

Association of urine CC16 and lung function and asthma in Chinese children

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ABSTRACT

Background: Many studies have shown the relationship between serum Club cell secretory protein-16 (CC16) and respiratory diseases. However, little research has been done to study urinary CC16 in relation to respiratory diseases. Our objective was to examine the association of urinary CC16 and physician-diagnosed asthma or lung function measurements in Chinese children.

Methods: A total of 147 physician-diagnosed children with asthma, ages 9–15 years, were recruited from our cross-sectional study population in northeast China. The 390 healthy children who were not asthmatic and not smokers were selected at random from the population according to 10% proportional sampling. Lung function values, including forced expiratory volume in 1 second and forced vital capacity were measured with two portable spirometers. Urine CC16 was determined by using an enzyme-link immunoassay kit. The relationships between urine CC16 levels and asthma, lung function were assessed by multiple regression models.

Results: The geometric mean (95% confidence interval [CI]) creatinine-adjusted urine CC16 level was, for creatinine, 9.77 ng/mg (95% CI, 8.12–12.02 ng/mg). After adjustments for sex, age, body mass index, parental education, and smoking status, lower urine CC16 levels were found to be associated with asthma (odds ratio 0.782 [95% CI, 0.617–0.990]). A positive association was found between urine CC16 and forced vital capacity (beta 0.064 [95% CI, 0.008–0.119]).

Conclusion: Our study demonstrated lower levels of urine CC16 and lung function in patients with asthma than in those patients without asthma. CC16 in urine may be a useful tool or biomarker for investigating lung epithelium integrity among children with asthma or lung injury.

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Allergic diseases and asthma are chronic inflammatory diseases of the airways that involve complex interactions of cells and mediators. Modulation of inflammatory processes occurs as a result of sensitization to both endogenous events and exogenous environmental oxidants.¹ Results of recent studies indicate that the primary cause of allergic diseases might lie in

some functional or structural defects of the airways epithelium.^{2,3} Although changes in airways epithelium are yet to be elucidated, the possible mechanism might be the destruction or the dedifferentiation of epithelial cells secreting immunomodulatory proteins.⁴

Biomarkers are useful molecular tools that are used to assess the pathobiologic mechanisms that underlie respiratory disorders and are indicative of early exposure or effects of environmental pollutants before the onset of symptoms.⁵ Lung surfactant proteins, e.g., the 16-kDa Club cell secretory protein-16 (CC16) (also known as SCGB1A1, CC10, CCSP, and uteroglobin), have served as a sensitive biologic marker of lung epithelium integrity.^{6,7} CC16 is a small protein mainly secreted by the Club cell, a nonciliated cell known for its high vulnerability to inhaled or systemic lung toxicants, which is found predominantly in the respiratory bronchioles but also in the prostate,⁷ endometrium, and kidney.^{6,8} Increased epithelial permeability may result in higher rates of passive diffusion and a transient increase in the concentration of CC16 protein in blood and, subsequently, in urine after CC16 elimination through glomerular filtration.⁵ Because of its anti-inflammatory properties, immunoregulation, and immunosuppression effects, CC16 has been proposed as a biomarker in the study of asthma.^{9–13}

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A variety of studies showed the levels of serum and/or urinary CC16 to be associated with poorer respiratory health in children and adults. Bernard *et al.*¹⁴ indicated that the poorer respiratory health of children who swam as infants could be linked to distal airways damage detected by the decrease of serum CC16. Kippelen *et al.*¹⁵ and Simpson *et al.*¹⁶ found that urinary CC16 with epithelial integrity in exercise-induced bronchoconstriction was associated with a rise in urinary excretion of CC16. In addition, several studies reported that acute exposure to smoke from factories, environmental tobacco smoke, or air pollutants (e.g., ozone) could cause a transient increases in serum CC16 levels, but several other studies showed chronic exposures to pulmonary irritants, such as environmental tobacco smoke, particulate matter, nitrogen oxides, and smoke from fire, were associated with decreased serum CC16 levels.^{5,10,17,18}

Due to its small size and water solubility, CC16 is eliminated by renal excretion and, therefore, can also be found and measured in urine.¹⁹ Because there is a very high correlation between serum and urinary concentrations of CC16 and CC16 is eliminated by renal excretion in subjects with no renal failure, urine CC16 as a marker for effects on the airways have been recommended as a replacement for serum CC16.^{20,21} However, few studies have been conducted on urinary CC16, and only a limited number of published studies investigated the associations of urine CC16 levels with lung function and asthma in children. The objective of this study was to assess the utility of urinary CC16 as a biomarker of asthma and lung function phenotypes in children from northeast China.

