Elsevier Editorial System(tm) for Annals of Epidemiology
Manuscript Draft

Manuscript Number: 11-288R1

Title: Gene-Environment Interaction between Interleukin-4 Promoter and Molds in Childhood Asthma

Article Type: Full Length Article

Keywords: Interleukin-4 promoter, molds, damp housing, asthma, interaction, effect modification

Corresponding Author: Dr. Bing-Fang Hwang, Ph.D.

Corresponding Author's Institution: China Medical University

First Author: Bing-Fang Hwang, Ph.D.

Order of Authors: Bing-Fang Hwang, Ph.D.; I-Ping Liu, M.Sc; Tzu-Pi Huang, Ph.D.

Manuscript Region of Origin: TAIWAN

Abstract: PURPOSE: To assess the role of gene-environment interaction between interleukin-4 (IL-4) promoter and mold exposure on the development of asthma.
METHODS: We conducted a cohort based, incident case-control study. The case group consisted of 188 children with new asthma and the control group (n=376) was matched for age and sex. The outcome of interest was the development of asthma during the 6-year study period. The studied determinants were three polymorphisms of IL-4 promoter (TT; CT and CC) and three indicators of exposure including histories of water damage, presence of visible molds, and perceived mold odor in the home.
RESULTS: Apparent joint effects between IL-4 promoter and mold exposure were observed on both additive and multiplicative scales. Specially, the risk of asthma was significantly associated with children carrying CT genotype and visible mold exposure comparing with those carrying TT genotype without any exposure indicator (adjusted OR 2.14, 95% CI 1.05-4.34) (modified Rothman synergy index for directly use of odds and odds ratios (s) =1.41; p for interaction=0.03). A similar tendency was found (s=1.30; p for interaction=0.04) for children who were exposed to mold odor and carried CT genotype (adjusted OR 1.99, 95% CI 1.03-4.41)
CONCLUSIONS: The results of this study suggest gene-environment interaction between IL-4 promoter and indoor mold problem may play an important role in childhood asthma.

Dear Professor Richard Rothenberg,

Thank you for offering us an opportunity to address the comments of the reviewers. We think we were able to respond to all the comments, as indicated in the point-by-point response. We would like to submit the revised manuscript for your consideration. The study protocol was approved by the Institutional Review Board of College of Public Health, China Medical University, and it complies with the principles outlined in the Helsinki Declaration.

The article is original, does not infringe upon any other copyright or other proprietary right of any third party, is not under consideration by another journal, and has not been previously published. The authors confirm that they have read and are in agreement that the work is ready for submission to Annals of Epidemiology and that they accept the responsibility for the manuscript contents.

Sincerely yours,

Bing-Fang Hwang I-Ping Liu Tzu-Pi Huang
(corresponding author)

POINT BY POINT RESPONSE

Ms. No.: 11-288 Title: Gene-Environment Interaction between Interleukin-4 Promoter and Molds in Childhood Asthma

Reviewers' comments:

Reviewer #1: General comments

Using a matched case-control study, the authors found that possible exposure to mold in home is associated with increased asthma risk and IL-4 promoter polymorphism imparts an additive interactive effect. There are several major concerns that need to be addressed as described below.

Major

1. Based on the paper by Kalilani and Atashili (Epidemiol Perspect Innov. 2006; 3: 5.), replacing odds ratio with risk ratios for calculation of synergy index can lead to erroneous results and may not be reliable. The authors need to provide some information why they did not test interaction on multiplicative scale as used in most genetic epidemiology literature. The results from multiplicative interaction testing should be presented. The authors could get an unbiased estimate of the additive interaction from the unadjusted model using the codes provided by Kalilani and Atashili.

Response: The results from multiplicative interaction testing have been added on pages 9-10. The unbiased estimate of the additive interaction (AP and Rothman index calculated by substituting odds ratios for risk ratios) suggested by Kalilani and Atashili have been performed on pages 8 and 10.

2. It is not clear why the authors chose to use the terms "incidence odds ratio"? Shouldn't this be matched odds ratio? Also, given the case-control study design, the use of the words "cumulative incidence" is incorrect, as this does not represent the incidence in the entire cohort.

Response: We agree that the effect estimates should be matched odds ratios. We have corrected the words as matched odds ratios throughout the text.

3. It is unclear how the authors identified the cases if they had not attempted to recruit all subjects in the follow-up study. In other words, do the 188 represent all cases that develop from the 1,922 asthma-free children at cohort entry? Also, how many subjects were excluded that had no asthma diagnosis but had one of the five symptoms? The authors need to provide details about the subject recruitment. As it stands now, it appears that they attempted to recruit all 1,922 subjects and developed a case-control sample based on some criteria.
Response: Yes, the all new 188 cases that developed from the 1,922 asthma-free and non-asthmatic symptoms children at cohort entry during the study periods. We excluded 331 children with asthma (n=150), no asthma but with at least one asthmatic symptoms (n=160), or incomplete questionnaire (n=21) at the baseline survey (i.e. cohort entry) (page 4).

4. Some earlier studies have documented significant association between IL-4 promoter polymorphism and asthma. The authors need to reference those and evaluate them along with their findings to provide some explanations why a significant association was not observed in this study population. What is the study powered to detect a main effect given the study design and minor allele frequency?
Response: We have provided some explanations "the differences in direction of effects with IL-4 promoter genotypes may be due to chance, insufficient power, different populations or ethnic origins, variations of study design and different phenotypes studies" on page 13. The study power was around 0.92 to detect a main effect given the study design (188 cases and 376 controls, α=0.05, minor allele frequency=0.24).

