ORIGINAL ARTICLE

Circulating CC16 and Asthma

A Population-based, Multicohort Study from Early Childhood through Adult Life

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Abstract

Rationale: Club cell secretory protein (CC16) is an antiinflammatory protein highly expressed in the airways. CC16 deficiency has been associated with lung function deficits, but its role in asthma has not been established conclusively.

Objectives: To determine *1*) the longitudinal association of circulating CC16 with the presence of active asthma from early childhood through adult life and *2*) whether CC16 in early childhood predicts the clinical course of childhood asthma into adult life.

Methods: We assessed the association of circulating CC16 and asthma in three population-based birth cohorts: the Tucson Children's Respiratory Study (years 6–36; total participants, 814; total observations, 3,042), the Swedish Barn/Children, Allergy, Milieu, Stockholm, Epidemiological survey (years 8–24; total participants, 2,547; total observations, 3,438), and the UK Manchester Asthma and Allergy Study (years 5–18; total participants, 745; total observations, 1,626). Among 233 children who had asthma at the first survey in any of the cohorts, baseline CC16 was also tested for association with persistence of symptoms.

Measurements and Main Results: After adjusting for covariates, CC16 deficits were associated with increased risk for the presence of asthma in all cohorts (meta-analyzed adjusted odds ratio per 1-SD CC16 decrease, 1.20; 95% confidence interval [CI], 1.12–1.28; $P < 0.0001$). The association was particularly strong for asthma with frequent symptoms (meta-analyzed adjusted relative risk ratio, 1.40; 95% CI, 1.24–1.57; $P < 0.0001$), was confirmed for both atopic and nonatopic asthma, and was independent of lung function impairment. After adjustment for known predictors of persistent asthma, children with asthma in the lowest CC16 tertile had a nearly fourfold increased risk for having frequent symptoms persisting into adult life compared with children with asthma in the other two CC16 tertiles (meta-analyzed adjusted odds ratio, 3.72; 95% CI, 1.78–7.76; $P < 0.0001$).

Conclusions: Circulating CC16 deficits are associated with the presence of asthma with frequent symptoms from childhood through midadult life and predict the persistence of asthma symptoms into adulthood. These findings support a possible protective role of CC16 in asthma and its potential use for risk stratification.

Keywords: asthma; CC16; birth cohorts

Club cell secretory protein (CC16, also known as CC10 and CCSP) is receiving growing attention as a potential protective factor against lung function deficits and obstructive lung diseases (1–3). This homodimeric pneumoprotein is produced mainly by club cells and other epithelial cells in distal airways and is one of the most abundant proteins in normal airway secretions, with measurable concentrations in circulation (4). CC16 is encoded by the *SCGB1A1* gene on chromosome 11q12.3,

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and the SNP rs3741240, which is located in the 5′ untranslated region of *SCGB1A1*, has consistently been found to be by far the strongest protein quantitative trait locus for serum CC16 concentrations (5–7). Although the biological functions of CC16 have not been determined conclusively, this protein has been shown to possess antiinflammatory, immunomodulatory, and antitoxicant properties in the lungs (8, 9), which, in turn, may play a protective role against development and progression of lung diseases (1–3).

In line with this scenario, recently we reported low circulating CC16 concentrations to be related to impaired lung function (10) and decreased baseline serum CC16 to be a predictor for subsequent FEV_1 deficits in childhood and accelerated lung function decline in adulthood in the general adult population (11). Individuals with chronic obstructive pulmonary disease (COPD) were shown to have decreased CC16 concentrations in the airways (4, 12, 13) and circulation (4, 14, 15), and low serum concentrations of CC16 were associated with subsequent accelerated FEV_1 decline (16, 17), suggesting CC16 as a potential serum biomarker for disease progression in COPD.

The role of circulating CC16 in asthma, however, has not been established conclusively. Although cross-sectional clinical studies have reported lower concentrations of circulating CC16 in children (18, 19) and adults (20, 21) with asthma than in healthy control subjects, epidemiological studies are scarce. The few population-based studies, which were limited by the cross-sectional nature of the data and included only adult populations, failed to observe significant associations between serum CC16 concentrations and asthma (11, 15, 22). Whether these discrepancies are due to heterogeneous effects of CC16 on different asthma endotypes and severity stages or across different ages remains unknown. In addition, although several studies have shown that circulating concentrations of CC16 predict subsequent lung function decline and COPD risk (11, 16, 17), no study has addressed the potential use of CC16 in early stratification of childhood asthma to forecast the persistence of symptoms into adult life, a progression for which there are currently neither established prediction models nor available biomarkers (23).

In this study, we used data from three independent prospective birth cohorts to determine, in the first aim, the longitudinal relationship of circulating CC16 to the presence of asthma from childhood through adult life. In the second aim, we examined whether low CC16 concentrations in children with asthma are associated with the persistence of symptoms into adulthood. Some of the results of these studies were previously reported in the form of an abstract (24).

Methods

Study Participants

We used data from three population-based birth cohorts: TCRS (Tucson Children's Respiratory Study), BAMSE (Swedish Barn/Children, Allergy, Milieu, Stockholm, Epidemiological survey), and MAAS (UK Manchester Asthma and Allergy Study). The study design and enrollment process for each of the cohorts are described elsewhere (25–27) and summarized in the Methods section of the online supplement. For the present study, we included 814 TCRS, 2,547 BAMSE, and 745 MAAS participants who had both CC16 and asthma data available from the same year from at least one of the following surveys: years 6, 11, 16, 22, 26, 32, and 36 for TCRS; years 8 and 24 for BAMSE; and years 5, 8, and 11 and in early adulthood at age ⩾18 years (mean, 19.4; SD, 0.76) for MAAS. Participants who did not have CC16 and asthma data available in any of the survey years were excluded from the present study.

