

Environmental tobacco smoke exposure, urine CC-16 levels, and asthma outcomes among Chinese children

Y.-N. Ma¹, Z. Qian², J. Wang³, E. Rodemich², Y. L. Lee⁴, X.-F. Lv⁵, Y.-Q. Liu¹, Y. Zhao¹, M.-M. Huang¹, Y. Liu¹, J. Sun¹, Q.-C. He¹ & G.-H. Dong⁶

¹Department of Biostatistics and Epidemiology, School of Public Health, China Medical University, Shenyang, Liaoning, China; ²Department of Epidemiology, College for Public Health and Social Justice, Saint Louis University, Saint Louis; ³Department of Biostatistics, College for Public Health and Social Justice, Saint Louis University, Saint Louis, MO, USA; ⁴Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei, Taiwan; ⁵Atmosphere pollution control center of Liaoning province, Shenyang, Liaoning; ⁶Department of Preventive Medicine, School of Public Health, Sun Yat-sen University, Guangzhou, Guangdong, China

To cite this article: Ma Y-N, Qian Z, Wang J, Rodemich E, Lee YL, Lv X-F, Liu Y-Q, Zhao Y, Huang M-M, Liu Y, Sun J, He Q-C, Dong G-H. Environmental tobacco smoke exposure, urine CC-16 levels, and asthma outcomes among Chinese children. *Allergy* 2014; DOI: 10.1111/all.12559.

Keywords

asthma; children; Clara cell secretory protein-16; environmental tobacco smoke exposure.

Correspondence

Qin-Cheng He, Department of Biostatistics and Epidemiology, School of Public Health, China Medical University, 92 North 2nd Road, Heping Districts, Shenyang 110001, China.

Tel.: +86-24-23256666-5394

Fax: +86-24-23265492

E-mail: qche@mail.cmu.edu.cn

and

Guang-Hui Dong, Department of Preventive Medicine, School of Public Health, Sun Yat-sen University, 74 Zhongshan 2nd Road, Yuexiu District, Guangzhou 510080, China.

Tel.: +86-20-87333409

Fax: +86-20-87330446

E-mail: donggh5@mail.sysu.edu.cn

Abstract

Background: Previous studies have shown the relationship between club cell secretory protein (Clara) (CC-16) and respiratory diseases. However, few studies have explored the associations between urine CC-16 levels and environmental tobacco smoke (ETS) exposure in children. The objective of this study was to evaluate whether ETS exposure is associated with CC-16 when stratified by asthma status.

Methods: In our study, CC-16 was measured on 537 children aged 9–15 from northeast China in 2011–2012 using the Human Clara Cell Protein ELISA kits. Doctor-diagnosed asthma was defined as having ever been diagnosed with asthma by a physician. The relationship between ETS exposure and urine CC-16 level was assessed using linear regression.

Results: When stratified by asthma status, a negative association between ETS exposure and urine CC-16 was observed after adjusting for the effects of the related covariates, with an adjusted β coefficient [P value] = -0.31 [0.006] in the first 2 years of life and with an adjusted β coefficient [P value] = -0.68 [0.004] in the first 2 years of life and current.

Conclusions: Our study shows long-term exposure to ETS was associated with urinary CC-16 among children without asthma.

Accepted for publication 9 December 2014

DOI:10.1111/all.12559

Edited by: Bodo Niggemann

Asthma is a chronic inflammatory airway disease that involves a complex interaction between mediators and cells. Modula-

Abbreviations

ANOVA, analysis of variance; ATS, American Thoracic Society; BMI, body mass index; CC-16, Clara cell secretory protein-16; CIS, confidence intervals; ETS, environmental tobacco smoke exposure; MDD, mean minimum detectable dose; NIH, National Institutes of Health; OR, odds ratio; SD, standard deviation.

tion of inflammatory processes occurs as a result of endogenous asthmatic events as well as from inhalation of exogenous environmental oxidants (1). Human (2) and animal (3) models have suggested that environmental tobacco smoke (ETS) exposure was associated with an elevated pro-inflammatory cytokine, which in turn may be associated with the development and/or exacerbation of chronic lung diseases.