Minor
1. Need to provide a correlation table for the exposures so that the reader can assess how correlated these exposures are.
Response: There was a positive association between visible mold and mold odor (r=0.30). Water damage was not associated with visible mold, but positively with mold odor (r=0.22) (page 9 and Table 3, page 20).

Table 3. Spearman correlation coefficients of visible mold, mold odor and water damage.

	Visible mold	Mold odor	Water damage
Visible mold	1.00	0.30*	0.07
Mold odor		1.00	0.22*
Water damage			1.00

* Correlation is significant at the 0.05 level.

2. Is the odds ratios (ORs) in Table 1 represent matched ORs?
Response: Yes, they are. The odds ratios represent matched odds ratios.

3. Rather than the population size, provide an estimate of how many children were born in Taoyuan between 1995 and 2002.
Response: There were approximately 44,000 children born in Taoyuan city between 1995 and 2002 (page 4).

4. Were data available on parental asthma and allergy? If so, that needs to be considered as a potential confounder.
Response: We also considered parental atopy as a potential confounder in the conditional logistic regression. The revised results were shown in the results (pages 9-10) tables 4-6 (pages 21-23).

5. A revision from a native English speaker will improve readability of the paper.
Response: We have asked English native speaker for proofreading in the revised version.

Reviewer #2: This study was conducted to assess the gene-environment interaction between interleukin-4 (IL-4) promoter and molds exposure on the development of asthma. The authors recruited 188 children with new asthma in a 6-year study period and a control group of 376 children who were matched for age and sex. Three indicators of exposure--water damage, presence of visible molds, and perceived mold odor-were used, and an apparent additive interaction between IL-4 promoter and molds exposure was observed. This is a prospective study, which is quite valuable. While the topic and results are interesting, I have some concerns:

1. Please discuss more about the biological effect of IL-4 promoter polymorphisms -589C/T; at least, a reference should be provided to support the statement "One of the IL-4 promoter polymorphisms -589C/T (also referred to rs number 2243250) is close to the glucocorticoid response element that positively stimulates IL-4 expression."

Response: We have added reference to support our statement on pages 3 and 15 (reference 4).

2. The covariate "cockroaches" was not mentioned in the Methods. Please provide its definition as well as the definition of environmental tobacco smoke (ETS).

Response: The definitions of cockroaches and ETS were added on page 7.

3. The title of Table 3 is "Incidence rates of asthma in the different exposure categories and incidence rate ratios calculated contrasting the reference category and adjusted for confounding in Poisson regression analysis." However, logistic regression was applied to analyze the data. Please clarify.

Response: It should be conditional logistic regression rather than Poisson regression. We have corrected it now in the table 4 (page 21).

4. The total number of cases varies across the tables, indicating some participants did not provide in formation on certain exposure indicators. Such information should be provided. In Table 4, it seems that the case with CC genotype did not report data on visible mold; it this correct?

Response: Because small size of CC genotype, we did not include them to elaborate the joint effect between visible mold or mold odor and IL-4 promoter.

5. Please explain why the gene-environment interaction between IL-4 promoter polymorphisms -589C/T and water damage was not evaluated like the other two exposure indicators.

Response: After consider parental atopy as confounder, the risk of asthma was not related to water damage. It’s not reasonable to evaluate the joint effects of water damage and IL-4 promoter like visible mold and mold odor.

6. It seems that an interaction also existed in the multiplicative scale. Are there any reasons why the authors believe that the Rothman synergy index is better than adding genotype as a covariate in the regression models?

Response: We provided the reasons why we assessed the interaction in additive scale by using Rothman synergy index on page 12. We also performed the multiplicative scale by adding interaction term in the regression models (page 9).

7. The changes in the living environment during the 6-year period should be addressed. If there is no way to address it in this study, it should be listed as a limitation.

Response: We did not have the information on the living environment change over time based on two surveys (baseline and follow-up). We listed this limitation on page 11.

8. The English should be polished. For example, in the 1st paragraph of the Methods, I wonder the statement "We excluded 331 children with asthma and incomplete questionnaire." should be "We excluded 331 children with asthma or incomplete questionnaire." In the 2nd paragraph, I wonder the statement "We identified 188 new cases that were developed asthma during the study period between October 15, 2002 and October 31, 2008." should be "We identified 188 new cases who developed asthma during the study period between October 15, 2002 and October 31, 2008."

Response: We have corrected these errors and asked English native speaker for proofreading.

Gene-Environment Interaction between Interleukin-4 Promoter and Molds in Childhood Asthma

Running Head: Interleukin-4 promoter and Molds and asthma

Bing-Fang Hwang, Ph.D., I-Ping Liu, M.Sc., Tzu-Pi Huang, Ph.D.

Bing-Fang Hwang, Ph.D.
Department of Occupational Safety and Heath
College of Public Health
China Medical University
Taichung, Taiwan

I-Ping Liu, M.Sc.
Department of Occupational Safety and Heath
College of Public Health
China Medical University
Taichung, Taiwan

[2]**Tzu-Pi Huang, Ph.D**
Department of Plant Pathology
National Chung Hsing University
Taichung, Taiwan
Tzu-Pi Huang

Correspondence to:
Dr. Bing-Fang Hwang,
Department of Occupational Safety and Health,
College of Public Health, China Medical University
No 91 Hsueh-Shih Rd, Taichung, Taiwan, 40402 R.O.C.
E-mail: bfhwang@mail.cmu.edu.tw
Telephone: +886-4-22053366 ext 6208
Telefax: +886-4-22071861

ABSTRACT

PURPOSE: To assess the role of gene-environment interaction between interleukin-4 (IL-4) promoter and mold exposure on the development of asthma.

