

Accepted Manuscript

Associations of early life exposures and environmental factors with asthma among children in rural and urban areas of Guangdong, China

Mulin Feng, MD, Zhaowei Yang, PhD, Liying Pan, MD, Xuxin Lai, PhD, Mo Xian, MD, Xiafei Huang, MD, Yan Chen, MD, Paul C. Schröder, PhD, Marjut Roponen, PhD, Bianca Schaub, MD, Gary W.K. Wong, MD, Jing Li, MD

PII: S0012-3692(16)00450-5

DOI: [10.1016/j.chest.2015.12.028](https://doi.org/10.1016/j.chest.2015.12.028)

Reference: CHEST 231

To appear in: *CHEST*

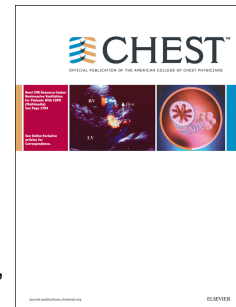
Received Date: 12 January 2015

Revised Date: 13 December 2015

Accepted Date: 21 December 2015

Please cite this article as: Feng M, Yang Z, Pan L, Lai X, Xian M, Huang X, Chen Y, Schröder PC, Roponen M, Schaub B, Wong GWK, Li J, Associations of early life exposures and environmental factors with asthma among children in rural and urban areas of Guangdong, China, *CHEST* (2016), doi: 10.1016/j.chest.2015.12.028.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



**Associations of early life exposures and environmental factors with asthma among children
in rural and urban areas of Guangdong, China**

Mulin Feng, MD^{a,*}, Zhaowei Yang, PhD^{a,*}, Liying Pan, MD^a, Xuxin Lai, PhD^b, Mo Xian, MD^a,
Xiafei Huang, MD^a, Yan Chen, MD^c, Paul C Schröder, PhD^d, Marjut Roponen, PhD^e, Bianca
Schaub, MD^d, Gary W.K. Wong, MD^f and Jing Li, MD^{a, g}

^aDepartment of Allergy and Clinical Immunology, State Key Laboratory of Respiratory Disease,
The First Affiliated Hospital, Guangzhou Medical University, Guangzhou, China

^bResearch Asia Pacific, ALK A/S, Hong Kong, China

^cDepartment of Internal Medicine, Guangdong Pharmaceutical University, Guangzhou, China

^dUniversity Children's Hospital Munich, Department of Pulmonary and Allergy, LMU Munich,
Munich, Germany and Member of the German Center for Lung Research (DZL)

^eDepartment of Environmental Science, University of Eastern Finland, Kuopio, Finland

^fDepartment of Paediatrics, Prince of Wales Hospital, The Chinese University of Hong Kong,
Hong Kong, China

^gGuangdong Provincial Key Laboratory of Allergy & Clinical Immunology, Guangzhou Medical
University, Guangzhou, China

*These authors contributed equally to the work described in this paper.

Corresponding authors

Jing Li, MD

Department of Allergy and Clinical Immunology
State Key Laboratory of Respiratory Disease,
The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, 510120, China
Email: lijing@gird.cn

Gary W.K. Wong, MD

Department of Pediatrics,
Prince of Wales Hospital,
The Chinese University of Hong Kong,
Hong Kong Special Administrative Region, China.
Email: wingkinwong@cuhk.edu.hk

35 **Abstract word count:** 250

36 **Text word count:** 2450

37

Abstract

Background: Environmental factors may play important roles in asthma, but findings were inconsistent.

Objective: To determine the associations between early life exposures, environmental factors and asthma in urban and rural children in southeast China.

Methods: A screening questionnaire survey was performed in 7164 children from urban Guangzhou and 6087 from rural Conghua. In the second stage, subsamples of 854 children (419 from Guangzhou, 435 from Conghua) were recruited for a case-control study including detailed questionnaire enquiring family history, early life environmental exposures, dietary habits, and testings including histamine airway provocation, skin prick test, and serum antibody analysis. House dust samples from 76 Guangzhou and 80 Conghua families were obtained to analyze levels of endotoxin, house dust mite and cockroach allergens.

Results: The prevalence of doctor-diagnosed-asthma was lower in children from Conghua (3.4%) than Guangzhou (6.9%, $p<0.001$) in the screening survey. A lower percentage of asthma was found in rural compared to urban subjects (2.8% vs. 29.4%, $p<0.001$) in case-control study. Atopy (odds ratio 1.91, 95% confidence interval 1.58-2.29), parental atopy (2.49, 1.55-4.01), hospitalization before age 3 (2.54, 1.37-4.70), high milk product consumption (1.68, 1.03-2.73) and dust *Dermatophagoides farinae* 1 level (1.71, 1.34-2.19) were positively, while living in a crop-farming family before age 1 (0.15, 0.08-0.32) and dust endotoxin level (0.69, 0.50-0.95) were negatively associated with asthma.

Conclusions: Rural children from an agricultural background showed a reduced risk of asthma. Exposure to crop farming at early life and high environmental endotoxin levels might protect the children from asthma in southern China.

Keywords: asthma; rural; endotoxin; house dust mite; environmental factors.

ABBREVIATIONS

AHR: Airway Hyperresponsiveness

FEV₁: Forced Expiratory Volume in One Second

FVC: Forced Vital Capacity

ISAAC: The International Study of Asthma and Allergic disease in Childhood

INTRODUCTION

The prevalence of asthma and allergies has increased around the world in the past few decades¹. One recent study showed a doubling of the prevalence of asthma in schoolchildren in Guangzhou, China over the last 15 years². China is experiencing a rapid transition from low to high prevalence of allergic diseases, possibly related to changes in environmental exposures³.

Both genetic and environmental factors contribute to the development of asthma. Accumulating evidence indicates that the pregnancy and early-life are critical time periods involved in the early programming of asthma and allergy. Important exposures including breastfeeding, diet, antibiotic use, and exposure to indoor allergens and microbes and microbial infections as well as air pollutions in early life may alter infant immune profiles and are potential stimuli for the early programming of asthma and allergy⁴⁻⁹. Some of these exposures may be protective. In particular, studies confirmed that children from a farming background had a reduced risk of asthma¹⁰⁻¹⁴. Environmental exposure to microbial agents might be protective^{3,15,16}. Endotoxin levels, assessed in dust samples, were found higher in homes of children living on farms compared to homes of non-farming families¹⁷. Studies also showed individuals seropositive to different microorganisms, including hepatitis A virus¹⁸, *Salmonella typhi*¹⁹ and *Helicobacter pylori*²⁰ were protected against asthma.