The 16-kDa club cell secretory protein (Clara) (CC-16) is a lung surfactant protein, mainly secreted by type II

pneumocytes in the lungs, and serves as a sensitive biological marker of lung epithelial integrity in the study of asthma because of its protective effects against oxidative stresses on the respiratory tract as well as its anti-inflammatory properties. It has been reported that the decreased expression of CC-16 could increase the risk of developing asthma (4–7). Experimental and human studies have shown that CC-16 is also found in extrapulmonary fluids such as serum and urine (8–10). Urine samples are often used as a replacement for serum samples due to the high correlation between serum and urinary concentrations of CC-16 and the use of CC-16 as a biomarker for assessing the serum concentrations in subjects without significant renal impairment (11, 12). In addition, because urine collection is less invasive, more readily accepted, and less expensive than serum collection, urine samples have been considered as a biomarker that are particularly suitable for epidemiological or experimental studies on young children (13).

China has seen a high ETS exposure in children in recent years; however, very few studies have been conducted in the ETS exposure effect on asthma in Chinese children. Moreover, little research has been carried out to study the effect of ETS exposure on urine CC-16 levels and asthma in children. In this study, we attempted to evaluate whether ETS exposure was associated with urine CC-16 levels of school-aged children stratified by asthma status.

Methods

Study population and design

We conducted a cross-sectional survey design in 2011–2012 in full compliance with the ethical standards of the Responsible Committee on Human Experimentation of China Medical University. Samples were selected from the urban population consisting of more than 20 million residents from across 14 cities in Liaoning province in northeast China. These 14 cities were stratified into three socioeconomic zones, that is, low, medium, and high by gross domestic product (GDP) from 2008 to 2010 provided by Liaoning Provincial Bureau of Statistics. Five cities were randomly selected from these three socioeconomic zones, including Benxi (low zone), Dandong (medium zone), Anshan (medium zone), Dalian (high zone), and Shenyang (high zone). Details about the design have been described elsewhere (14). A total of 4542 children selected at random from 36 randomly selected schools from the five cities in the study. Information about the active smoking status, defined as having ever smoked more than 100 cigarettes during the participant's lifetime (15), was obtained from a confidential interview with the participant by an experienced field worker during the school visit. To eliminate the potential confounding effect of active tobacco use, we excluded the children who had reported to be active smokers during the personal interview from the study sample. A total of 147 children were reported to be nonsmokers and were ever diagnosed with asthma. A total of 390 (10%) children were selected at random from those without asthma.

Questionnaire

Each of the 4542 children was asked to give a questionnaire handout attached to an endorsed consent form and a return envelope to their parents to fill out. After reviewing the questionnaire and completing the endorsed consent form, parents were invited to the school to learn detailed information about the survey, including the objective of the survey and instructions on filling out the questionnaire. Parents who wished to complete the questionnaire at home would have their children return the completed questionnaire in an envelope to the teacher the next day.

Information about children's respiratory health, demographic characteristics, and environmental exposures was collected using an American Thoracic Society (ATS) Epidemiologic Standardization Project questionnaire in Chinese translation (16). The personal and familial characteristics of participants included gender, age, BMI (body mass index), parental education levels, and residential district where they lived. Doctor-diagnosed asthma was defined as having been diagnosed with asthma by a doctor. The reported ETS exposure was classified into three categories (17): (i) current ETS exposure, (ii) passive ETS exposure during the first 2 years of the child life, defined as child's ETS exposure from birth until age 2, and (iii) ETS exposure in the first 2 years of the child's life and current ETS exposure in the home, defined as daily ETS exposure in the home, each of which was measured on 4 scales: (0 = never being exposed to ETS, 1 = being exposed only in the first 2 years of life, 2 = being currently exposed only, and 3 = being exposed in the first 2 years of life and currently). To predict the effect of exposure at various time-points, we included one exposure variable in each model and measured the number of current smokers in the home and the number of cigarettes smoked in the home per day on 3 scales: no cigarette smoking, 1–10 cigarettes smoked per day, and ≥ 10 cigarettes smoked per day.