METHODS: We conducted a cohort based, incident case-control study. The case group consisted of 188 children with new asthma and the control group (n=376) was matched for age and sex. The outcome of interest was the development of asthma during the 6-year study period. The studied determinants were three polymorphisms of IL-4 promoter (TT; CT and CC) and three indicators of exposure including histories of water damage, presence of visible molds, and perceived mold odor in the home.

RESULTS: Apparent joint effects between IL-4 promoter and mold exposure were observed on both additive and multiplicative scales. Specially, the risk of asthma was significantly associated with children carrying CT genotype and visible mold exposure comparing with those carrying TT genotype without any exposure indicator (adjusted OR 2.14, 95% CI 1.05-4.34) (modified Rothman synergy index for directly use of odds and odds ratios (s) =1.41; p for interaction=0.03). A similar tendency was found (s=1.30; p for interaction=0.04) for children who were exposed to mold odor and carried CT genotype (adjusted OR 1.99, 95% CI 1.03-4.41)

CONCLUSIONS: The results of this study suggest gene-environment interaction between IL-4 promoter and indoor mold problem may play an important role in childhood asthma.

Key words**: Interleukin-4 promoter, molds, damp housing, asthma, interaction, effect modification**

INTRODUCTION

Asthma is the most common chronic childhood disease (1). It is characterized by recurrent respiratory symptoms, reversible airway obstruction, airway inflammation and increase airway responsiveness (2). One of the most important pathways on development of asthma involves IgE antibody responses to ubiquitous allergens. Interleukin-4 (IL-4) plays an important role in IgE synthesis by activating the pre-T helper cells that trigger isotype switching from IgM/IgG to IgE in B cells by promoting Th2 cell development (3). It is reasonable to expect that increase expression of IL-4 might lead to the development of asthma and therefore the IL-4 promoter region (chromosome 5q31) seems to be the most likely site for polymorphisms. One of the IL-4 promoter polymorphisms -589C/T (also referred to rs number 2243250) is close to the glucocorticoid response element that positively stimulates IL-4 expression (4). Few studies of genetic epidemiology have assessed the relation between IL-4 promoter and childhood asthma in different populations, but these studies provided inconsistent results (5-8).

In our systematic Medline search, we identified only two previous longitudinal studies (9-10) and a population-based incident case-control study (11) which investigated exposure prior to the onset of asthma in children. All the three studies report that children exposure to dampness problems and molds increase the incidence of asthma (9-11). However, potential modification of the association between exposure to molds and the development of asthma by IL-4 promoter (i.e. gene-environment interaction) has not yet investigated.

We conducted a cohort based, incident case-control study to assess the independent and joint effect of IL-4 promoter and molds exposure on the risk of childhood asthma.

METHODS

Data Collection and Study Design

The source population included 44,000 children born in Taoyuan City between January 1, 1995 and December 31, 2002. Taoyuan is an urban-suburban municipality located across the north-western boarder of Taiwan. In the October 2002 baseline survey, a modified Chinese version of The International Study of Asthma and Allergies in Childhood (ISAAC-C) questionnaire was used to collect information on children's health, environmental exposures, and other relevant factors. The parents or other guardians were asked to provide information on children's personal characteristics, health, details of the environment, and other relevant factors (Table 1). We approached a random sample of 2,253 children aged 1-7 from the source population. The response rate was 85.3%. We excluded 331 children with asthma (n=150), without asthma but with at least one asthmatic symptoms (n=160), or incomplete questionnaire (n=21). The study population included 1,922 children free of asthma and non-asthmatic symptoms at the cohort entry (baseline). The study design has been described in detail previously (12).

In October 2008, we conducted a cohort-based incidence case-control study. We identified 188 new cases who developed asthma during the study period between October 15, 2002 and October 31, 2008. The definition of new asthma subjects was determined by a positive response to the question "Has a physician ever diagnosed your child as having asthma during 2002-2008?" at the follow-up survey. Five questions related to current asthmatic symptoms were also asked at both baseline and follow-up surveys:

1. In the past 12 months, has your child dyspnoea with wheezing in the chest? (wheezing)
2. In the past 12 months, has your child's sleep been disturbed because of wheezing? (night wheezing)
3. In the past 12 months, has whizzing ever been severe enough to limit your child's

speech to only one or two words at a time between breaths? (dyspnoea at rest)

4. In the past 12 months, has your child's sounded wheezy during or after exercise? (exercise wheeze)

5. In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection? (night cough)

Control subjects were selected without asthma or asthmatic symptoms applying one-to-two matching for age and sex from 2002 baseline survey of 1,922 children. The criteria of control subjects was (1) no physician diagnosed asthma or dyspnoea with wheezing in the past; (2) no positive response to any of the five questions concerning current asthmatic symptoms. The final study population constituted 188 case and 376 control subjects. The study protocol was approved by the Institutional Review Board of China Medical University, and it complied with the principles outlined in the Helsinki Declaration.

IL-4 promoter Genotyping

Cotton swabs containing oral mucosa were collected and were immediately maintained at −80°C throughout the transfer and storage. Genomic DNA will be isolated using phenol/chloroform extraction method.