Guangzhou is one of the biggest cities in southeast China. Conghua is an agricultural area producing the fruit Lychee and other related crops and located 130 kilometers northeast to Guangzhou. The two areas share the same geography and climate, and offer the opportunity to investigate environmental influences on the development of asthma. The aim of this study is to examine if early life exposures and environmental factors may explain the disparity of the prevalence of asthma between urban and rural populations in southeast China.

METHODS

Study Design and Population

The initial random population sample consisted of 7164 children aged 13-14 from 10 secondary schools in Guangzhou and 6087 same age children from 4 secondary schools of Conghua. They were invited for the International Study of Asthma and Allergies in Childhood (ISAAC) with written and video questionnaires. We used the ISAAC questionnaire (<http://isaac.auckland.ac.nz>)

as validated in written Chinese²¹⁻²³. Questionnaires were conducted in validated Chinese Children with positive response to at least one of two questions, ‘Have you had wheezing in your chest when you breathe during the past 12 months?’ and ‘Have you ever been diagnosed with asthma by a doctor?’ (n=250, each area), and negative response to these two questions and without other allergic diseases (Guangzhou: n=250, Conghua: n=500) were invited for a second stage case-control study. All subjects completed a detail questionnaire along with histamine airway provocation, allergen skin prick test, and serum antibody analysis. Random samples of one hundred families in the second stage from each region were invited for home dust samples for analysis. Informed consent was obtained from children’s parents. The study was approved by the Ethics Review Board of the First Affiliated Hospital of Guangzhou Medical University (#2012049).

Detail Questionnaire

The interviewer administered questionnaire (online material) included specific questions related to possible risk and protection factors including family history, number of siblings, indoor or outdoor farm and nonfarm animal exposures, parental participation of agricultural or livestock farming activities, cigarette smoking, cooking fuels, bedroom environment and dietary habits in early life and at time of survey.

Spirometry and Histamine Bronchial Provocation Test

Forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) were measured using Cosmed Microquark Spirometer (Italy), which met with standards of the American Thoracic Society and European Respiratory Society²⁴, and shown as percentage predicted value (FVC%, FEV₁%). The presence of airway hyperresponsiveness (AHR) was assessed by rapid histamine inhalation provocation test²⁵. AHR was defined as FEV₁ decreasing $\geq 20\%$ of its baseline level when $\leq 7.8\mu\text{mol}$ of cumulative dose of histamine is administered.

Skin Prick Tests

Sensitization to 8 common aeroallergens (Soluprick SQ, ALK, Denmark) was assessed, including *Dermatophagoides pteronyssinus* (*Der p*), *Dermatophagoides farina* (*Der f*), *Blomia tropicalis*, cat and dog dander, cockroach, ragweed and mugwort pollen. Positive skin reaction was defined as a wheal size ≥ 3 mm after subtraction of the negative control. Atopic index was sum of the number of allergens that give positive responses plus a constant added based on the largest skin

wheal size (1, <4mm; 2, $\geq$ 4mm)²⁶.

Serum Antibody Analysis

Serum IgG antibodies to *Salmonella typhi*, *Helicobacter pylori* and total antibodies to hepatitis A virus were detected using commercial ELISA Kits from Calbiotech (ST093G, United States), Demeditec Diagnostics (DEHEL01, Germany) and DiaSorin (N0136, Italy).

Dust Collection and Analysis

Dust samples were collected from bed-sheet, pillowcase, pillow, quilt and mattresses of each child's bedding by the study staff. Endotoxin and allergen levels were analyzed as described in our previous studies^{27,28}. Endotoxin level was measured following the manufacturer's instructions of the kinetic chromogenic Limulus Amebocyte Lysate assay (Hycult Biotech, Netherlands) and expressed as Endotoxin Units per square meter of dust collection area (EU/m²). Measurement of dust mite and cockroach allergens was performed using ELISA kits (ALK, Denmark). Allergen levels were expressed as microgram per square meter of dust collection area (μ g/m²) for mite allergens and arbitrary unit²⁹ per square meter of dust collection area (U/m²) for cockroach allergens.

Case Definitions

Cases were defined as subjects with asthma, which is having a positive response of 'diagnosed asthma' in the second stage detailed questionnaire or confirmed by coexistence of current wheeze and AHR. Controls were defined as subjects without asthma and other allergic symptoms.

Statistical Analyses

The Chi-squared test was used to compare prevalence of allergic symptoms and percentage of each categorical variable between groups. Comparison of age, body mass index, FVC% and FEV1% were determined by the Student's t-test. Mann-Whitney U-test was employed to compare levels of endotoxin and allergens. All analysis used a two tailed *p* value with significance level <0.05.

Association between each potential factor (the independent variable) and asthma (the dependent variable) was performed by using univariate logistic regression analyses. Crude odds ratios (OR) with 95% confidence intervals (CI) were calculated. Factors with *p* value <0.10 were conducted in further univariate logistic regression analyses adjusted for confounders including age, gender, and region. Those with *p* value <0.05 were included in multivariate logistic regression analyses with forward stepwise method. Only independent variables with *p* value <0.05 in

multivariate logistic regression were shown in the final model. All statistical analyses were performed with SPSS Statistics (version 22.0, New York, USA).

RESULTS

Prevalence of Wheezing in the Screening Survey between Rural and Urban Area

A total of 13251 screening questionnaires were distributed, of which 6928 (96.7%) from Guangzhou and 5841 (96.0%) from Conghua were returned (Table 1). Guangzhou children were younger than Conghua children (13.3 ± 0.64 vs. 14.2 ± 0.83). The prevalence rates of doctor-diagnosed-asthma (6.9% vs. 3.4%) and current wheeze (6.1% vs. 1.5%) were significantly higher in Guangzhou than Conghua ($p < 0.001$).