Measurement of students and urine CC-16 level detection

Measurements of the child's height, weight, waist-to-hip ratio, blood pressure, and lung function were obtained from 8 am to 1 pm in schools prior to the experiment. Children were asked to prepare their first morning mid-stream urine samples in their homes and keep them frozen until experiment started. Afterward, 100-ml urine sample was obtained from each boy to avoid contamination by prostatic secretions (12, 13), collected in a dry tube, and allowed to clot overnight at 4°C. All samples were then shipped with dry ice to the laboratory and were stored at -80°C until ready for analysis. Repeated freeze–thaw cycles were avoided. Creatinine concentrations of the urine sample were determined using commercially available ELISA (Oxford Biomedical Research, Rochester Hills, MI, USA). The CC-16 level of urine was measured by a commercially available Human Clara Cell Protein ELISA kit from Quantikine (R&D Systems, Minneapolis, MA, USA). The limit of detection of this assay for CC-16 was 0.07 ng/ml. Concentrations of CC-16 and biomarkers below the mean minimum detectable dose

(MDD) were set to the MDD. To adjust for possible diurnal variations, the duration of the experiment or sample collection was recorded. The concentration of CC-16 was repeatedly measured twice, and the average of the two measures was used as the concentration of CC-16 protein.

Statistical analysis

Because the CC-16 measurements were found to follow a log-normal distribution, we performed a logarithm transformation on the ratio of urinary CC-16 to urinary creatinine and reported the geometric means and their 95% confidence

intervals (CIS) for the log-transformed CC-16, denoted by LGCC-16, in Table 1. For the other variables, we calculated their mean $\pm$ standard deviation (SD) or the percentages in Table 1. The chi-square test was used to test for the difference in a categorical variable between the two groups of children by asthma. The *t*-test was used to test for the difference in the group means of a continuous variable by asthma. Table 2 presents the means of the creatinine-adjusted urine CC-16 and their confidence intervals by ETS exposure for children with and without asthma separately. Table 3 shows the linear associations between ETS exposure and urine CC-16, as stratified by asthma. Table 4 shows

Table 1 Characteristics of children with and without asthma in the study population

Characteristics	Children without asthma (<i>n</i> = 390)	Children with asthma (<i>n</i> = 147)	<i>P</i> value
Age (year)*, <i>n</i> (%)	11.86 $\pm$ 2.35	11.59 $\pm$ 2.21	0.232
9–11	201 (51.54)	82 (55.78)	0.433
11–13	63 (16.15)	26 (17.69)	
13–15	126 (32.31)	39 (26.53)	
Study area			
1	177 (45.38)	40 (27.21)	0.0004
2	46 (11.79)	25 (14.29)	
3	68 (17.44)	26 (17.69)	
4	61 (15.64)	30 (20.41)	
5	38 (9.74)	30 (20.41)	
BMI (kg/m ²)*	19.45 $\pm$ 4.37	20.23 $\pm$ 4.69	0.071
Gender, <i>n</i> (%)			
Male	201 (51.54)	60 (40.82)	0.027
Female	189 (48.46)	87 (59.18)	
Parental education, <i>n</i> (%)			
<High School	114 (29.23)	56 (38.10)	0.049
$\geq$ High School	276 (70.77)	91 (61.90)	
LGCC-16 (ng/mg)†	9.90 (7.90–12.43)	9.74 (6.85–13.84)	0.938
ETS exposure in the first 2 years of life			
No	316 (81.03)	104 (70.75)	0.010
Yes	74 (18.97)	43 (29.25)	
Current ETS exposure			
No	202 (51.79)	57 (38.78)	0.007
Yes	188 (48.21)	90 (61.22)	
ETS exposure in the first 2 years of life and current			
None	188 (48.21)	52 (35.37)	0.014
In the first 2 years only	14 (3.59)	5 (3.40)	
First 2 years and current	60 (15.38)	38 (25.85)	
Current only	128 (32.82)	52 (35.38)	
Number of cigarettes smoked during workdays (cigarettes/day)‡			
0	237 (60.77)	72 (48.98)	0.036
1–10	111 (28.46)	51 (34.69)	
$\geq$ 10	42 (10.77)	24 (16.33)	
Number of cigarettes smoked during weekends (cigarettes/day)§			
0	251 (64.36)	79 (53.74)	0.077
1–10	92 (23.59)	44 (29.93)	
$\geq$ 10	47 (12.05)	24 (16.33)	

BMI, body mass index.

*Mean $\pm$ SD.

†Data are in ng/mg creatinine, geometric mean (95% confidence interval).

‡The number of cigarettes smoked inside the house per day during workday when the child is at home.

§The number of cigarettes smoked inside the house per day during weekends when the child is at home.

the effect of interaction of ETS exposure and asthma on CC-16. Estimates were adjusted for study district, gender, age, body mass index, and parental education level. All analyses were performed using the SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA). The statistical significance level was defined as when a *P* value is less than the $\alpha = 0.05$.