Measurement of Circulating CC16 and Genotyping of rs3741240

Circulating CC16 concentrations were measured by ELISA. Assay information is available in the Methods section of the online supplement. To minimize any potential impact of intercohort and intersurvey heterogeneity, CC16 values at each survey were $\log_{10}$ transformed, and standardized concentrations were generated to test effects related to a 1-SD decrease in CC16 concentrations. Standardized CC16 concentrations were also categorized into tertiles (low, medium, and high) to evaluate nonlinear associations. Because rs3741240 had previously been established as the strongest protein quantitative trait locus for circulating CC16 (5–7), we used genotype data for this SNP (*see* the Methods section of the online supplement).

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This article has a related editorial.

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

At a Glance Commentary

Scientific Knowledge on the Subject: Club cell secretory protein (CC16) is a major antiinflammatory protein highly expressed in the airways, with measurable concentrations in circulation. Studies have reported diminished circulating CC16 to be associated with lower lung function and accelerated decline in the general population and have identified CC16 as a possible biomarker in chronic obstructive pulmonary disease. However, research to date has been inconclusive regarding whether circulating CC16 deficits are related to asthma.

What This Study Adds to the Field: In this study, for the first time, to our knowledge, we characterized the longitudinal relationship of circulating CC16 to asthma. Using data from three independent population-based birth cohorts, we found a consistent association of circulating CC16 deficits with asthma from childhood through young adult life, independent of lung function impairment. The association was particularly strong for asthma with frequent symptoms and was present for both atopic and nonatopic asthma. Among children with asthma, low baseline circulating CC16 concentrations were associated with the persistence of frequent symptoms into adulthood. Results remained significant after adjustment for known risk factors for persistent asthma. These findings, together with existing evidence supporting a causal association, indicate a possible protective role of CC16 in asthma and its potential use for risk stratification and as a therapeutic target.

Study Variables

For the first aim of the study, consistent with previous work (28), asthma status at each survey year was defined as follows. Active asthma was defined as participants who had physician-diagnosed asthma with active symptoms (one or more asthma attacks or wheezes during the previous year). No active asthma was defined as participants who had physician-diagnosed asthma without active symptoms during the previous year (inactive asthma) and participants who did not have physician-diagnosed asthma (no asthma).

Because of limited information on medication use, rather than classifying asthma by severity, we characterized active asthma with symptom frequency, as done previously (29). Active asthma was further classified into asthma with infrequent symptoms (one to three episodes per year) and asthma with frequent symptoms (four or more episodes per year). Because of incomplete information on symptom frequency at Year 18 in MAAS, for this cohort, only active asthma was evaluated at that age (*see* the Methods section of the online supplement for details).

For the second aim, in analyses of symptom persistence among children with asthma at the first survey (Year 6 in TCRS, Year 8 in BAMSE, and Year 5 in MAAS), the persistence of symptoms was defined as having active symptoms in at least 50% of follow-up surveys with available data, a cutoff we used in previous work (29) (*see* the Methods section of the online supplement). In TCRS and BAMSE, where complete information on symptom frequency was available, we further defined children with asthma who reported having four or more episodes per year in at least 50% of follow-up surveys as having persistence of frequent symptoms. In secondary analyses, active asthma at each survey year was classified into atopic and nonatopic asthma on the basis of childhood atopic status, as described in the Methods section of the online supplement.

Lung Function Measurements

Spirometry was performed at multiple surveys. In each of the cohorts, we defined low lung function based on evidence of airway obstruction using at least two measurements of FEV_1/FVC percent predicted. For sensitivity analyses, low lung function was also defined using lung function trajectory classes previously reported (30–32). Details on spirometry procedures and definitions of low lung function are provided in the Methods section of the online supplement.

Asthma–Lung Function Groups

To evaluate independent relationships of circulating CC16 with asthma and lung function, in each of the cohorts, participants were categorized into four mutually exclusive groups on the basis of whether they had asthma with frequent symptoms at any of the surveys and/or low lung function during the study follow-up as follows: normal, low lung function only, asthma with frequent symptoms only, and asthma with frequent symptoms and low lung function (*see* the Methods section of the online supplement).

Statistical Analysis

One possible directed acyclic graph representing the main hypothesis of the study is presented in Figure E1 in the online supplement. For the first aim of the study, a longitudinal model using generalized estimating equations was used to assess the relationship of circulating CC16 to active asthma. For models with three-category asthma outcomes (no active asthma, asthma with infrequent symptoms, asthma with frequent symptoms; and no active asthma, atopic asthma, nonatopic asthma), we used multinomial logistic regression with subject-clustered sandwich estimators of standard errors. Survey year, sex, race or ethnicity, parental asthma, and childhood atopy were included as covariates in primary analyses. Comprehensive multivariate analyses to assess the effects of covariates on the association between CC16 and asthma were also conducted. Associations of the asthma–lung function groups with circulating CC16 across ages were evaluated using random coefficient models, adjusted for age and sex.

Genetic analyses were restricted to participants with both non-Hispanic White (Caucasian) parents for TCRS and to White participants for BAMSE and MAAS. Additive genetic models were used to determine the relationship of rs3741240 to circulating CC16 across ages in random effects models adjusted for survey year. The association of rs3741240 with ever asthma (a positive report of active asthma at any survey) was tested using logistic regression. Because power computations indicated that our study was not powered to detect additive odds ratios (ORs) less than 1.19, to increase statistical power, we further assessed rs3741240 for association with asthma using publicly available genome-wide association study (GWAS) databases with large sample sizes (www.ebi.ac.uk/gwas/) from the Trans-National Asthma Genetic Consortium (TAGC) (33) and the UK Biobank (34).