Baseline Characteristics and Clinical Examinations in Case-Control Study

In the second stage study, 188 subjects from Guangzhou and 129 from Conghua with positive response to at least one of two questions related to asthma, while 231 from Guangzhou and 306 from Conghua with negative response to the two questions and other allergic diseases agreed to participate in a detailed questionnaire and lab testings (Fig.1). Based on response to the question 'diagnosed asthma' in the detailed questionnaire or coexistence of current wheeze and AHR, asthma cases and controls were then defined. Of these participants, a significantly lower percentage of asthma was found in rural compared to urban subjects (2.8% vs. 29.4%, $p < 0.001$).

Guangzhou cases had significantly higher body mass index, atopic index, percentage of sensitization to cat, dog dander and lower $FEV_1/FVC\%$, when compared with Conghua cases. For control groups, body mass index, atopic index, percentage of overweight, obesity, sensitization to *Dermatophagoides farina* and cat dander and $FVC\%$ were significantly higher whereas age, prior infection to *Helicobacter pylori*, and sensitization to *Blomia tropicalis* and cockroach and $FEV_1/FVC\%$ were significantly lower in Guangzhou compared to Conghua. Guangzhou cases had significantly higher percentage of overweight, obesity, atopy and higher atopy index but lower $FEV_1\%$ and $FEV_1/FVC\%$ when compared to its controls (Table 2).

Family History and Early Life Exposures in Case-Control Study

Guangzhou cases and controls had a higher percentage of parental allergic diseases, daycare attending, using air conditioners in bedroom, consumption of milk products and meats, but lower percentage of any family member smoking compared to Conghua counterparts. However,

significantly higher percentage of Conghua children (cases and controls) had consumed well water, exposed to farming and animal environments from early life, while less likelihood to have no siblings than Guangzhou children. Guangzhou cases had significantly higher percentage of parental allergic diseases, hospitalization due to lung infections and use of antibiotics <age 3 than its controls, while Conghua cases had higher percentage for one-child family, hospitalization due to lung infections, cat inside the house <age 1, and lower percentage for living in a crop-farming family before age 3 (Table 2).

Indoor Dust Analysis

For dust analysis, 76 subjects in Guangzhou and 80 in Conghua agreed to participate (Table 3). Guangzhou subjects were exposed to lower levels of endotoxin, *Der p* 1 and cockroach but higher level of *Der f* 1 compared to Conghua ones.

Risk Factors for Asthma

Associations between each independent variable and asthma with univariate logistic regression analyses are presented in Table 4. With adjustment for age, gender and region, atopy, parental allergic diseases, one-child family, daycare attending, hospitalization due to lung infections, use of antibiotics, using air conditioners, consumption of milk product and meat, breastfeeding and high environmental *Der f* 1 level were associated with a higher risk for asthma, while consumption of well water, dog inside house, crop farming and high environmental endotoxin level were associated with a lower risk for asthma.

Table 5 shows the final models of multivariate regression analyses. Atopy (OR 1.91, 95%CI 1.58-2.29), parental allergic diseases (2.49, 1.55-4.01), hospitalization due to lung infections <age 3 (2.54, 1.37-4.70), high milk product consumption (1.68, 1.03-2.73) and high dust *Der f* 1 level (1.71, 1.34-2.19) were associated with a higher risk for asthma, whereas living in a crop-farming family before age 1 (0.22, 0.12-0.43) and high dust endotoxin level (0.69, 0.50-0.95) were associated with a lower risk for asthma.

DISCUSSION

Our study found, consistent with the hygiene hypothesis, exposure to crop farming and higher endotoxin levels were associated with decreased risk for asthma. Consistent with other research we found atopy, parental atopy, hospitalization for lung infection before age three, high

consumption of milk products and exposure to higher levels of dust mite allergen to be associated with increased risk for asthma.

Asthma is more common in urban areas compared to their rural counterparts¹⁴. We confirmed that prevalence of doctor-diagnosed-asthma was two-fold higher in Guangzhou as compared to Conghua using the validated ISAAC questionnaire³⁰. A similar difference was reported in north China comparing urban and neighboring rural region of Beijing using the same ISAAC methodology¹¹. Although far from each other, the two rural regions share general agriculture-dominated environments. Given the same ethnic background, these disparities are likely due to exposure to different environmental and lifestyle factors.

Our final multivariate logistic models showed that atopy, diet and environmental factors were related to childhood asthma. First, the genetic influence has been well recognized in population-based twin studies³¹. Second, atopy is a consistent risk factor for childhood asthma and house dust mite sensitization is associated with the increase in prevalence of wheeze in Guangzhou². Supporting this, the atopic index and percentage of atopy were higher in Guangzhou subjects. Third, presence of lung infections during early life has also been reported as a possible risk factor for the development of asthma⁶. Fourth, Guangzhou children consumed more milk products and meat. Consistent with our previous finding, high frequency of milk product (mainly processed milk) consumption was associated with an increased risk of asthma in our children⁸. The findings of this and our previous studies agree with the fact that milk was one of the major food allergens and milk sensitization and hypersensitivity were mainly found in urban children⁸.

Three environmental factors were found to be related to asthma. First, exposure to agricultural-farming has been reported to confer the same protection as livestock-farming in Beijing¹¹. The protective effect of a livestock-farming environment against allergy/asthma was well established in European studies. However, Conghua subjects were exposed to primarily crop-farming environment. Moreover, exposure to a crop-farming environment appeared to be particularly important in the first year of life, consistent with the idea of hygiene hypothesis that the protection effect may be time dependent³². The relation of exposure to livestock-farming and the protection against asthma/allergies is believed to be related to high level of exposure to microbes¹⁴. It is important to disentangle whether these components of livestock-farming environments hold true for rural areas of China in further studies. Second, our results suggested a

protective effect of high endotoxin exposure against asthma which was consistent with studies in children in from rural areas of Austria, Germany, and Switzerland¹⁵. These differences of endotoxin exposure on clinical outcomes may be time and dose-dependent and could be modified by gene-environment interactions³³, or by co-exposure to other indoor pollutants³⁴. The high percentage of dog ownership in our rural families can partly explain high level of dust endotoxin³⁵. Besides, large family size, higher level of cockroach allergens and percentage of drinking well water reflected unhygienic conditions in our rural families, suggesting extensive contact with microorganism, a key protective component that may shape the development of the immune system³⁶. Third, *Der f 1* was identified as a risk factor. Mite allergen in dust is associated with the prevalence of sensitization and level of asthma severity in sensitized patients³⁷. Besides, it was well known that mite allergen concentrations vary across regions attributed to differential geographic distribution and housing characteristics³⁸. Supporting this, the *Dermatophagoides farinae* populations were reported to thrive in filters of air-conditioners in similar warm moist environments (Shenzhen, China) close to Guangzhou³⁹.