The cotton swabs directly immersed in 300 μL cell lyses buffer (50 mm Tris-HCl, 1 mm EDTA, 0.1 m NaCl, pH 8.0) containing 2% SDS and 20 μg/mL proteinase K in a 1.5 mL micro centrifuge tube. After incubation overnight at 55°C, the swabs were discarded and the DNA in supernatants were purified by phenol/chloroform extraction and then precipitated with ethanol. DNA fragments including the -589C/T variant were amplified a 198 base pair (bp) long by allele-specific polymerase chain reaction (PCR). The forward primer sequence from -683 to -633 nucleotides is 5' TGG GTA AGG ACC TTA TGG ACC 3' whereas the reverse primer sequence from -486 to -505 is 5' GGT GGC ATC TTG GAA ACT GT 3'. The reverse primer

was designed to contain a base substitution C→T at the fourth last nucleotide from its 3'end. The individual PCR reaction vial contains a final volume of 20 µl solution. Two-hundred nanograms of DNA samples was added to 8 µl of PCR master mix consisting of 2.0 µl of 10X Mg Free PCR Buffer, 2.0 µl of 10 µM forward primer, 2.0 µl of 10 µM reverse primer and 2.0 ml of 10 mM dNTP. An appropriate amount of sterile ultrapure water (which totals up to 20 µl) was added to each of the microfuge tube. One micro liter of 5 units/µl *Taq* DNA polymerase was added to the reaction vial only after 5 minutes of pre-denaturation process prior to performing 'hot start' PCR. The PCR was performed using the Thermal Cycler (Applied Biosystems 9800®) for 35 cycles. The temperature for the initial denaturation of DNA was 94 °C for 1 minute, annealing at 60 °C for 1 minute and extension 72°C for 1 minute and a final extension at 72 °C for 5 minutes following the last cycle. The PCR product was subjected to *BsmF*I digestion for 2 hours at 37°C and electrophoresed on a 2.5% agarose gel with ethidium bromide staining. The IL-4 promoter -589 C/T polymorphisms was visualized as 198 bp fragments for TT genotype, 120 pb and 78 pb fragments for CC genotype and 198 bp, 120 bp, 78 pb fragments for CT genotype. All assays were performed by a laboratory worker unaware of the clinical status of individual subjects, and genotype assignments were based on two consistent experimental results. About 15% of randomly selected samples were directly sequenced, and all of them were concordant with the initial genotyping results.

Environmental Determinants of Interest

We used three indicators of exposure defined from the answers to following structured questions at baseline:

Mold odor. "Have you perceived mold odor in your dwelling during the past 12 months?" (No; Yes, almost daily; Yes, 1-3 days a week; Yes, 1-3 days a month)

Visible mold. "Have you ever had visible mold in your dwelling?"(No; Yes, during the past 12

months; Yes, only earlier.)

Water damage. "Have you ever had water damage in your dwelling?" (No; Yes, during the past 12 months; Yes, only earlier.)

Any exposure indicator. Presence of any of the three exposure indicators.

We decided to focus on exposures documented prior to the study period to ensure a plausible temporal sequence between exposure and the studied outcome for the causal inference.

Covariates

Information on potential confounders was obtained from the baseline questionnaire. The covariates in the present analyses included parental education, duration of breastfeeding, daily activity, furry/feathery pets and environmental tobacco smoke (ETS) (Table 1). The duration of breastfeeding was categorized into i) less than 1 months, ii) 1 to 5 months and iii) 6 months or longer. Daily activity was counted hours per day spent at activity levels corresponding to high (>4 hour/day), medium (2-4 hours/day) and low (<2 hours/day) as the reference category. Parents education was categorized into i) $\leq$ 9 years, ii) 10-12 years, iii) 13-16 years and iv) $\geq$ 17 years, and four indicators variables were formed with i) as a reference category. ETS exposures were defined as paternal smoke only, maternal smoke only, or both paternal and maternal smoke exposure (yes) and none (no). Other covariates, such as cockroaches noted monthly, furry/feathery pets were dichotomous. Parental atopy was defined as the father or mother of the index child ever having been diagnosed as having asthma, or allergic rhinitis, or atopic eczema.

Statistical Methods

First, we estimated the incidence rate of asthma during the 6-year study period according to polymorphisms of IL-4 promoter and indicators of exposure to dampness and molds. In the crude analysis, matched odds ratio of the relations between exposure and outcome relations were estimated. We estimated adjusted odds ratios applying conditional logistic regression analysis. The matched odds ratios were adjusted for the aforementioned covariates.

Second, we studied the additive joint effects of IL-4 promoter and the two most relevant exposure indicators, namely 'mold odor' and 'visible mold' on the risk of asthma. Because of small number of CC genotype, we compared the odds ratios (OR) of asthma in four exposure categories: 1) TT genotype and no exposure ($OR_{00,}$ reference category); 2) CT genotype and no exposure (OR_{10}); 3) TT genotype and exposure (OR_{01}); and 4) CT genotype and exposure (OR_{11}). Then their odds (O) in four exposure categories (O_{00}, O_{01}, O_{10} and O_{11}) were derived from the same conditional logistic regression model adjusting for the covariates. On an additive scale, the attributable proportion due to interaction calculated by substituting odds ratios for risk ratios (AP) was suggested by Kalilani and Atashili (13):

$$AP = \left(\frac{1 + O_{11}}{OR_{11}}\right) * \left(\frac{OR_{11}}{1 + O_{11}} - \frac{OR_{10}}{1 + O_{10}} - \frac{OR_{01}}{1 + O_{01}} - \frac{1}{1 + O_{00}}\right)$$

Finally, the Rothman synergy index calculated by substituting odds ratios for risk ratios and its 95% CI were used to assess the joint effect of the two factors (14). The synergy index (S) modified for direct use of odds and odds ratios was employed for more accurate assessment of additive interaction (13). The synergy index was calculated using the following formula:

$$S = \left(\frac{\left(\frac{OR_{11}\,(1 + O_{00})}{1 + O_{11}} - 1\right)}{\left(\frac{OR_{10}(1 + O_{00})}{1 + O_{10}} - 1\right) + \left(\frac{OR_{01}(1 + O_{00})}{1 + O_{01}} - 1\right)}\right).$$

An observed synergy index value that departs substantially from the expected additive null, i.e., synergy index not equal to 1, suggests an additive interaction effect. The IR values and their variance covariance matrix were then used to calculate values for synergy index and 95% CIs (15). SAS version 9.2 was used for all statistical analyses. Furthermore, we studied the multiplicative joint effect of IL-4 promoter and exposure by introducing interaction terms in the model. All tests assumed a two-sided alternative hypothesis and a 0.05 significance level.