For the second aim of the study, only children with asthma at the first survey were

Table 1. Characteristics of TCRS, BAMSE, and MAAS Participants Included in Present Study

Characteristics	TCRS (*n* = 814)	BAMSE (*n* = 2,547)	MAAS (*n* = 745)
Male, % (*n*/total)	49% (397/814)	47% (1,193/2,547)	55% (408/745)
Maternal age at child's birth, mean ± SD (*n*)	28 ± 4.6 (814)	31 ± 4.4 (2,546)	31 ± 4.7 (706)
Maternal smoking during pregnancy, % (*n*/total)	16% (131/813)	12% (296/2,546)	13% (97/744)
Parental smoking at birth, % (*n*/total)	34% (273/802)	21% (521/2,535)	29% (215/744)
Parental asthma, % (*n*/total)	22% (179/807)	22% (567/2,547)	30% (224/745)
Parental education, % (*n*/total)			NA
Both >12 yr	63% (505/798)	28% (704/2,543)	
Mother or father ⩽12 yr	37% (293/798)	72% (1,839/2,543)	
Characteristics at first survey	Year 6	Year 8	Year 5
Age, mean ± SD (*n*)	6 ± 0.8 (804)	8 ± 0.5 (2,032)	5 ± 0.1 (712)
BMI, mean ± SD (*n*)	16 ± 2.0 (760)	17 ± 2.1 (2,027)	16 ± 1.5 (705)
BMI category, % (*n*/total)			
Normal weight	74% (566/760)	70% (1,410/2,027)	72% (509/705)
Underweight	5% (35/760)	1% (11/2,027)	1% (8/705)
Overweight	13% (99/760)	16% (332/2,027)	18% (125/705)
Obese	8% (60/760)	13% (274/2,027)	9% (63/705)
Active asthma, % (*n*/total)	10% (76/796)	6% (146/2,459)	14% (97/716)
Childhood atopy*, % (*n*/total)	27% (189/707)	26% (516/1,951)	31% (210/682)

Definition of abbreviations: BAMSE = Swedish Barn/Children, Allergy, Milieu, Stockholm, Epidemiological survey; BMI = body mass index; MAAS = UK Manchester Asthma and Allergy Study; NA = not available; TCRS = Tucson Children's Respiratory Study.
*Assessed by skin prick test in TCRS and MAAS and by Phadiatop in BAMSE.

included in analyses, and their baseline circulating CC16 was tested for association with persistence of symptoms. To examine a potential use of circulating CC16 for risk stratification, we compared children with asthma in the lowest CC16 tertile with those in the remaining tertiles combined (medium-high). Sensitivity, specificity, positive predictive value, and negative predictive value were calculated. Univariate and multivariate logistic regression models were performed. Sex, baseline symptom frequency, and childhood atopy were included as *a priori* covariates on the basis of their known influences on asthma persistence. The area under the curve was compared between models with and without baseline CC16.

For both aims, fixed effects meta-analyses were conducted to generate a pooled estimate of the effect across the three cohorts. All analyses were done with Stata SE version 15.0 (StataCorp). Additional methods for statistical and sensitivity analyses are provided in the Methods section of the online supplement.

Results

Characteristics of participants included in the present study from TCRS ($n = 814$), BAMSE ($n = 2,547$), and MAAS ($n = 745$) are summarized overall in Table 1 and by survey in Table E1. The distribution of participants by the total number of surveys with available CC16 and asthma data is shown for each of the cohorts in Figure E2. For each of the cohorts, characteristics of participants included in and excluded from the study are compared in Table E2. The only consistent difference across cohorts was that, as compared with participants excluded, those included were more likely to have parents of older age. As previously described (35), circulating CC16 concentrations increased from childhood into adult life and, although boys had lower concentrations than girls in childhood, this effect by sex reversed during adulthood in all cohorts (Table E1).

Circulating CC16 Deficits Are Associated with Asthma, Particularly Asthma with Frequent Symptoms

In generalized estimating equation models from childhood to adult life, after adjusting for survey year, sex, race or ethnicity, parental asthma, and childhood atopy, we found circulating CC16 concentrations to be inversely related to active asthma (Figure 1 and Table E3). The associations were consistent across all cohorts. A 1-SD decrease in CC16 concentrations increased the odds of having active asthma by 20% (meta-analyzed adjusted OR [adjOR], 1.20; [95% confidence interval, 1.12–1.28; $P < 0.0001$) (Figure 1A). To put this effect estimate in context, in TCRS, a 1-SD decrease in CC16 concentrations was equivalent to a difference of 2.67 ng/ml at age 6.

When active asthma was classified into asthma with infrequent symptoms and asthma with frequent symptoms, the relationship with circulating CC16 was more pronounced for the latter. Across the three cohorts, the risk of asthma with frequent symptoms increased by 40% for each 1-SD decrease in CC16 concentration (meta-analyzed adjusted relative risk ratio, 1.40 [1.24–1.57]; $P < 0.0001$) (Figure 1C), whereas the risk of asthma with infrequent symptoms, despite being statistically significant, was substantially smaller (Figure 1B). These results were in line with the finding that low CC16 showed a stronger and more consistent association with the presence of active asthma than inactive asthma (i.e., asthma without active symptoms during the previous year; Table E4), suggesting a dose–response relationship.

Data on the prevalence of active asthma, asthma with infrequent symptoms, and asthma with frequent symptoms by concurrent CC16 tertiles at each survey are shown in Figure E3. In all surveys and in all cohorts, participants with low CC16 had higher rates for asthma with frequent symptoms than participants with high CC16. Consistent with these findings, overall