The strength of our study is the comparative design of a large population sampled in both rural and urban environments in southern China using a validated methodology. There are several limitations. First, air pollution was not assessed. Second, the environmental assessment was not based on personal exposure. We, therefore, were unable to determine if there were differences in actual inhalation of endotoxin and allergens that might contribute to the associations. Finally, the numbers of asthma patients recruited from the rural region and dust samples were rather small. Further studies with larger sample size are needed to confirm our preliminary findings related to the dust analyses.

In conclusion, our findings that early life exposures to crop farming environment and high dust endotoxin are associated with a reduced risk of asthma lend further support to the hygiene hypothesis and may explain the lower prevalence of asthma in the rural environment.

ACKNOWLEDGMENTS

Declaration of all sources of funding: Supported by International (Regional) Cooperation and Exchange Program (Cooperation Research-NSFC-AF-DFG) by National Natural Science

Foundation of China (81261130023), the Academy of Finland (grant 256375, MR), Hong Kong Research Grant Council CUHK 477110 and German Research Foundation (DFG, SCHA 997/3-1)

Disclosure of potential conflict of interest: All authors have declared that they have no relationship to declare.

Author contributions: JL and GW mainly designed the study, performed the survey, collected the data, performed the statistical analysis and drafted the manuscript. MLF performed the survey, collected the data and drafted the manuscript. ZWY collected the data, performed the statistical analysis and drafted the manuscript. LYP, XXL, MX, XFH and YC mainly performed the survey, and collected the data. GWKW, PS, MR and BS participated in designing the study, performed the statistical analysis and drafted the manuscript.

REFERENCES

- 1 Anandan C, Nurmatov U, van Schayck OC, et al. Is the prevalence of asthma declining? Systematic review of epidemiological studies. *Allergy* 2010; 65:152-167
- 2 Li J, Wang H, Chen Y, et al. House dust mite sensitization is the main risk factor for the increase in prevalence of wheeze in 13- to 14-year-old schoolchildren in Guangzhou city, China. *Clin Exp Allergy* 2013; 43:1171-1179
- 3 von Mutius E, Braun-Fahrlander C, Schierl R, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000; 30:1230-1234
- 4 Paaso EM, Jaakkola MS, Rantala AK, et al. Allergic diseases and asthma in the family predict the persistence and onset-age of asthma: a prospective cohort study. *Respir Res* 2014; 15:152
- 5 Matheson MC, Allen KJ, Tang ML. Understanding the evidence for and against the role of breastfeeding in allergy prevention. *Clin Exp Allergy* 2012; 42:827-851
- 6 Lee E, Kwon JW, Kim HB, et al. Association Between Antibiotic Exposure, Bronchiolitis, and TLR4 (rs1927911) Polymorphisms in Childhood Asthma. *Allergy Asthma Immunol Res* 2015; 7:167-174
- 7 Pelucchi C, Galeone C, Bach JF, et al. Pet exposure and risk of atopic dermatitis at the pediatric age: a meta-analysis of birth cohort studies. *J Allergy Clin Immunol* 2013; 132:616-622.e617
- 8 Yang Z, Zheng W, Yung E, et al. Frequency of food group consumption and risk of allergic disease and sensitization in school children in urban and rural China. *Clin Exp Allergy* 2015;10.1111/cea.12532
- 9 Kozyrskyj AL, Bahreinian S, Azad MB. Early life exposures: impact on asthma and allergic disease. *Curr Opin Allergy Clin Immunol* 2011; 11:400-406
- 10 von Mutius E, Vercelli D. Farm living: effects on childhood asthma and allergy. *Nat Rev Immunol* 2010; 10:861-868
- 11 Ma Y, Zhao J, Han ZR, et al. Very low prevalence of asthma and allergies in schoolchildren from rural Beijing, China. *Pediatr Pulmonol* 2009; 44:793-799
- 12 Endara P, Vaca M, Platts-Mills TA, et al. Effect of urban vs. rural residence on the association between atopy and wheeze in Latin America: findings from a case-control analysis. *Clin Exp Allergy* 2015; 45:438-447

