·033	2·12 (0·89–5·05), p=0·091	4·18 (1·37–12·8), p=0·012	3·35 (1·02–10·9), p=0·045	3·06 (0·84–11·2), p=0·090
p_{trend}	0·0070	0·032	0·085	0·0072	0·036	0·079
Effect associated with 1 SD decrease in CC16 concentration	1·47 (1·17–1·85), p=0·0011	1·39 (1·08–1·80), p=0·012	1·38 (1·00–1·92), p=0·051	1·75 (1·27–2·40), p=0·0005	1·64 (1·13–2·40), p=0·010	1·53 (1·00–2·34), p=0·049
Area under curve (for models including CC16 tertiles)	0·60	0·70	0·92	0·65	0·81	0·91

ECRHS-Sp=European Community Respiratory Health Survey. FEV_1=forced expiratory volume in 1 s. FVC=forced vital capacity. *Total population n=514, but 19 had missing information on smoking status, intensity, or both, and were excluded from analyses. †Adjusted for centre, sample type (random *vs* enriched), sex, age, smoking status and intensity, pack-years, and asthma at baseline. ‡Adjusted for centre, sample type (random *vs* enriched), sex, age, smoking status and intensity, pack-years, asthma, and FEV_1/FVC ratio at baseline.

Table 4: **Risk of incident airflow limitation associated with baseline CC16 concentration in serum in ECRHS-Sp**

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Figure 2: Figure 2: Relation between tertiles of circulating CC16 concentrations at age 4–6 years and FEV1 at 8‐16 years Results are from random eff ects models adjusted for sex, age, height, survey, maternal smoking, ethnic origin (CRS and MAAS), and baseline FEV1 (MAAS). Error bars indicate 95% CIs. The dependent variable of the models was FEV1. CRS=Tucson Children’ s Respiratory Study. MAAS=UK Manchester Asthma and Allergy Study. BAMSE=Swedish Barn/ children, Allergy, Milieu, Stockholm, Epidemiological survey. FEV1=forced expiratory volume in 1 s. Low=<6・68 ng/mL. Medium=6・68‐9・79 ng/mL. High=>9・79 ng/mL. obs=observations.