Results

Children with asthma tended to have more ETS exposure in the first 2 years of life, more current ETS exposure, and more ETS exposure during the first 2 years of life and current exposure than children without asthma (Table 2). In addition, the number of cigarettes smoked per day during workdays and weekends indicates a dose-response relationship between asthma and ETS exposure; however, there was no statistical difference in CC-16 between the two groups of children by asthma (Table 1).

The geometric mean (95% CI) creatinine-adjusted urine CC-16 level was 9.77 (8.12, 12.02) ng/mg creatinine. Among asthmatic children, CC-16 urine level appeared to increase with passive smoking exposure; however, the *t*-test did not show a significant positive relationship between these two variables. Additionally, among children without asthma, ETS exposure in the first 2 years of life was significantly associated with CC-16 (Table 2). The significance of the association was also observed for ETS exposure in the first 2 years of life and currently.

Among children without asthma, urine CC-16 was found to be negatively associated with ETS exposure in the first 2 years of life (adjusted β coefficients [*P* value] = -0.31 [0.006]) and ETS exposure in the first 2 years of life and currently (adjusted β coefficients [*P* value] = -0.68 [0.004]). Such association was not observed among children with asthma (Table 3).

No significant interaction effect of asthmatic and ETS exposure was observed (Table 4).

Discussion

In this study, we found a negative association between ETS exposure and urinary CC-16 among children without asthma. We also found indoor chronic ETS exposure was associated with a decrease in lung epithelial permeability in children.

To the best of our knowledge, there is limited information in the literature on the utility of CC-16 in assessing the impact of lung damage in children with passive ETS exposure. Our results are consistent with those reported by Van Miert et al. (18) who reported that ETS exposure was associated with a low CC-16 level. In addition, Berthoin et al. (19) showed that CC-16 in serum decreased significantly with ETS exposure, indicating the chronic effect of ETS exposure on respiratory epithelium. Recently, Singh et al. (20) showed that CC-16 was negatively associated with exposure to secondhand cigarette smoke during gestation, and therefore, they hypothesized that a decreased CC-16 level would be reflective of the toxic effects of secondhand tobacco smoke

Table 2 Means of the creatinine-adjusted urine CC-16 and their confidence intervals by ETS exposure for children with and without asthma separately

ETS exposure	<i>n</i>	Children without asthma* (<i>n</i> = 390)	Children with asthma* (<i>n</i> = 147)
ETS exposure in the first 2 years of life			
No	420	11.31 (8.86–14.44)†	9.28 (6.10–14.11)
Yes	117	5.59 (3.13–9.96)	10.93 (5.58–21.41)
Current ETS exposure			
No	259	9.19 (6.72–12.57)	8.34 (4.64–15.01)
Yes	278	10.74 (7.71–14.96)	10.77 (6.90–16.81)
ETS exposure in the first 2 years of life and current			
None	240	10.16 (7.40–13.94)†	8.81 (4.76–16.32)
In the first 2 years only	19	2.46 (0.54–11.13)	4.72 (0.25–89.69)
First 2 years and currently	98	6.81 (3.62–12.81)	12.20 (5.98–24.90)
Currently only	180	13.27 (9.01–19.55)	9.79 (5.45–17.60)
Number of cigarettes smoked during workdays (cigarettes/day)‡			
0	309	9.61 (7.23–12.77)	9.32 (5.70–15.27)
1–10	162	10.75 (6.82–16.95)	10.86 (5.84–20.19)
≥10	66	9.49 (4.70–19.19)	8.81 (3.29–23.54)
Number of cigarettes smoked during weekends (cigarettes/day)§			
0	330	9.55 (7.22–12.62)	9.31 (5.75–15.08)
1–10	136	10.79 (6.56–17.73)	12.91 (6.60–25.25)
≥10	71	10.18 (5.26–19.69)	6.72 (2.78–16.28)

*Data are in ng/mg creatinine, geometric mean (95% confidence interval).

†Significant *P* < 0.05.

‡The number of cigarettes smoked inside the house per day during workday when the child is at home.

§The number of cigarettes smoked inside the house per day during weekends when the child is at home.