- 13 Genuneit J. Exposure to farming environments in childhood and asthma and wheeze in rural populations: a systematic review with meta-analysis. *Pediatr Allergy Immunol* 2012; 23:509-518
- 14 Schroder PC, Li J, Wong GW, et al. The rural-urban enigma of allergy: what can we learn from studies around the world? *Pediatr Allergy Immunol* 2015; 26:95-102
- 15 Braun-Fahrlander C, Riedler J, Herz U, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; 347:869-877
- 16 Schaub B, Vercelli D. Environmental protection from allergic diseases: From humans to mice and back. *Curr Opin Immunol* 2015; 36:88-93
- 17 von Mutius E. The environmental predictors of allergic disease. *J Allergy Clin Immunol* 2000; 105:9-19
- 18 McIntire JJ, Umetsu SE, Macaubas C, et al. Immunology: hepatitis A virus link to atopic disease. *Nature* 2003; 425:576
- 19 Pelosi U, Porcedda G, Tiddia F, et al. The inverse association of salmonellosis in infancy with allergic rhinoconjunctivitis and asthma at school-age: a longitudinal study. *Allergy* 2005; 60:626-630
- 20 Reibman J, Marmor M, Filner J, et al. Asthma is inversely associated with *Helicobacter pylori* status in an urban population. *PLoS One* 2008; 3:e4060
- 21 Wong GW, Ko FW, Hui DS, et al. Factors associated with difference in prevalence of asthma in children from three cities in China: multicentre epidemiological survey. *BMJ* 2004; 329:486
- 22 Wong GW, Li ST, Hui DS, et al. Individual allergens as risk factors for asthma and bronchial hyperresponsiveness in Chinese children. *Eur Respir J* 2002; 19:288-293
- 23 Lai CK, Chan JK, Chan A, et al. Comparison of the ISAAC video questionnaire (AVQ3.0) with the ISAAC written questionnaire for estimating asthma associated with bronchial hyperreactivity. *Clin Exp Allergy* 1997; 27:540-545
- 24 Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. *Eur Respir J* 2005; 26:948-968
- 25 Sovijarvi AR, Malmberg LP, Reinikainen K, et al. A rapid dosimetric method with controlled tidal breathing for histamine challenge. Repeatability and distribution of bronchial reactivity in a clinical material. *Chest* 1993; 104:164-170
- 26 Boulet LP, Turcotte H, Laprise C, et al. Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clin Exp Allergy* 1997; 27:52-59
- 27 Zhang C, Gjesing B, Lai X, et al. Indoor allergen levels in Guangzhou city, southern China. *Allergy* 2011; 66:186-191
- 28 Weiland SK, Bjorksten B, Brunekreef B, et al. Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. *Eur Respir J* 2004; 24:406-412
- 29 Gore JC, Schal C. Cockroach allergen biology and mitigation in the indoor environment. *Annu Rev Entomol* 2007; 52:439-463
- 30 Leung R, Wong G, Lau J, et al. Prevalence of asthma and allergy in Hong Kong schoolchildren: an ISAAC study. *Eur Respir J* 1997; 10:354-360
- 31 Koppelman GH, Los H, Postma DS. Genetic and environment in asthma: the answer of twin studies. *Eur Respir J* 1999; 13:2-4
- 32 Brooks C, Pearce N, Douwes J. The hygiene hypothesis in allergy and asthma: an update. *Curr Opin Allergy Clin Immunol* 2013; 13:70-77
- 33 Simpson A, John SL, Jury F, et al. Endotoxin exposure, CD14, and allergic disease: an interaction

between genes and the environment. *Am J Respir Crit Care Med* 2006; 174:386-392

34 Matsui EC, Hansel NN, Aloe C, et al. Indoor Pollutant Exposures Modify the Effect of Airborne Endotoxin on Asthma in Urban Children. *Am J Respir Crit Care Med* 2013; 15:1210-1215

35 Gereda JE, Klinnert MD, Price MR, et al. Metropolitan home living conditions associated with indoor endotoxin levels. *J Allergy Clin Immunol* 2001; 107:790-796

36 Hansel TT, Johnston SL, Openshaw PJ. Microbes and mucosal immune responses in asthma. *Lancet* 2013; 381:861-873

37 Bousquet J, Khaltaev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008; 63 Suppl 86:8-160

38 Zock JP, Heinrich J, Jarvis D, et al. Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. *J Allergy Clin Immunol* 2006; 118:682-690

39 Liu Z, Bai Y, Ji K, et al. Detection of *Dermatophagoides farinae* in the dust of air conditioning filters. *Int Arch Allergy Immunol* 2007; 144:85-90

Figure legends

FIGURE 1. Study population and design. ISSAC, International Study of Asthma and Allergies in Childhood.

Table 1. Comparison of prevalence of asthma and allergic symptoms among children between urban Guangzhou and rural Conghua

	Guangzhou N=7164	Conghua N=6087	<i>p</i> value
Subjects, response rate (n, %)	6928 (96.7)	5841 (96.0)	-
Ethnicity (n, %)			
Han Chinese	6928 (100)	5841 (100)	-
Gender (n, %)			
Boys	3504 (50.6)	3434 (50.6)	-
Girls	2953 (49.4)	2888 (49.4)	0.999
Age (years) ^Δ	13.3±0.64	14.2±0.83	<0.001
Written questionnaire [†]			
Doctor-diagnosed-asthma	475, 6.9(6.5-7.2)	198, 3.4(3.0-3.8)	<0.001
Wheeze ever	1012, 14.6(14.1-15.0)	248, 4.2(3.8-4.7)	<0.001
Current wheeze	423, 6.1(5.8-6.4)	89, 1.5(1.3-1.8)	<0.001
Current sleep-disturbing wheeze	97, 1.4(1.3-1.5)	42, 0.7(0.5-0.9)	<0.001
Current speech-limiting wheeze	84, 1.2(1.1-1.3)	25, 0.4(0.3-0.6)	<0.001
Current exercise-induce wheeze	1903, 27.5(26.9-28)	1375, 23.5(22.6-24.5)	<0.001
Current night waking with cough	1451, 21.0(20.5-21.4)	907, 15.5(14.7-16.3)	<0.001
Video questionnaire [†]			
Wheeze at rest ever	547, 7.9(7.6-8.2)	175, 3.0(2.6-3.4)	<0.001
Current wheeze at rest	340, 4.9(4.6-5.2)	100, 1.7(1.4-2.0)	<0.001
Wheeze after exercise ever	1271, 18.4(17.9-18.8)	586, 10.0(9.4-10.7)	<0.001
Current wheeze after exercise	948, 13.7(13.3-14.1)	324, 5.5(5.1-6.0)	<0.001
Wheeze at night ever	166, 2.4(2.2-2.6)	57, 1.0(0.8-1.2)	<0.001
Current wheeze at night	72, 1.0(0.9-1.2)	19, 0.3(0.2-0.5)	<0.001
Cough at night ever	1073, 15.5(15.1-15.9)	522, 8.9(8.3-9.6)	<0.001
Current cough at night	621, 9.0(8.6-9.3)	275, 4.7(4.3-5.2)	<0.001
Severe wheeze attack ever	356, 5.3(4.6-5.9)	96, 1.6(1.4-1.9)	<0.001
Current severe wheeze attack	208, 3.0(2.8-3.2)	47, 0.8(0.6-1.0)	<0.001

Results are expressed as [†]number of positive response, percentage (95%CI) or ^Δmean ± standard deviation.