MATERIALS AND METHODS

Ethics Statement

The procedures used in this study were in accordance with the ethical standards of the committee on human experimentation at China Medical University. All experiments were consistent with the principles outlined in National Institutes of Health (NIH) guidelines on the ethical conduct of human research. Written informed consent was obtained from the parent or guardian of each child before data collection.

Study Characteristics

This study was based on a large-scale epidemiologic study in Liaoning in northeast China during 2011–2012. Details about the design were described by Ma *et al.*²² A total of 147 parental-reported physician-diagnosed children with asthma and who did not smoke, ages 9–15 years, were recruited from our cross-sectional study population in 36 public schools of northeast China. The 390 healthy children without asthma and who did not smoke were selected at random from

the population according to 10% proportional sampling. Information about demographic variables, environmental exposures, and asthma outcomes was collected from questionnaires. Spot urine samples were collected from each child during our morning visits at each school.

Urine CC16 Level Detection

Measurements of the child's height, weight, and lung function were obtained from schools before the experiment. The participants were asked to provide their first morning mid-stream urine samples in their homes and to keep them frozen until the experiment started. Afterward, 100-mL urine sample was obtained from each boy to avoid contamination by prostatic secretions, as described in Andersson *et al.*,²¹ collected in a dry tube and allowed to clot overnight at 4°C. Urine samples were stored at 80°C until analysis. Creatinine concentrations of the urine sample were determined by using a commercially available enzyme-linked immunoassay kit (Oxford Biomedical Research, Rochester Hills, MI). CC16 in urine was determined by a commercially available Human Club Cell Protein enzyme-linked immunoassay kit from Quantikine (R&D Systems, Minneapolis, MN). The limit of detection of this assay for CC16 was 0.07 ng/mL. Concentrations of CC16 and biomarkers below the mean minimum detectable dose were set to the minimum detectable dose. All the tests were duplicated, and the average of the two measurements was calculated as the concentration of CC16 protein.

Lung Function

Lung function values, including forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC) were measured with two portable electronic type spirometers (Spirolab; Medical International Research S.r.l., Roma, Italy) by two experienced researchers and technicians who used the American Thoracic Society and European Respiratory Society standards.²³ The child was asked to stand comfortably and to wear a nose clip to stop air from moving through the nose during the test. Both FVC and FEV₁ should be the largest value obtained from any of three technically satisfactory curves, and the FVC and FEV₁ values on at least two of these three curves should vary by no more than 5%. All the measurements were automatically corrected for body temperature and pressure saturated.

Statistical Analysis

The lung function predicted values were obtained by the reference equations used in our previous study.²² A spirometric pattern was considered impaired if %FEV₁ was <85% or FVC was <85% of the predicted value of the respective parameter. Parental education was di-

Table 1 Characteristics of children in the study population

Characteristics	All Children, <i>n</i> = 537
Age, mean (SD), y	11.79 ± 2.31
Height, mean (SD), cm	151.48 ± 14.69
Weight, mean (SD), kg	46.35 ± 16.47
Body mass index, mean (SD), kg/m ²	19.66 ± 4.47
Sex, no. (%)	
Male	261 (48.6)
Female	276 (51.4)
Parental education, no. (%)	
<High school	170 (31.7)
≥High school	367 (68.3)
Environmental tobacco smoke exposure, no. (%)	
No	259 (48.2)
Yes	278 (51.8)
FEV ₁ , mean (SD), L	2.40 ± 0.82
FEV ₁ <85% predicted	
No	485 (90.3)
Yes	52 (9.7)
FVC, mean (SD), L	2.59 ± 0.88
FVC <85% predicted	
No	468 (87.2)
Yes	69 (12.8)

SD = Standard deviation.

chotomized according to high school attendance. Smoking status referred to current exposure to smoke by family members.

Continuous variables (*e.g.*, age) were reported as mean (standard deviation), and categorical variables (*e.g.*, sex) were reported as percentages in each subgroup, seen in Table 1. The χ^2 test was used to test for the difference in a categorical variable by asthma. The *t*-test was used to test for the difference in the group means of a continuous variable by asthma. Because the CC16 measurements were found to follow a log-normal distribution, we performed logarithm transformation on the ratio of urinary CC16 to urinary creatinine and reported the geometric means and their 95% confidence intervals (CI) for the log-transformed CC16. The geometric means of the creatinine-adjusted urine CC16 and their CIs by sociodemographic characteristics, asthma, spirometric patterns, and lung function separately are presented in Table 2. Statistical analysis was performed with the *t*-test. The results from the analyses of the relationships between urine CC16 levels (as an independent variable) and asthma, spirometric patterns, and lung function parameters (as the outcomes) by using logistic and linear regression models are shown in Table 3. Estimates were adjusted for sex,

age, body mass index, parental education, and smoking status. All analysis was performed by using the SAS software (version 9.2; SAS Institute Inc., Cary, NC). Each test was two-tailed and used a significance level of 5%.