Table 3 Adjusted association between creatinine-adjusted urine CC-16 and ETS exposure as stratified by asthma*

		Children without asthma		Children with asthma	
ETS exposure	<i>n</i>	β (95% CI)	<i>P</i> value	β (95% CI)	<i>P</i> value
ETS exposure in the first 2 years of life					
No	420	Referent		Referent	
Yes	117	−0.31 (−0.53, −0.09)	0.006	−0.09 (−0.42, 0.23)	0.574
Current ETS exposure					
No	259	Referent		Referent	
Yes	278	0.01 (−0.17, 0.19)	0.918	0.13 (−0.16, 0.41)	0.391
ETS exposure in the first 2 years of life and current					
None	240	Referent		Referent	
In the first 2 years only	19	−0.68 (−1.14, −0.22)	0.004	−0.29 (−1.10, 0.52)	0.480
First 2 years and current	98	−0.21 (−0.47, 0.05)	0.109	0.02 (−0.35, 0.39)	0.921
Current only	180	0.10−0.18, 0.21	0.868	0.15 (−0.18, 0.49)	0.372
Number of cigarettes smoked during workdays (cigarettes/day)†					
0	309	Referent		Referent	
1–10	162	0.00 (−0.20, 0.20)	0.966	0.16 (−0.16, 0.47)	0.331
≥10	66	−0.00 (−0.29, 0.29)	0.988	−0.04 (−0.45, 0.38)	0.866
Number of cigarettes smoked during weekends (cigarettes/day)†					
0	330	Referent		Referent	
1–10	136	−0.01 (−0.22, 0.20)	0.942	0.22 (−0.11, 0.54)	0.192
≥10	71	−0.04 (−0.31, 0.24)	0.800	−0.17 (−0.57, 0.23)	0.408

BMI, body mass index; CI, confidence interval.

*Estimates obtained with a linear regression model adjusted for gender, age, BMI, parental education level, and study area.

†Contrast with the subjects with no current exposure to ETS (1–10 vs zero; 10 vs zero).

Table 4 Adjusted effect of interaction of asthma and ETS exposure on urine LGCC-16*

ETS exposure*asthma	β (95% CI)	<i>P</i> value
ETS exposure in the first 2 years of life	0.29 (−0.09, 0.68)	0.132
Current ETS exposure	0.12 (−0.22, 0.46)	0.484
ETS exposure in the first 2 years of life and current	0.04 (−0.09, 0.17)	0.511
Number of cigarettes smoked during workdays (cigarettes/day)†	0.06 (−0.17, 0.29)	0.623
Number of cigarettes smoked during weekends (cigarettes/day)†	−0.01 (−0.24, 0.22)	0.931

BMI, body mass index; CI, confidence interval.

*Estimates obtained with a linear regression model adjusted for gender, age, BMI, parental education level, and study area.

†Contrast with the subjects with no current exposure to ETS (1–10 vs zero; 10 vs zero).

on Clara cells. One of the most noteworthy findings of our study is the negative association of ETS exposure in the first 2 years with urinary CC-16 among children without asthma, indicating the adverse impact of early-life ETS exposure, which was also consistent with our earlier findings that chronic irritant exposure was associated with a decreased level of CC-16.

This study showed an association between exposure to ETS in the first 2 years and urinary CC-16 levels among children without asthma and that no such association was found

among children with asthma, which may possibly be due to one or more of the following reasons. First, parents tend to protect their children who suffered from asthma from getting ETS exposure and children with asthma are more likely to avoid getting ETS exposure than nonasthmatic counterparts. One study revealed that the cumulative effects of smoking could play a key role in biomarker changes to the airways (18). Second, previous studies suggested that levels of CC-16 were relatively low in patients with asthma, which may be due to the epithelial cell dysfunction and disease activity (21). Children with asthma frequently have symptoms of increased airway permeability, which may possibly reduce the effect of ETS exposure on urine CC-16. Third, it has been shown that CC-16 A38G polymorphism might intrinsically influence the CC-16 expression. A decreased expression of the anti-inflammatory protein CC-16 was associated with the polymorphic allele of the CC-16 gene (22) and therefore may possibly cause the difference in the A38G polymorphism of the Clara cell gene between asthmatics and nonasthmatics. Prospective birth cohort studies would be necessary to study the polymorphic nature of genes controlling CC-16 expression to gain a further understanding of the ETS effect on CC-16 in asthmatic and nonasthmatic children.