RESULTS

Characteristics of Case and Control Subjects

Table 1 compares the demographic and environmental characteristics between the case and control subjects at baseline. The case subjects had lower duration of breastfeeding, more time spent outdoors and higher proportion of parental atopy compared with the control subjects, and were more commonly exposed to cockroaches (94.7 vs. 90.2%) and furry or feathery pets (30.3 vs. 27.1%) in the home.

Independent Effects of IL-4 Promoter and Exposure to Dampness and Mold problems

Hardy-Weinberg equilibrium tests showed non-significance ($p>0.05$) in both case and control groups. IL-4 promoter (CT vs. TT genotype) was not significantly associated with the risk of asthma with an adjusted odds ratio of 1.17 (95% CI 0.76-1.79) as shown in the Table 2.

There was a positive association between visible mold and mold odor ($r=0.30$). Water damage was not associated with visible mold, but positively associated with mold odor ($r=0.22$) (Table 3).Table 4 presents the matched odds ratios for asthma according to the three exposure indicators at baseline, as well as odds ratios contrasted to the reference category of no exposure. The risk of asthma was related to any indicator of exposure (adjusted OR 1.43, 95% CI 1.01-2.13), presence of mold odor (adjusted OR 1.61, 95% CI 1.02-2.68), and visible mold (adjusted OR 1.50, 95% CI 1.01-2.31).

Joint Effect of IL-4 Promoter and Visible Mold

Table 4 shows the incidence rates of asthma in four categories representing the reference, independent effects of IL-4 promoter and any exposure indicator, and their additive joint effect. Children carrying CT genotype without visible mold didn't have a significantly increased risk of asthma with an adjusted odds ratio (OR_{10}) of 0.97 (95% CI 0.45 – 2.07) (Table 5). The effect of visible mold exposure on children with TT genotype increased with an OR_{01} of 1.35 (95% CI 0.82-2.22). In children with both CT genotype and visible mold, the adjusted OR_{11} of asthma was 2.14 (95%CI 1.05-4.34), compared with children of the reference category. Thus the attributable proportion due to interaction of CT genotype and visible mold (AP) was 11.4%. Additionally, the Rothman synergy index (s) calculated by substituting odds ratios for risk ratios was 1.41 (95% CI 1.01-3.47) greater than 1. It suggests additive and multiplicative interactions (p for interaction =0.03) between IL-4 promoter and visible mold exposure (Table 5).

Joint Effect of IL-4 Promoter and Exposure to Mold Odor

One the basis of the table 5, the joint effect for CT genotype and exposure to mold odor (adjusted OR_{11} 1.99 95% CI 1.03-4.41), corresponded with the independent effects for CT genotype (adjusted OR_{01} 0.99 95% CI 0.45-2.22) and exposure to mold odor (adjusted OR_{10} 1.46 95% CI 0.83-2.59). Thus there was an apparent additive interaction (AP=8.9%) between IL-4 promoter and mold odor exposure (s=1.30 95% CI 1.03-2.35). In addition, a multiplicative interaction between IL-4 promoter and mold odor exposure (p for interaction =0.04) was also found (Table 6).

.

DISCUSSION

The results of present study, we found approximately 43%, 61%, and 50% increased the risk of development of asthma for children living in homes with any mold problem, mold odor, or visible mold respectively. Although the IL-4 promoter did not predict asthma, the results indicate that the joint effect of IL-4 promoter, representing genetic constitution, and exposure to visible mold and mold odor was stronger than expected on the basis of their independent effects in additive and multiplicative scales.

Validity of Results

A cohort-based incident case-control study offers an appropriate approach to assess the role of mold problems on the development of asthma. The prospective study design minimizes selection bias and information bias. A selection bias will be eliminated if the parents of children with asthma are more likely to change housing conditions after the first symptoms and signs of asthma compared with parents of healthy children. Information bias will not introduce if the parents of the symptomatic children report or recall similar exposure indicators differently from the parents of healthy children.

The exposure assessment was based parental reporting at baseline rather than objective measurements, which is a limitation of the present study. Objective measurements were not yet used in any of the epidemiologic studies conducted at the time of the data collection. Visual observation by a trained person would also have improved the exposure assessment (11, 16). However, our exposure information was collected before the onset of the asthma and therefore any bias due to awareness of the disease or exposure to molds was avoided. Another limitation was that it's not possible to address the changes in children's living environment during the 6-year period based on two surveys (baseline and follow-up).

We were able to take into account most of the known potential confounders related to individual characteristics, other environmental exposure in the condition logistic regression

analysis. However, dampness problems may also be related to other indoor environmental factors, such as dust mites. Dampness problems may also imply low ventilation rate and consequently increase the levels of indoor pollutants. We cannot rule out these indoor environmental factors will influence our results.