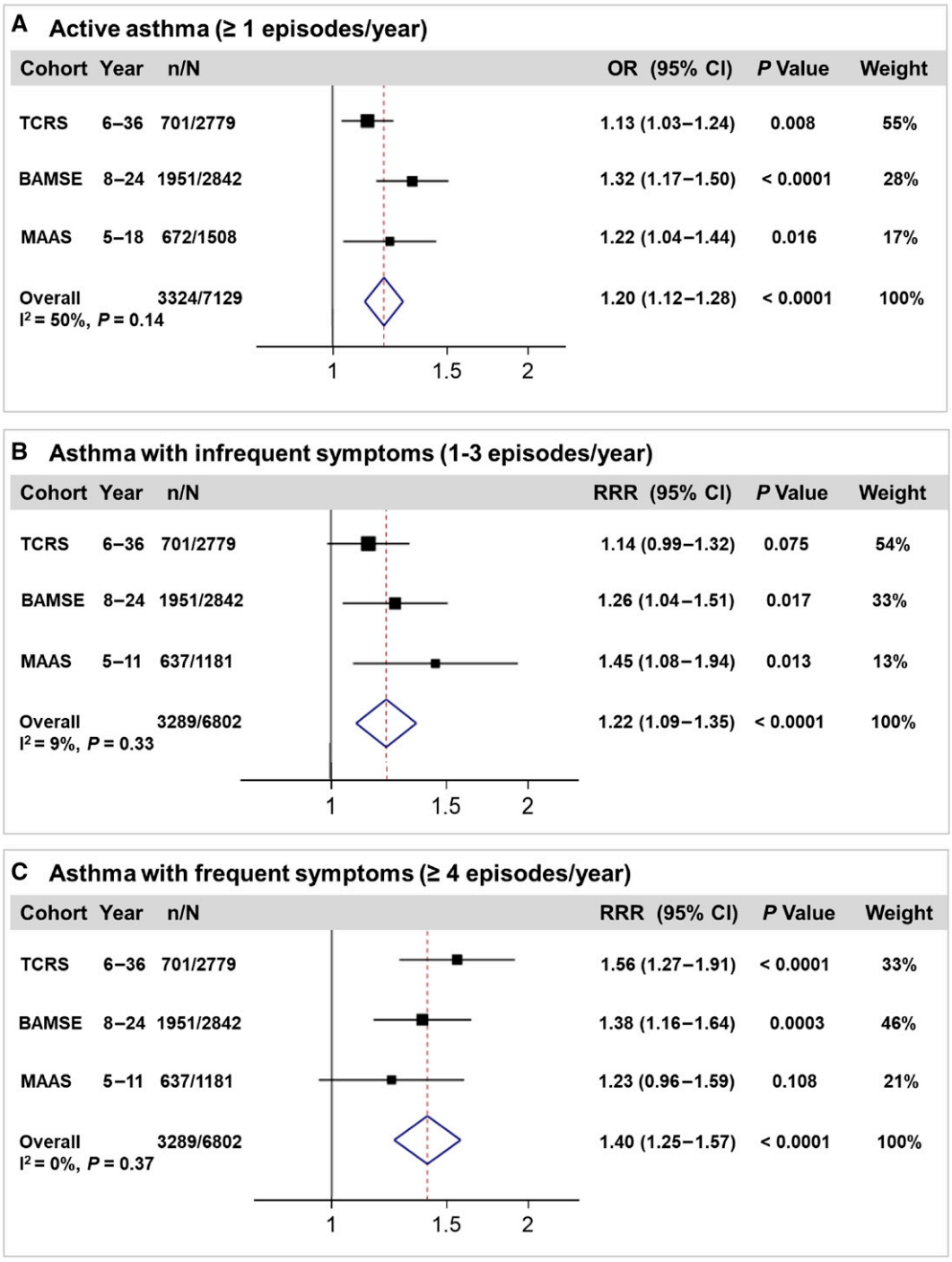

Figure 1. Longitudinal association of a 1-SD decrease in circulating club cell secretory protein concentrations with the presence of (*A*) active asthma, (*B*) asthma with infrequent symptoms, and (*C*) asthma with frequent symptoms. The no active asthma group was used as the reference group. Models were adjusted for survey year, sex, race or ethnicity, parental asthma, and childhood atopy (assessed by skin prick test at Year 6 in TCRS [Tucson Children's Respiratory Study] and Year 5 in MAAS [UK Manchester Asthma and Allergy Study] and by Phadiatop test at Year 8 in BAMSE [Swedish Barn/Children, Allergy, Milieu, Stockholm, Epidemiological survey]). In MAAS, only active asthma was evaluated at Year 18 because of incomplete information on symptom frequency. Totals of 113 TCRS, 596 BAMSE, and 73 MAAS participants had missing information for race or ethnicity, parental asthma, or childhood atopy and were excluded from the analyses. CI = confidence interval; *n* = number of participants; *N* = total number of observations; OR = odds ratio; RRR = relative risk ratio.

participants in the low CC16 tertile had 45% greater odds of having active asthma (meta-analyzed adjOR, 1.45 [1.22–1.72]; $P < 0.0001$) (Table E3 and Figure E4A) and were twice as likely to have asthma with frequent symptoms (meta-analyzed adjusted relative risk ratio, 1.97 [1.47–2.62]; $P < 0.0001$) (Table E3 and Figure E4C) than those in the high CC16 tertile. Being in the low tertile also increased the risk for asthma with infrequent symptoms, but to a lesser magnitude (meta-analyzed $P = 0.003$) (Table E3 and Figure E4B). There was no significant increase in asthma risk associated with the medium CC16 tertile.

The potential confounding effects of covariates (sex, race or ethnicity, parental asthma, childhood atopy, parental education, parental smoking at birth, early-life nitrogen gas exposure, early-life lower respiratory illnesses, active smoking, body mass index [BMI], and FEV_1 percent predicted) on the association between CC16 and asthma are shown in Table E5. CC16 effects were strikingly consistent and robust to adjustment for all covariates. Meta-analyzed estimates were virtually unchanged or only modestly altered (Table E5). There was no marked difference in the relationship of CC16 with asthma when data were stratified by sex (Figure E5) and by age group (childhood <18 y and adulthood ≥ 18 y) (Figure E6). Furthermore, when active asthma was classified into atopic and nonatopic asthma, we found relatively similar risk estimates associated with decreased CC16 for atopic and nonatopic asthma (Figure E7).