Urinary CC-16 levels appeared to be higher in children with current ETS exposure than those without. Van Miert et al. (23) observed a similar effect on female Sprague Dawley rats that were exposed to cigarette smoke for 2 h with a 30-min break after the first hour, and their CC-16 concentrations in serum showed a dose-dependent increase after mainstream cigarette smoke exposure. Sardella et al. (24, 25)

reported in their two studies that children exposed to parental smoking had increased serum CC-16 levels. St Helen et al. (13) reported a positive association between outdoor second-hand smoke exposure and increased lung epithelial permeability, and a positive association between cigarette count and urinary CC-16 among females. CC-16 is one biomarker which may reflect both the pulmonary epithelial barrier integrity and number of club cells (Clara) lining the terminal bronchioles or the permeability of the alveolar–capillary barrier (26). In fact, the positive association between current ETS exposure and CC-16 in children observed in this study may reflect the effect of an acute exposure, which may in turn lead to increased CC-16 levels in serum/urine due to a transient increase in pulmonary epithelial ‘leakiness’, increased production of CC-16, or decreased renal clearance (7, 8). CC-16 is a useful biomarker that has many biological mechanisms, pulmonary effects, and potential value (7). A chronic decrease in CC-16 could reflect decreased production of the club cells (Clara) due to irritation/cytotoxicity, whereas the driving mechanism in the case of short-term exposure is most likely lung hyperpermeability and not a reduced production of CC-16 (27).

Although this study does not allow for the elucidation of the mechanisms underlying the association between CC-16 and ETS exposure, some main pathways have been suggested based on experimental and epidemiological evidence. Clara cell-specific protein (CC-16) is a small protein mainly secreted by Clara cells, nonciliated cells known for their high vulnerability to systemic or inhaled lung toxicants, which is predominantly found in the respiratory bronchioles and in the prostate (5). CC-16 was thought to protect the respiratory tract from inflammatory reactions, due to its potential inhibitory effect on several cytokines, including IL-4, IL-5, and IL-13 (28). CC-16 also has direct anti-inflammatory properties due to the fact that it inhibits the protein prostaglandin D₂ (PGD₂) and the enzyme phospholipase A₂, both of which are involved in the arachidonic acid inflammatory cascade (29). Van Miert et al. (18) reported cigarette smoking reduces the concentration of CC-16 in bronchoalveolar lavage by reducing the number of Clara cells and therefore hypothesized that cigarette smoke exposure would cause similar epithelial damage in the upper airways to that in the lower airways. These findings support the hypothesis that the Clara cell is sensitive to the destructive potential of cigarette smoke.

There are some limitations to the present study. First, our study was based on a cross-sectional study, which might be subject to selection bias. The relationship of the ETS exposure and transient and chronic changes of urine CC-16 needs further investigation. Second, information about ETS exposure and health measurements were obtained from retrospective questionnaires, which might be subject to recall bias. Accurate exposure measurements and health outcomes or biomarkers of tobacco smoke exposure measured in urine such as salivary cotinine or urinary NNAL (13) to ascertain SHS exposure or tobacco use are needed to validate our study. Moreover, no ETS exposure information from age 2 to the study time was available in

the study. Prospective studies may be needed to overcome the limitations of the retrospective design. Third, renal function may affect the urinary CC-16 concentrations, which may limit the use of urinary CC-16 as a biomarker of lung epithelial changes in men. However, several studies have demonstrated that there is consistent correlation between serum and urinary CC-16 concentrations (12). Fourth, several underlying factors, including temperature, humidity, outdoor air pollution, exercise, diurnal variation, and genotypic variation, that affect the magnitude of the effect of indoor ETS exposure on increasing lung epithelial permeability assessed by urine CC-16 levels were not sufficiently investigated. Future studies with these additional variables are needed. Fifth, there was no information in our study about the previous ETS exposure in children with asthma, which may possibly have a confounding effect on CC-16, especially after birth, due to the fact that children with asthma may tend to avoid the ETS exposure in their homes. Lastly, Alexis et al. (30) reported that the production of CC-16 can be suppressed by inhaled corticosteroids and information about the participants’ use of inhaled corticosteroids was not available in our study, which may have caused a potential confounding effect to our study.

In conclusion, the results of our study indicate that indoor ETS exposure may decrease the lung epithelial permeability among the nonasthmatic children, although the association between ETS and CC-16 needs to be further studied. A major strength of this study is that it was the first study that systematically examined the associations of urine CC-16 on indoor ETS exposure in children in China. Our study attempted to ascertain the change in urine CC-16 levels due to the exposure to ETS in children with asthma. The analyses stratified by asthma status indicated that asthma may act as an effect modifier in the association between ETS exposure and urine CC-16 levels in our study population. However, it should be kept in mind that the sample size for asthmatic subjects was relatively small in our study. Studies with sufficient statistical power are also warranted to address the causal relationship between biomarker changes and indoor ETS exposure.