Table 2. Characteristics of early life and environmental exposures and clinical testings between cases and controls in urban Guangzhou and rural Conghua

Variables	Cases		<i>p</i> value ¹	Controls		<i>p</i> value ²	<i>p</i> value ³	<i>p</i> value ⁴
	Guangzhou n=123	Conghua n=12		Guangzhou n=296	Conghua n=423			
Demographics								
Gender (girl) [†]	50 (40.7)	6 (50.0)	0.532	140 (47.3)	214 (50.6)	0.385	0.093	0.722
Age (years) ^Δ	13.6±0.8	13.5±1.1	0.620	13.8±0.7	14.1±0.9	<0.001	0.072	0.024
Body mass index ^Δ	19.6±3.5	15.6±3.5	0.001	19.7±3.2	18.3±2.2	<0.001	0.877	<0.001
Overweight [†]	22 (17.9)	2 (16.7)	0.916	45 (15.2)	24 (5.7)	<0.001	0.039	0.009
Obesity [†]	15 (12.2)	2 (16.7)	0.657	29 (9.8)	20 (4.7)	0.008	0.013	0.003
Serum antibodies								
<i>Helicobacter Pylori</i> [†]	24 (21.8)	2 (20.0)	0.894	60 (23.6)	127 (33.4)	0.008	0.467	0.388
<i>Salmonella Typhi</i> [†]	35 (28.9)	1 (10.0)	0.199	84 (29.4)	94 (22.5)	0.039	0.429	0.750
Hepatitis A virus [†]	94 (77.7)	10 (100.0)	0.095	227 (79.4)	316 (75.6)	0.242	0.338	0.170
Atopy								
Atopic index [§]	4.0 (3.0-5.0)	1.5 (1.0-3.8)	0.007	1.0 (1.0-4.0)	1.0 (1.0-2.0)	<0.001	<0.001	0.288
SPT to any allergens [†]	103 (83.7)	10 (83.3)	0.971	160 (54.1)	208 (49.2)	0.198	<0.001	0.259
SPT to <i>Dermatophagoides pteronyssinus</i> [†]	102 (82.9)	8 (66.7)	0.168	137 (46.3)	177 (41.8)	0.238	<0.001	0.990
SPT to <i>Dermatophagoides farina</i> [†]	95 (77.2)	9 (75.0)	0.861	143 (48.3)	155 (36.6)	0.002	<0.001	0.065
SPT to <i>Blomia Tropicalis</i> [†]	81 (65.9)	8 (66.7)	0.955	84 (28.4)	165 (39.0)	0.003	<0.001	0.871
SPT to cockroach [†]	14 (11.4)	2 (16.7)	0.590	19 (6.4)	132 (31.2)	<0.001	0.270	0.339
SPT to cat dander [†]	43 (35.0)	0 (0.0)	0.013	39 (13.2)	11 (2.6)	<0.001	<0.001	0.667
SPT to dog dander [†]	31 (25.2)	0 (0.0)	0.048	29 (9.8)	25 (5.9)	0.052	<0.001	0.510
SPT to ragweed pollen [†]	5 (4.1)	0 (0.0)	0.478	3 (1.0)	5 (1.2)	0.832	0.559	0.774
SPT to mugwort pollen [†]	2 (1.6)	2 (16.7)	0.003	4 (1.4)	6 (1.4)	0.940	0.969	0.715
Lung function								
FVC% ^Δ	98.4±12.7	99.7±15.8	0.755	99.9±14.0	96.7±12.0	0.002	0.329	0.444
FEV ₁ % ^Δ	97.0±13.4	104.3±14.2	0.102	102.6±12.6	101.6±13.2	0.332	<0.001	0.520

FEV ₁ /FVC% [§]	85.4(80.9-89.6)	91.2(89.7-95.0)	0.001	90.6(85.9-94.1)	92.7(88.5-96.1)	<0.001	<0.001	0.589
Family history								
Parental allergic diseases [†]	65 (52.8)	2 (16.7)	0.017	73 (24.9)	43 (10.2)	<0.001	<0.001	0.732
Siblings allergic diseases [†]	12 (33.3)	1 (8.3)	0.095	11 (13.3)	31 (7.8)	0.106	0.219	0.414
High parental education (≥high school) [†]	79 (64.8)	0 (0.0)	<0.001	147 (50.2)	20 (4.7)	<0.001	0.644	0.558
Early life exposures and habits								
One-child family [†]	87 (70.7)	1 (8.3)	<0.001	211 (72.0)	26 (6.1)	<0.001	0.311	0.014
Breastfeeding (≥ 6 months) [†]	46 (38.7)	8 (66.7)	0.055	125 (43.7)	264 (63.2)	<0.001	0.490	0.739
Daycare attending (≥ 6 months) [†]	107 (87.7)	4 (33.3)	<0.001	270 (93.1)	82 (19.4)	<0.001	0.128	0.546
Hospitalization due to lung infections (<age 3) [†]	34 (27.6)	4 (33.3)	0.677	32 (11.0)	16 (3.8)	0.003	<0.001	<0.001
Use of antibiotics (<age 3) [†]	102 (82.9)	8 (66.7)	0.168	165 (56.9)	200 (47.7)	0.021	<0.001	0.631
Drinking well water (<age 3) [†]	3 (2.4)	10 (83.3)	<0.001	16 (5.5)	346 (81.8)	<0.001	0.422	0.787
After-school physical activities (≥3 times/week) [†]	62 (50.4)	6 (50.0)	0.979	173 (58.4)	252 (59.6)	0.930	0.453	0.901
Cigarette smoking exposures								
Any household member who smokes [†]	52 (42.3)	11 (91.7)	0.001	126 (43.0)	283 (66.9)	<0.001	0.818	0.065
Indoor environment								
Using air conditioners in bedroom [†]	111 (90.2)	4 (80.0)	<0.001	255 (86.1)	60 (14.2)	<0.001	0.505	0.972
Using oil and gas to cook meals [†]	66 (53.7)	4 (33.3)	0.180	150 (50.7)	114 (27.0)	<0.001	0.938	0.350
Farming and Animal exposures								
Cat inside house [†]								
<age 1	5 (4.1)	3 (25.0)	0.003	11 (3.8)	20 (4.7)	0.529	0.602	0.006
Present	11 (8.9)	3 (25.0)	0.083	25 (8.5)	48 (11.3)	0.221	0.910	0.164
Dog inside house [†]								
<age 1	4 (3.3)	5 (41.7)	<0.001	14 (4.8)	98 (23.3)	<0.001	0.475	0.233
Present	9 (7.3)	6 (50.0)	<0.001	35 (11.9)	206 (48.8)	<0.001	0.051	0.749
Farm animals (pig or poultries) [†]								
<age 1	3 (2.4)	4 (33.3)	<0.001	5 (1.7)	110 (26.0)	<0.001	0.559	0.889