Association between Asthma with Frequent Symptoms and Circulating CC16 Deficits Is Independent of Lung Function Impairment

The relationship of longitudinal CC16 concentrations to the asthma–lung function groups is shown in Table 2. Consistent with our previous report (10), circulating CC16 concentrations were diminished in individuals with low lung function. However, notably, we found asthma with frequent symptoms to be associated with decreased circulating CC16 concentrations, regardless of the presence of lung function deficits. In all cohorts, among those with normal lung function, participants who had asthma with frequent symptoms had lower concentrations of CC16 from childhood to young adulthood than those who did not have such a condition (meta-analyzed

Table 2. Associations of Asthma–Lung Function Groups with Circulating Club Cell Secretory Protein from Childhood to Adult Life

Group	Z-Score Circulating CC16													
	TCRS Years 6–36			BAMSE Years 8–24			MAAS Years 5–18			Overall				
	n/N = 626/2,705			n/N = 1,861/2,694			n/N = 637/1,479			n/N = 3,124/6,878				
	n	Coefficient (95% CI)	P Value	n	Coefficient (95% CI)	P Value	n	Coefficient (95% CI)	P Value	n	Coefficient (95% CI)	P Value		
Group 1: Normal	472	ref		1,498	ref		498	ref		2,468	ref			
Group 2: Low lung function only	34	−0.39 (−0.67, −0.10)	0.008	154	−0.11 (−0.26, 0.04)	0.158	38	−0.27 (−0.56, 0.02)	0.073	226	−0.19 (−0.31, −0.07)	0.003		
Group 3: Asthma with frequent symptoms only	92	−0.34 (−0.52, −0.16)	0.0003	175	−0.16 (−0.30, −0.02)	0.030	73	−0.29 (−0.51, −0.07)	0.009	340	−0.24 (−0.34, −0.14)	<0.0001		
Group 4: Asthma with frequent symptoms and low lung function	28	−0.36 (−0.67, −0.05)	0.023	34	−0.42 (−0.72, −0.12)	0.006	28	−0.26 (−0.60, 0.08)	0.129	90	−0.35 (−0.53, −0.17)	<0.0001		

Definition of abbreviations: BAMSE = Swedish Barn/Children, Allergy, Milieu, Stockholm, Epidemiological survey; CC16 = club cell secretory protein; CI = confidence interval; MAAS = UK Manchester Asthma and Allergy Study; *n* = number of participants; *N* = total number of observations; ref = reference group; TCRS = Tucson Children's Respiratory Study.

Low lung function was defined using FEV_1/FVC percent predicted (*see* the METHODS section of the online supplement). Results were derived from random coefficient models, adjusted for age and sex.

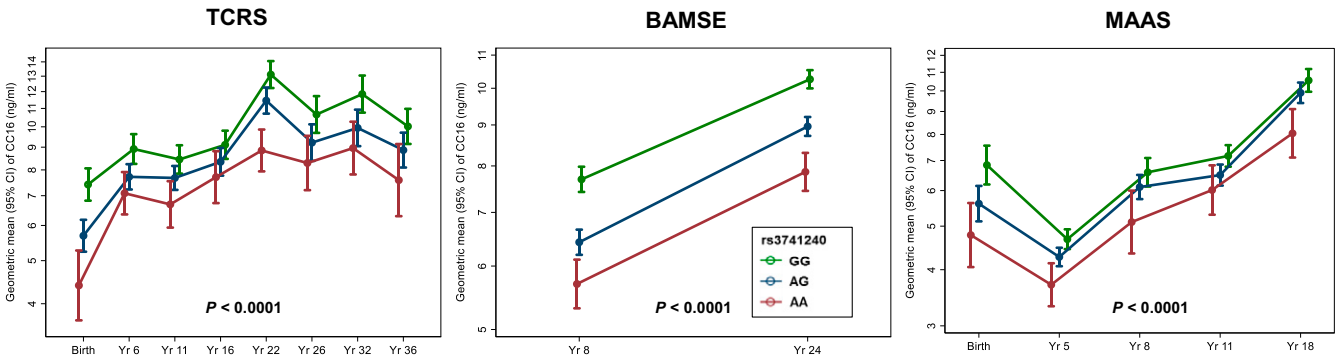

Figure 2. Geometric means of circulating club cell secretory protein concentrations by rs3741240 genotypes. Number of participants = 463, 2,023, and 671; number of observations = 2,179, 2,833, and 1,692 for TCRS (Tucson Children's Respiratory Study), BAMSE (Swedish Barn/Children, Allergy, Milieu, Stockholm, Epidemiological survey), and MAAS (UK Manchester Asthma and Allergy Study), respectively. *P* values were derived from random effects models, adjusted for survey year. CI = confidence interval.

$P < 0.0001$) (Table 2). The magnitude of CC16 deficits associated with having asthma with frequent symptoms but without low lung function was comparable to that of having low lung function but without asthma with frequent symptoms. As expected, individuals with both impaired lung function and asthma with frequent symptoms had the greatest reduction in circulating CC16 concentrations. Results from sensitivity analyses using previously described lung function trajectories to define low lung function (Table E6) supported those of the primary analyses.

Effects of rs3741240 on Circulating CC16 and Asthma

In genetic analyses, in accordance with our previous report (35), we first confirmed that circulating CC16 concentrations were strongly influenced by rs3741240 in TCRS and replicated this association in BAMSE and MAAS. The effects of rs3741240 on circulating CC16 were consistent from birth to young adulthood (Figure 2), resulted in diminished CC16 concentrations at all ages in individuals carrying the A allele (meta-analyzed $P < 0.0001$) (Table E7), and explained 4–6% of the variability of CC16 concentrations across ages (Table E7). Although we did not observe this SNP to be significantly associated with asthma across our three cohorts (meta-analyzed OR, 1.09; 0.96–1.23; $P = 0.182$) (Table E7), by using GWAS databases that provided stronger statistical power, we observed a significant association between rs3741240 and asthma in the UK Biobank ($P = 0.002$) and a trend in the same direction in TAGC ($P = 0.071$), with a meta-analyzed 2% increase in asthma risk with each additional A allele (OR, 1.02 [1.01–1.03]; $P = 0.0003$) (Table 3).