Acknowledgments

We are very grateful for the tremendous support of the participants from northeast China. We would also like to thank the reviewers and editor for their constructive suggestions and comments.

Author contributions

Q-CH and G-HD conceived and designed the experiments. Y-NM performed the experiments. Y-NM analyzed the data. Y-NM, JW, G-HD, Y-Q L, YZ, M-M H, Y L, J S, W-HR, X-F L, and Q-CH contributed reagents/materials/analysis tools. Y-NM wrote the paper. G-HD, JW, and YLL revised the paper. Y-NM, Y-Q L, YZ, M-M H, Y L, J S, W-HR, and X-F L contributed to the investigation. All authors provided critical review of the manuscript.

Funding

This work was supported by Grants from National Natural Science Foundation of China (No. 81302405), Shenyang City Science and Technology Foundation (No. F13-220-9-34) and China Environmental Protection

Foundation (CEPF2008-123-1-5; CEPF2010-123-1-3; CEPF2011-123-2-7).

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

- Comhair SA, Gaston BM, Ricci KS, Hammel J, Dweik RA, Teague WG et al. Detrimental effects of environmental tobacco smoke in relation to asthma severity. *PLoS One* 2011;**6**:e18574.
- Flouris AD, Metsios GS, Jamurtas AZ, Koutedakis Y. Sexual dimorphism in the acute effects of secondhand smoke on thyroid hormone secretion, inflammatory markers and vascular function. *Am J Physiol Endocrinol Metab* 2008;**294**:E456–E462.
- Castro P, Legora-Machado A, Cardilo-Reis L, Valenca S, Porto LC, Walker C et al. Inhibition of interleukin-1beta reduces mouse lung inflammation induced by exposure to cigarette smoke. *Eur J Pharmacol* 2004;**498**:279–286.
- Broekaert FCA, Knoop B, Hermans C, Bernard A. Clara cell secretory protein (CC16): features as a peripheral lung biomarker. *Ann N Y Acad Sci* 2000;**923**:68–77.
- Bernard A. Critical review of Clara cell protein: sound science? *Biomarkers* 2008;**13**:237–243.
- Broekaert FBA. Clara cell secretory protein (CC16): characteristics and perspectives as lung peripheral biomarker. *Clin Exp Allergy* 2000;**30**:469–475.
- Lakind JS, Holgate ST, Ownby DR, Mansur AH, Helms PJ, Pyatt D et al. A critical review of the use of Clara cell secretory protein (CC16) as a biomarker of acute or chronic pulmonary effects. *Biomarkers* 2007;**12**:445–467.
- Arsalane K, Broekaert F, Knoop B, Wiedig M, Toubeau G, Bernard A. Clara cell specific protein (CC16) expression after acute lung inflammation induced by intratracheal lipopolysaccharide administration. *Am J Respir Crit Care Med* 2000;**161**:1624–1630.
- Broekaert F, Arsalane K, Hermans C, Bergamaschi E, Brustolin A, Mutti A et al. Serum Clara cell protein: a sensitive biomarker of increased lung epithelium permeability caused by ambient ozone. *Environ Health Perspect* 2000;**108**:533–537.
- Halatek T, Hermans C, Broekaert F, Watzel R, Wiedig M, Toubeau G et al. Quantification of Clara cell protein in rat and mouse biological fluids using a sensitive immunoassay. *Eur Respir J* 1998;**11**:726–733.
- Jacquemin B, Lanki T, Yli-Tuomi T, Vallius M, Hoek G, Heinrich J et al. Source category-specific PM_{2.5} and urinary levels of Clara cell protein CC16. The ULTRA study. *Inhal Toxicol* 2009;**21**:1068–1076.
- Andersson L, Lundberg PA, Barregard L. Methodological aspects on measurement of Clara cell protein in urine as a biomarker for airway toxicity, compared with serum levels. *J Appl Toxicol* 2007;**27**:60–66.
- St Helen G, Holland NT, Balmes JR, Hall DB, Bernert JT, Vena JE et al. Utility of urinary Clara cell protein (CC16) to demonstrate increased lung epithelial permeability in non-smokers exposed to outdoor secondhand smoke. *J Expo Sci Environ Epidemiol* 2013;**23**:183–189.