Synthesis with Previous Knowledge

We identified only three previous prospective cohort or incident case-control studies with incident asthma in children as the outcome of interest (9-11). Wickman and colleagues conducted a population-based birth cohort study of 4089 children in Stockholm, where they reported an increased risk of asthma among children in damp home environment during the first two years of life compared with unexposed with an adjusted odds ratio of 1.75 (95% CI 1.26-2.43). The exposure was defined as smell and visible signs of mold, water damage inside construction, and persistent windowpane condensation in dwellings with double-glazing (9). Jaakkola and colleagues conducted a 6-year cohort study of 1916 children in Finland using self-reported exposure indicated an association between the risk of asthma and mold odor with an adjusted incidence rate ratio of 2.44 (95% CI 1.07-5.60), but not visible mold, and water damage (10). Pekkane and colleagues conducted an incident case-control study in Finland and found the presence of visible mold and moisture damage in main living parts increase the risk of asthma (11). The present study strengths the evidence that home dampness due to any problem of mold, mold odor, or visible mold increase the risk of development of asthma in childhood.

An important rationale for presenting the interaction on the additive scale is that it fits with the sufficient-component concept of causality (17). It has been suggested that the additive scale is more appropriate to assess "biologic interaction" which is implied by terms such as synergism or antagonism (18). We further performed test interaction on multiplicative scale as used in most genetic epidemiology literature. Our study shows apparent joint effects between IL-4 promoter and molds exposure on both additive and multiplicative scales.

The specific causal agents of asthma related to indoor dampness and mold problems are not well understood, and several potential causes have been suggested including molds, bacteria, house dust mites, and enhanced emission of chemicals from surface materials. Our results suggest that mold odor, and visible mold are important indicators of relevant exposure. Several biological mechanisms by which indoor molds, particular concerning Penicillium, Aspergillus, Cladosporium and Alternaria could induce asthma have been suggested including immunoglobulin E (IgE) or immunoglobulin G (IgG)-mediated hypersensitivity reactions, toxic reactions caused by mycotoxins, and nonspecific inflammatory reactions caused by irrigative volatile organic compounds produced by microbes (MVOCs) or cell wall components, such as 1, 3-ß-D-glucan and ergosterol (20-26).

We found IL-4 promoter might not be a determinant of developing asthma in childhood, which was inconsistent with a recent meta-analysis of 14 studies, representing a pooled total of 2,476 asthmatic cases and 2,339 controls (8). The differences in direction of effects with IL-4 promoter genotypes may be due to chance, insufficient power, different populations or ethnic origins, variations of study design and different phenotypes studies. This effect and these results warrant further investigation in larger studies. The results showed that the joint effect of IL-4 promoter, representing genetic constitution, and exposure to visible mold or mold odor was stronger than expected on the basis of their independent effects in additive scale. An apparent gene-environment interaction between IL-4 promoter and exposure to molds was found. It is possible that IgE antibody responses to ubiquitous allergens, such as molds. IL-4 plays an important role in IgE synthesis by activating the pre-T helper cells that trigger isotype switching from IgM/IgG to IgE in B cells by promoting Th2 cell development. Children with both IL-4 promoter (CT genotype) and molds exposure increase expression of IL-4 and make them more likely to develop asthma.

Concluding Remarks

Our results are consistent with the hypothesis that molds play an important role of childhood asthma. The results also provide further evidence that IL-4 promoter may influence sensitivity to molds on the development of asthma.

Funding

This study was supported by the National Science Council (NSC# 99-2314-B-039-021-MY3) and China Medical University (CMU#99-S-25).

Acknowledgments

We thank all the field workers who supported data collection, the school administrators and teachers, and especially the parents and children who participated in this study.

Conflict of interest

We have no conflicts to disclose.

REFERENCES

1. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjuntivitis, and atopic eczema: ISSAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet 1998; 351: 1225-32.
2. Global Initiative for Asthma, Updated from. NHLBI/WHO Workshop Report: Global Strategy for Asthma Management and Prevention Issued January 1995. NIH Publication No. 02-3659. 2002.
3. Holloway JW, Neghe B, Holgate ST. The genetic basis of atopic asthma. Clin Exp Allergy 1999; 29: 1023-32.
4. Rosenwasser LJ. Promoter polymorphism in the candidate genes, IL4, IL9, TGF-β1 for atopy and asthma. Int Arch Allergy Immunol 1999; 118: 268-270.
5. CuiT, Hu L, Pan S, et al. Association between interleukin-4 and interleukin-4 receptor gene polymorphism and atopic asthma in children. Chin J pathophysiol 2005; 21: 125-8.
6. Lee SG, Kim BS, Kim JH, et al. Gene-gene interaction between interleukin-4 and interleuin-4 receptor alpha in Korean children with asthma. Clin Exp Allergy 2004; 34: 1202-8.
7. Takabayashi A, Ihara K, Sasaki Y, et al. Children atopic asthma: positive association with a polymorphism of IL-4 receptor alpha gene but not with that of IL-4 promoter or Fc epsilon receptor I beta gene. Exp Clin Immunogenet 2000; 17: 63-70.
8. Li Y, Guo B, Zhang L, et al. Association between C-589T polymprophisms of interleukin-4 gene promoter and asthma: A meta-analysis. Respir Med 2008; 102: 984-992.
9. Wickman M, Melen E, Berglind N, et al. Strategies for preventing wheezing and asthma in small children. Allergy 2003; 58: 742-747.