Circulating CC16 Deficits in Children with Asthma Predict the Persistence of Frequent Symptoms

For the second aim of the study, we used a total of 233 children with asthma (TCRS, Year 6, $n = 52$; BAMSE, Year 8, $n = 121$; MAAS, Year 5, $n = 60$) who were prospectively followed to evaluate the persistence of symptoms up to adult life (TCRS, Years 8–36; BAMSE, Years 12–24; MAAS, Years 8–18). The distribution of participants by the number of follow-up surveys with available data is shown for each of the cohorts in Figure E8. Baseline characteristics of included participants are summarized in Table E8. Overall, 77%, 51%, and 62% of children with asthma had persistent symptoms during follow-up in TCRS, BAMSE, and MAAS, respectively. The corresponding percentages for persistence of frequent symptoms were 37% and 32% in TCRS and BAMSE, respectively. Compared with children with asthma in the medium and high CC16 tertiles combined, children with asthma in the lowest CC16 tertile were more likely to have persistent symptoms (unadjusted meta-analyzed OR, 1.97 [1.14–3.40]; $P = 0.016$) (Figure 3A) and particularly to have persistence of frequent symptoms (3.58 [1.83–7.01]; $P < 0.0001$) (Figure 3B) into adult life. We further determined whether this relationship was confounded by other factors and observed that, after adjustment for sex, baseline symptom frequency, and childhood atopy, being in the low CC16 tertile remained strongly associated with an increased risk for persistence of frequent symptoms (adjusted meta-analyzed OR, 3.72 [1.78–7.76]; $P < 0.0001$) (Table 4). We observed a similar discriminatory ability of low CC16 at baseline for persistence of frequent symptoms during follow-up in TCRS and BAMSE, with an overall 69% sensitivity, 62% specificity, 48% positive predictive value, and 80% negative predictive value (Table 4). The addition of baseline CC16 to a base model including other known predictors of persistent asthma symptoms improved the overall area under the curve by 5–7% (Table 4).

Sensitivity analyses were conducted, and the results were confirmed after adjusting for the number of follow-up surveys (Table E9, model B) and after redefining the persistence of symptoms to also include those with active

Table 3. Estimated Effects of rs3741240 (Additive Model; Effect Allele [A]) on Asthma in UK Biobank and Trans-National Asthma Genetic Consortium

	Odds Ratio (95% CI)	*P* Value	*n*
UK Biobank	1.02 (1.01–1.03)	0.002	393,859
TAGC	1.02 (1.00–1.05)	0.071	142,486
Meta-analysis of UK Biobank and TAGC	1.02 (1.01–1.03)	0.0003	536,345

Definition of abbreviations: CI = confidence interval; TAGC = Trans-National Asthma Genetic Consortium.

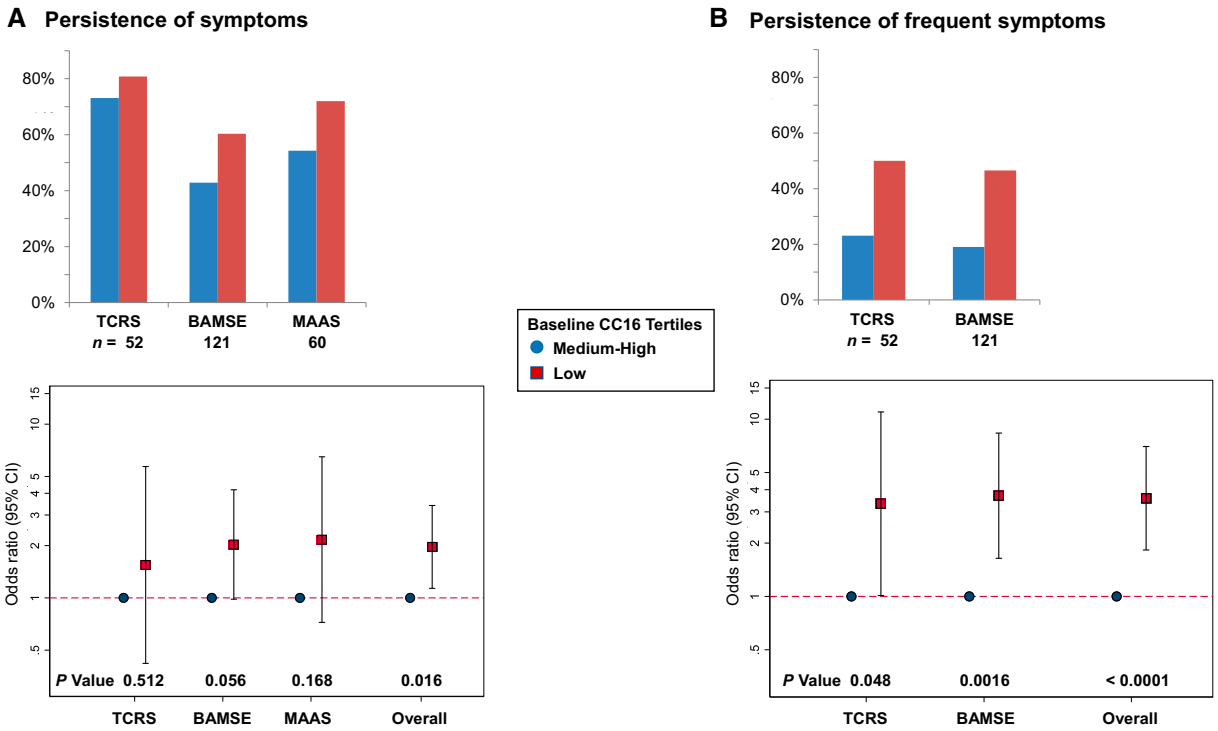

Figure 3. Association of baseline circulating club cell secretory protein with (*A*) persistence of symptoms and (*B*) persistence of frequent symptoms up to adult life among children with asthma at the first survey. *n* = number of participants. Results were derived from logistic regression (unadjusted). Persistence of frequent symptoms was evaluated only in TCRS (Tucson Children's Respiratory Study) and BAMSE (Swedish Barn/Children, Allergy, Milieu, Stockholm, Epidemiological survey) because of incomplete information on symptom frequency at Year 18 in MAAS (UK Manchester Asthma and Allergy Study). CI = confidence interval.