- Ma YN, Wang J, Dong GH, Liu MM, Wang D, Liu YQ et al. Predictive equations using regression analysis of pulmonary function for healthy children in Northeast China. *PLoS One* 2013;**8**:e63875.
- Chen JM, Hwang BF, Chen YC, Lee YL. Active smoking, environmental tobacco smoke and bronchitic symptoms among adolescents in Taiwan: a prospective cohort study. *Prev Med* 2014;**65C**:116–121.
- Salo PMXJ, Johnson CA, Li Y, Avol EL, Gong J, London SJ. Indoor allergens, asthma, and asthma-related symptoms among adolescents in Wuhan, China. *Ann Epidemiol* 2004;**14**:543–550.
- Dong GH, Wang D, Yang ZH, Zhang PF, Ren WH, Zhao YD et al. Gender-specific differences in effects of prenatal and postnatal environmental tobacco smoke exposure on respiratory symptoms in 23,474 children with and without allergic predisposition: results from 25 districts of northeast China. *Int J Environ Health Res* 2011;**21**:173–188.
- Van Miert E, Sardella A, Bernard A. Biomarkers of early respiratory effects in smoking adolescents. *Eur Respir J* 2011;**38**:1287–1293.
- Berthoin K, Broekaert F, Robin M, Haufroid V, De Burbure C, Bernard A. Serum pneumoproteins and biomarkers of exposure to urban air pollution: a cross-sectional comparison of policemen and foresters. *Biomarkers* 2004;**9**:341–352.
- Singh SP, Gundavarapu S, Smith KR, Chand HS, Saeed AI, Mishra NC et al. Gestational exposure of mice to secondhand cigarette smoke causes bronchopulmonary dysplasia blocked by the nicotinic receptor antagonist mecamylamine. *Environ Health Perspect* 2013;**121**:957–964.
- Shijubo N, Itoh Y, Yamaguchi T, Sugaya F, Hirasawa M, Yamada T et al. Serum levels of Clara cell 10-kDa protein are decreased in patients with asthma. *Lung* 1999;**177**:45–52.
- Laing IA, Hermans C, Bernard A, Burton PR, Goldblatt J, Le Souef PN. Association between plasma CC16 levels, the A38G polymorphism, and asthma. *Am J Respir Crit Care Med* 2000;**161**:124–127.
- Van Miert E, Dumont X, Bernard A. CC16 as a marker of lung epithelial hyperpermeability in an acute model of rats exposed to mainstream cigarette smoke. *Toxicol Lett* 2005;**159**:115–123.
- Sardella A, Voisin C, Nickmilder M, Dumont X, Annesi-Maesano I, Bernard A. Nasal epithelium integrity, environmental stressors, and allergic sensitization: a biomarker study in adolescents. *Biomarkers* 2012;**17**:309–318.
- Sardella A, Voisin C, Dumont X, Marcucci F, Bernard A. Nasal epithelium biomarkers in young children: associations with allergic sensitization and environmental stressors. *Pediatr Pulmonol* 2013;**48**:571–578.
- Hermans C, Bernard A. Pneumoproteinaemia: a new perspective in the assessment of lung disorders. *Eur Respir J* 1998;**11**:801–803.
- Van Miert E, Sardella A, Nickmilder M, Bernard A. Respiratory effects associated with wood fuel use: a cross-sectional biomarker study among adolescents. *Pediatr Pulmonol* 2012;**47**:358–366.
- Wang SZ RC, Espindola TM, Barrett EG, Tesfaigzi Y, Bice DE, Harrod KS. CCSP modulates airway dysfunction and host responses in an Ova-challenged mouse model. *Am J Physiol Lung Cell Mol Physiol* 2001;**281**:L1303–L1311.
- Mandal AK ZZ, Ray R, Choi MS, Chowdhury B, Pattabiraman N, Mukherjee AB. Uteroglobin represses allergen-induced inflammatory response by blocking PGD₂ receptor-mediated functions. *J Exp Med* 2004;**199**:1317–1330.
- Alexis NE, Lay JC, Haczku A, Gong H, Linn W, Hazucha MJ et al. Fluticasone propionate protects against ozone-induced airway inflammation and modified immune cell activation markers in healthy volunteers. *Environ Health Perspect* 2008;**116**:799–805.