RESULTS

Children with asthma were more likely to be male, have low education levels, report environmental tobacco smoke exposure and abnormal lung function, and to have significantly lower FEV₁ and FVC than children without asthma (Table 1). The geometric mean (95% CI) of the creatinine-adjusted urine CC16 level was, for creatinine, 9.77 ng/mg (95% CI, 8.12–12.02 ng/mg). Urine CC16 levels were higher in boys than in girls. The asthma and spirometric restrictive patterns were not significantly associated with urine CC16 levels (Table 2). When adjusted for confounding variables, a negative association was found between urine CC16 levels and asthma in logistic regression (odds ratio 0.782 [95% CI, 0.617–0.990]). In linear regression, positive associations were observed between urine CC16 levels and FVC (β 0.064 [95% CI, 0.008–0.119]) (Table 3).

DISCUSSION

Based on this study of a sample of children living in northeast China, we showed that urinary CC16 could be developed and validated as a biomarker of early effects on the lung epithelium injury and pulmonary permeability. We also observed that decreased urine CC16 levels were associated with asthma and lower lung function in children. Our findings of decreased levels of urine CC16 in children with asthma were consistent with other studies that showed that levels of the CC16-kd protein in adults or children with asthma were lower in plasma and bronchoalveolar lavage fluid than their counterparts without asthma.^{24–26} Moreover, Shijubo *et al.*²⁴ found that patients with chronic asthma had lower serum CC16 levels than patients without chronic disease. In a recent case-control study, Ku *et al.*²⁷ reported that the serum CC16 levels in children with rhinitis and asthma were significantly lower than those who had no asthma. The primary causes of these altered levels may be the remodeling of the small airways and alteration of CC16 turnover by the respiratory epithelium.²⁶ In all, these findings confirmed that a decreased expression of the anti-inflammatory protein CC16 and that the polymorphic allele of the CC16 gene could play an important role in studying the pathogenic mechanism of asthma. Chronic airways disease may damage the Club cells and decrease excretion of CC16 in airways.²⁸ The decreased urinary excretion of CC16 may be the result of the decreased urine CC16 levels.

Table 2 Association between creatinine-adjusted urine CC16 levels and sociodemographic characteristics, asthma, spirometric patterns, and lung function

	No.	Urine CC16 Level, Geometric Mean (95% CI), ng/mg Creatinine	<i>p</i> Value
Sex			<0.001
Female	261	4.38 (3.42–5.61)	
Male	276	18.07 (13.94–23.42)	
Parental education			0.538
<High school	170	9.81 (6.92–13.90)	
≥High school	367	8.63 (6.89–10.81)	
Environmental tobacco smoke exposure			0.334
No	259	8.16 (6.22–10.72)	
Yes	278	9.83 (7.56–12.79)	
FEV ₁ , <85% predicted			0.831
No	485	9.04 (7.72–11.02)	
Yes	52	8.45 (4.41–16.18)	
FVC, <85% predicted			0.661
No	468	8.84 (7.22–10.82)	
Yes	69	10.03 (5.80–17.32)	
Asthma			0.938
No	390	9.90 (7.90–12.43)	
Yes	147	9.74 (6.85–13.84)	

Table 3 Unadjusted and adjusted estimates (95% CI and *p*) for the associations among the creatinine-adjusted urine CC16 level (ng/mg) and asthma, spirometric patterns, and lung function

Outcome	Unadjusted		Adjusted	
	Estimate (95% CI)	<i>p</i> Value	Estimate (95% CI)	<i>p</i> Value
Asthma ^a	OR 0.830 (0.673–1.025)	0.083	OR 0.782 (0.617–0.990)	0.041
Spirometric patterns ^a				
FEV ₁ , <85% predicted	OR 0.967 (0.713–1.313)	0.831	OR 0.931 (0.666–1.300)	0.674
FVC, <85% predicted	OR 1.064 (0.808–1.400)	0.660	OR 1.133 (0.837–1.535)	0.419
Lung function ^b				
FEV ₁ , L	β 0.265 (0.195–0.336)	<0.001	β 0.047 (–0.002 to 0.096)	0.063
FVC, L	β 0.288 (0.213–0.364)	<0.001	β 0.064 (0.008–0.119)	0.025

BMI = Body mass index; OR = odds ratio.