Table 4. Ability of Low Club Cell Secretory Protein at Baseline (Lowest Tertile vs. Medium-High Tertiles Combined) to Risk Stratify Children with Asthma at First Survey for Persistence of Frequent Symptoms from Ages 8 to 36 in TCRS and from Ages 12 to 24 in BAMSE

	TCRS (*n* = 52)	BAMSE (*n* = 121)	Overall (*n* = 173)
Sensitivity	68% (43–87)	69% (52–83)	69% (57–84)
Specificity	61% (42–77)	62% (51–73)	62% (53–72)
PPV	50% (30–70)	47% (33–60)	48% (37–61)
NPV	77% (56–91)	81% (69–90)	80% (71–90)
AUC			
Base model: sex, baseline symptom frequency, childhood atopy	0.80 (0.67–0.92)	0.66 (0.55–0.76)	0.72 (0.65–0.81)
Base model + baseline CC16 tertiles	0.84 (0.73–0.95)	0.73 (0.64–0.82)	0.78 (0.72–0.86)
OR unadjusted	3.33 (1.01, 10.99), *P* = 0.048	3.70 (1.64, 8.35), *P* = 0.002	3.58 (1.83, 7.01), *P* < 0.0001
OR adjusted*	4.53 (0.98, 21.02), *P* = 0.054	3.50 (1.51, 8.11), *P* = 0.003	3.72 (1.78, 7.76), *P* < 0.0001

Definition of abbreviations: AUC = area under the receiver operating characteristic curve; BAMSE = Swedish Barn/Children, Allergy, Milieu, Stockholm, Epidemiological survey; CC16 = club cell secretory protein; MAAS = UK Manchester Asthma and Allergy Study; NPV = negative predictive value; OR = odds ratio; PPV = positive predictive value; TCRS = Tucson Children's Respiratory Study.
Persistence of frequent symptoms was defined as having four or more episodes per year in at least 50% of follow-up surveys with available data. Results were derived from logistic regression models (*see* Figure E9 for longitudinal models). Numbers in parentheses are 95% confidence intervals.
*Adjusted for sex, baseline symptom frequency, and childhood atopy (assessed by skin prick test at Year 6 in TCRS and by Phadiatop at Year 8 in BAMSE). Analyses were conducted only in TCRS and BAMSE because of incomplete information on symptom frequency at Year 18 in MAAS.

symptoms at the last childhood survey, regardless of the proportion of positive follow-up surveys (model C). Results from analyses using longitudinal models that assessed subsequent asthma outcomes at each of the follow-up surveys also supported those of the primary analyses (Figure E9A). These sensitivity analyses also confirmed that the association of low CC16 at baseline with subsequent persistence of asthma symptoms was robust to further adjustment for baseline BMI and lung function (Figure E9B) and was comparable for persistence of frequent symptoms in childhood and adult life (Figures E9C and E9D).

Discussion

In this study, for the first time, to our knowledge, we characterized the longitudinal relationship of circulating CC16 to asthma. In three population-based birth cohorts, we found low circulating concentrations of CC16 to be consistently associated with increased odds of the presence of asthma from childhood to midadult life. The associations were particularly evident for asthma with frequent symptoms and confirmed for both atopic and nonatopic asthma. In addition, the relationships were present in groups of subjects with asthma both with and without concomitant lung function impairment. We further identified circulating CC16 as a promising biomarker in predicting the clinical course of childhood asthma. Among children with asthma, participants with circulating CC16 in the lowest tertile were at greater risk for having frequent symptoms persisting into adult life compared with their peers with medium-high CC16, even after adjustment for other known predictors of persistent asthma.

Low CC16 concentrations in the circulation of children with asthma (18, 19) and adults with asthma (20, 21) have been observed in several clinical studies. A previous clinical study (36) reported an OR of ~2 for the association of a 1-SD decrease in circulating CC16 with asthma, which was greater than ours, as expected because asthma in the subjects from that clinical study was substantially more severe than in the subjects in our population-based study. Earlier epidemiological reports, however, did not find significant relationships between CC16 and the presence of asthma (11, 15, 22). These epidemiological studies were cross-sectional, did not address asthma severity or frequency, and included only older populations in which multiple exposures (e.g., smoking) and declining renal function (22, 37) could have predominant effects on circulating CC16. By overcoming these limitations, in the context of a longitudinal study from childhood up to age 36 years, we observed remarkably consistent associations of circulating CC16 with risk for the presence of asthma across all three population-based birth cohorts, with evidence of a dose–response relationship between CC16 deficits and asthma symptom frequency. Although this risk was stronger for asthma with frequent symptoms, it was similarly increased for atopic and nonatopic asthma, suggesting a possible relationship of CC16 to multiple asthma endotypes. Importantly, the relationship of CC16 deficits to asthma cannot be ascribed simply to impaired lung function, which has previously been reported to be associated with decreased circulating CC16 concentrations (10). We found only minimal changes in risk estimates for CC16 and asthma after additional adjustment for lung function levels. This was further confirmed by the finding of reduced CC16 concentrations among individuals who had asthma with frequent symptoms but normal lung function, confirming that the association between low CC16 and asthma was partly independent of lung function impairment. Notably, the association held true even after controlling for several factors that could have a confounding impact on CC16 concentrations and/or asthma.

Our study also provides evidence that low concentrations of circulating CC16 are associated with subsequent persistence of asthma symptoms, particularly frequent symptoms, from childhood into adult life. Among children with asthma, we found those in the lowest CC16 tertile to be nearly four times as likely to have frequent symptoms persisting into adulthood compared with their peers with asthma with medium-high CC16. This association was independent of baseline factors known to contribute to asthma persistence, including sex, childhood atopy, symptom frequency, BMI, and lung function deficits. These findings are in line with those of a recent study in which reduced CC16 mRNA expression levels in bronchial epithelial cells among adults with asthma were predictive of an increased number of prospective exacerbations (38), although no circulating CC16 data were available and exacerbations were evaluated only over 3 years in that study. Altogether, our findings suggest circulating CC16 as a promising candidate biomarker for early risk stratification in children with asthma. In this context, although a single baseline CC16 measurement is likely to yield only limited predictive ability, future studies will need to address whether serial CC16 evaluations and/or the addition of CC16 to other clinical and laboratory assessments may enhance accuracy for predicting asthma persistence.