10. Jaakkola JJ, Hwang BF, Jaakkola N. Home dampness and molds, parental atopy and asthma in children: a six year population-based cohort study. Envrion Health Perspect 2005; 113: 357-361.
11. Pekkanen J, Hyvarinen A, Haverinen-Shaughnessy U, Korppi M, Putus T, Nevalainen A. Moisture damage and childhood asthma: a population-based incident case-control study. Eur Respir J 2007; 209: 509-515.
12. Hwang BF, Liu I-Ping, Huang TP. Molds, Parental Atopy and Pediatric Incident Asthma. Indoor Air 2011 in press.
13. Kalilani L, Atashili J. Measuring additive interaction using odds ratios. Epidemiol Perspect Innov 2006; 3: 5.
14. Rothman KJ. The estimation of synergy or antagonism. Am J Epidemiol 1976; 103:506-11.
15. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992; 3:452-456.
16. Nafstad P, Øie L, Mehl R et al. Residential dampness problems and symptoms and signs of bronchial obstruction in young Norwegian children. Am J Respir Crit Care Med 1998; 157: 410-14.
17. Knol MJ, Egger M, Scott P, Greerlings MI, Vandenbroucke JP. When one depends on the other: reporting of interaction in case-control and cohort studies. Epidemiology 2009; 20: 161-166.
18. Kaufman JS. Interaction reaction. Epidemiology 2009; 20: 159-160.
19. VanderWeele TJ, Robins JM. The identification of synergism in the sufficient-component-cause framework. Epidemiology 2007; 18, 329-339.
20. Norbäck D, Björnsson E, Janson C, Palmgren U, Boman G. Current asthma and biochemical signs of inflammation in relation to building dampness in dwellings. Int J Tuberc Lung Dis 1999; 3: 368-76.

21. Husman T. Health effects of indoor-air microorganisms. Scand J Work Environ Health1996; 22: 5-13.

22. Thorn J, Rylander R. Airway's inflammation and glucan in a row house area. Am J Respir Crit Care Med 1989; 157: 1798-1803.

23. Johanning E, Landsbergis P, Gareis M, Yang CS, Olmsted E. Clinical experience and results of a sentinel health investigation related to indoor fungal exposure. Environ Health Perspect 1999; 107 (Suppl 3): 489-94.

24. Etzel RA. How environmental exposures influence the development and exacerbation of asthma. Pediatric 2003; 112: 233-239.

25. Mutius E, Martinez FD, Fritzsch C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. Am J Respir Crit Care Med 1994; 149: 358-364.

26. Laitinen T, Räsänen M, Kaprio J, Koskenvuo M, Laitinen LA. Importance of genetic factors in adolescent asthma: a population-based twin-family study. Am J Respir Crit Care Med 1998; 157: 1073-1078.

Table 1. Demographic and environmental characteristics of case and control subjects.

Categories	Case subjects n=188 (%)	Control subjects n=376 (%)	Matched odds ratio (95% CI)
Highest level of parental education (years)			
≤ 9	13 (6.9)	23 (6.2)	Reference
10-12	76 (40.4)	173 (46.4)	0.88 (0.43-1.80)
13-16	87 (46.3)	158 (42.4)	1.10 (0.54-2.25)
≥l7	12 (6.4)	19 (5.1)	1.26 (0.47-3.37)
Daily activity (hours per day)			
<2	21 (11.2)	34 (9.0)	Reference
2-4	107 (56.9)	202 (53.7)	0.86 (0.47-1.55)
>4	60 (31.9)	140 (37.2)	0.69 (0.37-1.29)
Duration of Breastfeeding (months)			
< 1	147 (78.2)	264 (70.2)	Reference
1-5	29 (15.4)	81 (21.5)	0.66 (0.41-1.04)
≥ 6	12 (6.4)	31 (8.3)	0.69 (0.34-1.40)
Cockroaches noted monthly			
No	10(5.3)	37(9.8)	Reference
Yes	178(94.7)	339(90.2)	1.94(0.94-4.00)
Furry/feathery pets			
No	131(69.7)	274(72.9)	Reference
Yes	57(30.3)	102(27.1)	1.17(0.80-1.72)
Environmental Tobacco Smoke			
No	76(40.4)	152(40.4)	Reference
Yes	112(59.6)	224(59.6)	1.00 (0.70-1.43)
Parental atopy			
No	84(44.7)	268(71.3)	Reference
Yes	104(55.3)	108(28.7)	3.30 (2.24-4.90)

Table 2. Genotype of IL-4 promoter in the relation to asthma.

.

Genotype	**Size of the group**	**No of new asthma cases**	**Crude matched odds ratio (95% CI)**	**Adjusted* matched odds ratio (95% CI)**
TT	441	136	1.00	1.00
CT	140	51	1.16 (0.78-1.73)	1.17 (0.76-1.79)
CC	13	1	0.17 (0.02-1.31)	0.20 (0.03-1.66)

*Conditional logistic regression controlling for parents' highest education, parental atopy, duration of breastfeeding, daily activity, presence of hairy or feathery pets at home and exposure to environmental tobacco smoke (ETS).

Table 3. Correlations of visible mold, mold odor and water damage.

	Visible mold	Mold odor	Water damage
Visible mold	1.00	0.30*	0.07
Mold odor		1.00	0.22*
Water damage			1.00

* Spearman correlation coefficient is significant at the 0.05 level.

Table 4. Matched odds ratios of asthma calculated contrasting the reference category and adjusted for confounding in conditional logistic regression analysis.

Exposure at baseline	**Size of the group**	**No of new asthma cases**	**Crude matched odds ratio (95% CI)**	**Adjusted*matched odds ratio (95% CI)**
Total	564	188	-	-
No exposure (reference)	244	63	1.00	1.00
Any exposure indicator	320	125	1.78 (1.25-2.57)	1.43 (1.01-2.13)
Mold odor	200	83	2.20 (1.41-3.43)	1.61 (1.02-2.68)
Visible mold	137	54	1.82 (1.25-2.64)	1.50 (1.01-2.31)
Water damage	20	10	2.57 (0.65-10.1)	2.28 (0.40-13.1)

*Conditional logistic regression controlling for parents' highest education, parental atopy, duration of breastfeeding, daily activity, presence of hairy or feathery pets at home and exposure to environmental tobacco smoke (ETS).