^aORs (95% CIs) were estimated with logistic regression models unadjusted and adjusted for sex, age, BMI, parental education, and smoking status.

^bEstimates were obtained with a linear regression model unadjusted and adjusted for sex, age, BMI, parental education, and smoking status

Analysis of the results of our study confirmed the association between lung function and the integrity of the lung epithelium assessed based on CC16 as reported by other studies. One of the most noteworthy implications of our study is that we observed these findings in children. All associations remained after adjusting for confounding factors, including sex, age, body mass index, parental education, and smoking status. Hence, our findings provided evidence that the urine CC16 effects on lung function parameters may be partially independent of demographics and smoking at

the population level. Similar findings were reported by other epidemiology and clinical studies. Rava *et al.*²⁹ found a positive association between serum CC16 levels and FEV₁% predicted and FEV₁/FVC, and that, when serum CC16 levels were categorized into quartiles, the lowest quartile was consistently associated with the lowest levels of FEV₁, FVC, and FEV₁/FVC. Greven *et al.*¹⁷ found that serum CC16 in firefighters were positively associated with FEV₁ and FVC (β = 0.034 [p = 0.04] and β = 0.023 [p = 0.03], respectively). In the recent Evaluation of COPD Longitudinally to

Identify Predictive Surrogate Endpoints study, Vestbo *et al.*³⁰ showed that CC16 levels were associated with the rate of change in FEV₁ and that such association was not modified by age, sex, the Global Initiative for Obstructive Lung Disease stage, current smoking status or smoking history, or patient subgroup.

Although the mechanisms that underlie the associations between the biomarker of CC16 and lung function or asthma are yet to be elucidated, some main pathways have been indicated based on experimental and epidemiologic evidence. The exact function of CC16 is not well known, but experimental evidence showed that it involved the mechanisms of immunoregulation¹¹ and immunosuppression in epithelia exposed to toxic agents.^{12,13} CC16 in the epithelial lining fluid was known to protect the respiratory tract from inflammatory reactions and had an inhibitory potential on several cytokines, including interleukin (IL)-4, IL-5, and IL-13.³¹ CC16 also had direct anti-inflammatory properties because it inhibited the enzyme phospholipase A₂ and the protein prostaglandin D₂, which were both involved in the arachidonic acid inflammatory cascade.^{32,33} In addition, Johansson *et al.*³⁴ reported that CC16 did not have a direct effect on differentiated human neonatal T helper type 2 (TH₂) cells but inhibited TH₂ cell differentiation through neonatal monocyte-derived dendritic cells. Analysis of these results indicates that a high level of CC16 may modulate the immune response because it inhibits TH₂ differentiation, which might result in allergy and asthma development. These findings supported the hypothesis that CC16 levels impact the immunopathogenic mechanisms of allergic inflammation.³⁴

The current study has some limitations. First, the nature of the case-control study determined that our study was subject to selection or recall bias. Prospective birth cohorts studies would be necessary to study the polymorphic nature of genes controlling CC16 expression, which may lead to a better understanding of the relationship of CC16 to asthma. In addition, prospective studies are also needed to learn whether altered CC16 levels precede clinical asthma or if alterations are the result of changes induced during asthma. Second, several articles showed that there is a large interindividual variation in urine among subjects. However, because there is a very high correlation between serum and urinary concentrations of CC16, we believe that the possible interindividual variation in urine will be alleviated or compromised by a large sample size, which is one of the strengths of our study. We believe that more studies based on large samples are needed to further investigate the use of urine samples in measuring airway inflammation. Third, the renal function may affect the urinary CC16 concentrations, which may result in lower power to detect changes in lung permeability.²¹ Fourth, the possible

effects of prostatic CC16 on male urine samples variability may limit the use of urinary CC16 as a biomarker of lung epithelial changes in men.⁵ However, to minimize random and systematic errors, we used optimal methods in collecting, sampling, shipping, storing, and testing urine CC16 samples. Fifth, confounding is unavoidable for biomarker research because many factors can affect a biologic system. Several factors could affect CC16 levels, including exercise and diurnal variation.⁷ Future studies are needed to collect biomarker data on exercise and diurnal variation.

CONCLUSION

The results of our study provided evidence that reduced urine CC16 levels were associated with asthma and lower lung function. The implication from these results is that the biomarker of urine CC16 may reflect disease activity in established asthma. A major strength of this study is that it was one of the limited studies that systematically examined the effects of the urine CC16 biomarker on asthma and lung function in Chinese children. However, it should be noted the sample size for subjects with asthma was relatively small in our study. Larger and longitudinal studies are warranted to evaluate the temporal relationship between the biomarker and the lung function or asthma status.

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