Our findings are consistent with increasing evidence that implicates CC16 deficits in the pathogenesis of several pulmonary diseases. In asthmatic airways, where type 2 inflammation is typically predominant, decreased CC16 expression was found to be associated with increased type 2 inflammatory biomarkers, suggesting CC16 as a potential nontraditional type 2 biomarker for asthma (38). A number of studies, however, have demonstrated CC16 deficiency to be associated with other lung diseases not primarily driven by type 2–mediated immune responses. Most notably, CC16 has been identified as a potential marker for disease severity and progression in COPD (16, 17). A recent study also revealed low serum CC16 to be associated with the severity of cystic fibrosis lung disease and to have synergistic effects with markers of neutrophilic inflammation (7). These observations suggest that CC16 deficits may not exclusively reflect dysregulation of a specific type of immune response. Given its wide range of biological properties (8, 9), it is reasonable to postulate that CC16 may mitigate airway inflammation and/or injury associated with various pathologies in a context-dependent manner.

Whether loss of CC16 precedes the development of asthma or asthmatic airway inflammation leads to diminished CC16 production is yet to be elucidated. CC16 may play a direct role in asthma inception and progression, or decreased CC16 concentrations may simply reflect the epithelial damage and airway remodeling that accompany asthma, in which the number of club cells is reduced (39). However, evidence from animal studies indicates that CC16 may be implicated directly in the pathophysiology of asthma. Recently, we have demonstrated that CC16 deficiency in mice resulted in enhanced airway hyperresponsiveness and structural alterations, including increased collagen deposition and enhanced smooth muscle

thickness, consistent with the characteristics of airway remodeling seen in chronic asthma (10). Further studies revealed that recombinant CC16 treatment abrogated exaggerated inflammatory responses to allergen in the lungs of CC16-knockout mice (40, 41). These findings indicate essential roles of CC16 in protecting murine lungs from the development of asthma-like phenotypes.

In support of a causal role of CC16 are also results from genetic analyses. In our study, we found that rs3741240A was consistently associated with reduced CC16 concentrations from birth into midadult life, and, when tested in sufficiently large populations, it was also associated with increased asthma risk. The association between rs3741240 and asthma reached statistical significance only in the large UK Biobank–TAGC meta-analysis but not in our substantially smaller cohort consortium, and, despite being significant ($P = 3 \times 10^{-4}$), the final OR for this association was relatively small (OR, 1.02). Because this SNP explains only ~5% of the variability in circulating concentrations of CC16, these results are not surprising, and although the magnitude of this genetic association is unlikely to provide any additional value of rs3741240 for risk stratification over what is already provided by circulating concentrations of CC16, its importance resides in supporting the possibly causal nature of the relationship between CC16 deficits and asthma. In line with these observations, previous studies have reported associations between rs3741240A and accelerated decline of lung function in smokers (42) and patients with cystic fibrosis (7), and a recent Mendelian randomization analysis supported a protective effect of genetically increased serum CC16 on both COPD risk and progression (6).

The underlying mechanisms by which CC16 could protect against asthma inception and progression remain to be determined. CC16 has been shown to possess antiinflammatory and immunomodulatory activities, through inhibition of nuclear factor-κB activation and phospholipase A2 activity, thereby suppressing arachidonic acid cascade and its downstream inflammatory mediators, such as prostaglandins and leukotrienes (41, 43–45). Studies have also demonstrated inhibitory effects of CC16 on serum amyloid A–driven inflammation via direct interaction with the lipoxin A4 receptor (46), which is present on airway epithelium and upregulated on macrophages in severe asthma (47). Recently, we identified a novel receptor for CC16, the integrin adhesion molecule very late antigen 4, whose binding may antagonize adhesion to endothelial cells and lung infiltration by activated leukocytes (48). Given these properties, it is reasonable to surmise that CC16 may play a direct protective role in asthma and that CC16 augmentation may prove beneficial in this disease. In this context, it is noteworthy that CC16 production has been shown to be upregulated both *in vitro* and *in vivo* by administration of retinoids in humans (49). Future research will need to address whether administration of recombinant human CC16 or drugs that augment endogenous CC16 production may influence the development and progression of asthma.

Among the limitations of our study is the relatively small number of children with asthma included in the analyses on prediction of symptom persistence. Although we had sufficient power to study rs3741240 in relation to circulating concentrations of CC16, analyses linking this SNP with asthma were underpowered in our three-cohort consortium. However, this limitation was overcome by using publicly available GWAS data. Circulating concentrations of CC16 were standardized within each cohort at each survey year to attain internal validity, but variation in blood collection protocols and sample type may have affected comparability across cohorts. However, we note that, despite this potential source of heterogeneity, our results were remarkably consistent across the three cohorts. The major strength of this study is the availability of three large, long-term, population-based birth cohorts, with extensive biorepositories, longitudinally assessed circulating CC16 concentrations, and phenotypic data on asthma collected from preschool age up to midadult life. The high consistency of results across the three independent cohorts also reinforces the validity of our findings.

In conclusion, we found circulating CC16 deficits to be strongly and independently associated with the presence of asthma, particularly asthma with frequent symptoms, from childhood through adult life. Furthermore, low circulating CC16 concentrations in children with asthma predicted the persistence of frequent symptoms into adulthood. Together with existing evidence for a causal relationship, these findings support a possible protective role of CC16 in asthma and warrant future studies to investigate systematically the potential use of this protein in risk stratification and as a therapeutic target in asthma. ■

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