age 1-3	3 (2.4)	4 (33.3)	<0.001	5 (1.7)	109 (25.8)	<0.001	0.559	0.878
Present	2 (1.6)	1 (8.3)	0.134	2 (0.7)	84 (19.9)	<0.001	0.681	0.722
Living in a crop-farming family (lychee or rice) [†]								
<age 1	6 (4.9)	8 (66.7)	<0.001	16 (5.4)	376 (88.9)	<0.001	0.487	0.009
age 1-3	4 (3.3)	8 (66.7)	<0.001	15 (5.1)	377 (89.1)	<0.001	0.862	0.010
Present	2 (1.6)	8 (66.7)	<0.001	7 (2.4)	353 (83.5)	<0.001	0.622	0.068
Eating habits								
Consumption of milk products (≥ 3 times/week) [†]	79 (64.2)	3 (25.0)	0.008	154 (52.0)	40 (9.5)	<0.001	0.328	0.696
Consumption of fruit (≥ 3 times/week) [†]	76 (61.8)	5 (41.7)	0.176	173 (59.0)	124 (29.3)	<0.001	0.593	0.435
Consumption of meat (≥ 3 times/week) [†]	114 (92.7)	6 (50.0)	<0.001	244 (83.0)	279 (66.0)	<0.001	0.256	0.642

Results are expressed as [†]number of positive response (percentage), ^Δmean $\pm$ standard deviation, or [§]median (lower and upper quartiles);

p value was calculated from the chi-square test, t-test or Mann-Whitney U test, accordingly; p value were compared between Guangzhou and Conghua in cases (p value¹) and controls (p value²) and difference between cases and controls in Guangzhou (p value³) or Conghua (p value⁴) subjects;

Cut points for overweight and obesity are 24 (overweight) or 28 (obesity) of body mass index in China by using the Working Group of Obesity in China criteria;

SPT represents skin prick response; FVC% represents percent of normal forced vital capacity; FEV₁% represents percentage of predicted value for forced expiratory volume in 1 second.

Table 3. Comparison of environmental dust endotoxin and allergens levels between urban Guangzhou and rural Conghua

Variables	Guangzhou	Conghua	<i>p</i> value
Subjects (n)	76	80	
Cases (%)	42.1	1.3	
Endotoxin (EU/m ²)	478.3(226.3-1185.1)	2267.8(846.3-4895.1)	<0.001
<i>Der p</i> 1 (μg/m ²)	0.02(0.002-0.14)	0.7(0.3-1.4)	<0.001
<i>Der f</i> 1 (μg/m ²)	1.6(0.8-5.9)	0.02(0.004-0.08)	<0.001
Cockroach (U/m ²) [¶]	30.5(6.3-109.4)	109.4(44.3-313.2)	<0.001

Results are expressed as median (lower and upper quartiles);

EU/m², Endotoxin Units per square meter of dust collection area; U/m², arbitrary unit per square meter of dust collection area;

[¶]For cockroach allergens, the sum of *Bla g* 1 and *Per a* 1 was calculated due to high cross-reactivity (>25%).

Table 4. Association between various factors and asthma by using univariate logistic regression analyses

Variables	Unadjusted analyses			Adjusted analyses [†]		
	Odds ratio	95% CI [¶]	<i>p</i> value	Odds ratio	95% CI	<i>p</i> value
Demographics						
Body mass index						
Normal	1.00			1.00		
Overweight	2.04	1.23-3.38	0.006	2.04	1.21-3.44	0.008
Obesity	1.97	1.10-3.54	0.023	1.95	1.06-3.58	0.033
Atopy						
Atopy index						
0	1.00			1.00		
1	0.67	0.25-1.76	0.416	NS		
2	1.88	1.35-2.61	<0.001	1.84	1.32-2.58	<0.001
≥3	2.26	1.94-2.64	<0.001	2.25	1.92-2.65	<0.001
SPT to any allergens	8.88	5.84-13.50	<0.001	10.34	6.52-16.40	<0.001
SPT to <i>Dermatophagoides pteronyssinus</i>						
Diameter <3 mm	1.00			1.00		
3 mm ≤ Diameter <6 mm [†]	3.36	1.72-6.56	<0.001	3.47	1.76-6.85	<0.001
Diameter ≥ 6 mm	4.22	3.29-5.41	<0.001	4.44	3.41-5.79	<0.001
SPT to <i>Dermatophagoides farina</i>						
Diameter <3 mm	1.00			1.00		
3 mm ≤ Diameter <6 mm [†]	2.78	1.82-4.23	<0.001	NS		
Diameter ≥ 6 mm	4.30	3.34-5.54	<0.001	3.42	2.70-4.32	<0.001
Family history						
Parental allergic diseases	5.10	3.45-7.54	<0.001	4.90	3.29-7.30	<0.001
Siblings allergic diseases	3.90	1.92-7.94	<0.001	4.12	1.99-8.52	<0.001
High parental education (≥high school)	4.71	3.21-6.93	<0.001	4.31	2.92-6.38	<0.001
Early life exposures and habits						
Number of children						
One-child	1.00			1.00		

2	0.44	0.29-0.69	<0.001	0.45	0.29-0.70	<0.001
3	0.40	0.29-0.57	<0.001	0.43	0.31-0.61	<0.001
≥4	0.44	0.30-0.65	<0.001	0.48	0.32-0.71	<0.001
Breastfeeding (≥ 6 months)	0.63	0.41-0.96	0.030	0.54	0.37-0.80	0.002
Daycare attending (≥ 6 months)	4.95	3.09-7.94	<0.001	4.37	2.71-7.05	<0.001
Hospitalization due to lung infections (<age 3)	4.22	2.47-7.20	<0.001	5.09	3.13-8.26	<0.001
Use of antibiotics (<age 3)	3.82	2.30-6.36	<0.001	3.91	2.46-6.21	<0.001
Drinking well water (<age 3)	0.10	0.06-0.18	<0.001	0.12	0.06-0.21	<0.001
After-school physical activities (≥3 times/week)	0.85	0.73-0.99	0.033	0.85	0.73-1.00	0.048
<i>Cigarette smoking exposures</i>						
Family member smoking						
None	1.00			1.00		
Mother	1.52	0.31-7.41	0.602	NS		
Parents	0.69	0.46-1.03	0.069	NS		
<i>Indoor environment</i>						
Use air conditioners in bedroom	1.87	0.91-3.85	0.089	NS		
Use oil and gas to cook meals	1.84	1.26-2.69	0.002	1.74	1.19-2.53	0.004
<i>Farming and Animal exposures</i>						
Cat inside house						
None	1.00			1.00		
<age 1	1.39	0.62-3.09	0.422	NS		
Present	1.00	0.54-1.87	0.996	NS		
Dog inside house						
None	1.00			1.00		
<age 1	0.38	0.19-0.78	0.008	0.41	0.20-0.83	0.014
Present	0.37	0.20-0.67	0.001	0.27	0.15-0.47	<0.001
Farm animals (pig or poultries)						
None	1.00			1.00		
<age 1	0.29	0.13-0.64	0.002	0.33	0.15-0.73	0.006
age 1-3	0.29	0.13-0.64	0.002	0.34	0.15-0.74	0.007
Present	0.17	0.05-0.54	0.003	0.19	0.06-0.62	0.006

Living in a crop-farming family (lychee or rice)						
None	1.00			1.00		
<age 1	0.08	0.04-0.15	<0.001	0.09	0.05-0.18	<0.001
age 1-3	0.10	0.05-0.17	<0.001	0.09	0.05-0.17	<0.001
Present	0.10	0.05-0.17	<0.001	0.11	0.06-0.19	<0.001
Eating habits						
Consumption of milk products (≥ 3 times/week)	2.74	1.78-4.23	<0.001	3.85	2.60-5.69	<0.001
Consumption of fruit (≥ 3 times/week)	2.12	1.45-3.08	<0.001	2.02	1.38-2.95	<0.001
Consumption of meat (≥ 3 times/week)	2.11	1.13-3.94	0.018	2.37	1.32-4.23	0.004
Dust analysis						
Endotoxin (EU/m ²) [†]						
Low (<478.3)	1.00			1.00		
Medial (between 478.3 and 2267.8)	0.86	0.37-2.03	0.733	NS		
High (>2267.8)	0.47	0.26-0.86	0.013	0.45	0.23-0.87	0.018
Der p 1 ($\mu\text{g}/\text{m}^2$) [†]						
Low (<0.02)	1.00			1.00		
Medial (between 0.02 and 0.7)	0.31	0.13-0.75	0.009	0.34	0.13-0.88	0.027
High (>0.7)	0.29	0.15-0.57	<0.001	0.26	0.12-0.53	<0.001
Der f 1 ($\mu\text{g}/\text{m}^2$) [†]						
Low (<0.02)	1.00			1.00		
Medial (between 0.02 and 1.6)	8.26	2.91-23.48	<0.001	12.00	3.33-43.28	<0.001
High (>1.6)	1.24	1.07-1.45	0.005	1.25	1.06-1.47	0.009
Cockroach (U/m ²) [†]						
Low (<30.5)	1.00			1.00		
Medial (between 30.5 and 109.4)	0.87	0.34-2.20	0.762	NS		
High (>109.4)	0.72	0.45-1.16	0.177	NS		

[†] Adjusted for age, gender and region; [†] 95% CI: 95% confidence interval; Diameter is the result of positive diameter minus that of negative control; The medial value of each variable was selected for hierarchy.

Table 5. Multivariate logistic regression analyses between selected factors and asthma

Variables [¶]	Multivariate analyses [§]		
	Odds ratio [†]	95% CI	p value
Case-control study model			
SPT to any allergens	1.91	1.58-2.29	<0.001
Parental allergic diseases	2.49	1.55-4.01	<0.001
Hospitalization due to lung infections (<age 3)	2.54	1.37-4.70	0.003
Crop farming (<age 1)	0.22	0.12-0.43	<0.001
Consumption of milk products (≥ 3 times/week)	1.68	1.03-2.73	0.038
Dust analysis model			
Endotoxin (>2267.8 EU/m ²)	0.69	0.50-0.95	0.021
<i>Der f 1</i> (between 0.02-1.6 $\mu\text{g}/\text{m}^2$)	1.71	1.34-2.19	<0.001

[†]Adjusted for age, gender and region.

[¶]A variety of factors were selected from those with $p < 0.05$ in univariate logistic regression analyses and were entered in the multivariable logistic analyses, including overweight, obesity, atopy index (2 and ≥ 3), SPT to *Dermatophagoides pteronyssinus* ($3 \text{ mm} \leq \text{diameter} < 6 \text{ mm}$ and $\text{diameter} \geq 6 \text{ mm}$), SPT to *Dermatophagoides farina* ($\text{diameter} \geq 6 \text{ mm}$), number of children, birth weight, breastfeeding, daycare attending, use of antibiotics, drinking well water, after-school physical activities, use oil and gas to cook meals, dog inside house (present and <age 1), crop farming (<age 1), farm animals (<age 1, age1-3 and present), consumption of fruit and meat as well as dust *Der p 1* (between 0.02 and 0.7 and $>0.7 \mu\text{g}/\text{m}^2$) and dust *Der f 1* ($>1.6 \mu\text{g}/\text{m}^2$).

[§]We use a forward stepwise method and only variables with a $p < 0.05$ were finally shown in the final model.