Table 5. Independent and joint effects of IL-4 promoter and exposure visible mold on the development of asthma.

Exposure category	**Size of the group**	**No of new asthma cases**	**Crude matched odds ratio (95% CI)**	**Adjusted* matched odds ratio (95% CI)**
TT, No exposure	175	46	1.00	1.00
CT, No exposure	69	17	1.01 (0.55-2.21)	0.97 (0.45-2.07)
TT, Exposure	191	76	1.68 (1.10-2.56)	1.35 (0.82-2.22)
CT, Exposure	72	31	2.01 (1.16-3.51)	2.14 (1.05-4.34)
Attributable proportion due to interaction (AP)				0.114
Rothman synergy index				1.41 (1.01-3.47)
P for interaction #				0.03

* Conditional logistic regression controlling for parents' highest education, parental atopy, duration of breastfeeding, daily activity, presence of hairy or feathery pets at home and exposure to environmental tobacco smoke (ETS).

p-value for an interaction term between IL-4 promoter (CT vs. TT) and exposure to visible mold exposure (yes vs. no any exposure indicator)

Table 6. Independent and joint effects of IL-4 promoter and exposure to mold odor on the development of asthma.

Exposure category	**Size of the group**	**No of new asthma cases**	**Crude matched odds ratio (95% CI)**	**Adjusted* matched odds ratio (95% CI)**
TT, No exposure	175	46	1.00	1.00
CC+CT, No exposure	69	17	0.99 (0.51-1.93)	0.99 (0.45-2.22)
TT, Mold odor	149	61	1.97 (1.22-3.18)	1.46 (0.83-2.59)
CC+CT, Mold odor	51	22	2.41 (1.22-4.74)	1.99 (1.03-4.44)
Attributable proportion due to interaction (AP)				0.089
Rothman synergy index				1.30 (1.03-2.35)
P for interaction[#]				0.04

[*] Conditional logistic regression controlling for parents' highest education, parental atopy, duration of breastfeeding, daily activity, presence of hairy or feathery pets at home and exposure to environmental tobacco smoke (ETS).

[#] p-value for interaction term between IL-4 promoter (CT vs. TT) and exposure to mold odor (yes vs. no any exposure indicator)

Gene-Environment Interaction between Interleukin-4 Promoter and Molds in Childhood Asthma

Running Head: Interleukin-4 promoter and Molds and asthma

Bing-Fang Hwang, Ph.D., I-Ping Liu, M.Sc., Tzu-Pi Huang, Ph.D.

Bing-Fang Hwang, Ph.D.
Department of Occupational Safety and Heath
College of Public Health
China Medical University
Taichung, Taiwan

I-Ping Liu, M.Sc.
Department of Occupational Safety and Heath
College of Public Health
China Medical University
Taichung, Taiwan

[2]**Tzu-Pi Huang, Ph.D**
Department of Plant Pathology
National Chung Hsing University
Taichung, Taiwan
Tzu-Pi Huang

Correspondence to:
Dr. Bing-Fang Hwang,
Department of Occupational Safety and Health,
College of Public Health, China Medical University
No 91 Hsueh-Shih Rd, Taichung, Taiwan, 40402 R.O.C.
E-mail: bfhwang@mail.cmu.edu.tw
Telephone: +886-4-22053366 ext 6208
Telefax: +886-4-22071861

ABSTRACT

PURPOSE: To assess the role of gene-environment interaction between interleukin-4 (IL-4) promoter and mold exposure on the development of asthma.

METHODS: We conducted a cohort based, incident case-control study. The case group consisted of 188 children with new asthma and the control group (n=376) was matched for age and sex. The outcome of interest was the development of asthma during the 6-year study period. The studied determinants were three polymorphisms of IL-4 promoter (TT; CT and CC) and three indicators of exposure including histories of water damage, presence of visible molds, and perceived mold odor in the home.

RESULTS: Apparent joint effects between IL-4 promoter and mold exposure were observed on both additive and multiplicative scales. Specially, the risk of asthma was significantly associated with children carrying CT genotype and visible mold exposure comparing with those carrying TT genotype without any exposure indicator (adjusted OR 2.14, 95% CI 1.05-4.34) (modified Rothman synergy index for directly use of odds and odds ratios (s) =1.41; p for interaction=0.03). A similar tendency was found (s=1.30; p for interaction=0.04) for children who were exposed to mold odor and carried CT genotype (adjusted OR 1.99, 95% CI 1.03-4.41)

CONCLUSIONS: The results of this study suggest gene-environment interaction between IL-4 promoter and indoor mold problem may play an important role in childhood asthma.

Key words**: Interleukin-4 promoter, molds, damp housing, asthma, interaction, effect modification**

INTRODUCTION

Asthma is the most common chronic childhood disease (1). It is characterized by recurrent respiratory symptoms, reversible airway obstruction, airway inflammation and increase airway responsiveness (2). One of the most important pathways on development of asthma involves IgE antibody responses to ubiquitous allergens. Interleukin-4 (IL-4) plays an important role in IgE synthesis by activating the pre-T helper cells that trigger isotype switching from IgM/IgG to IgE in B cells by promoting Th2 cell development (3). It is reasonable to expect that increase expression of IL-4 might lead to the development of asthma and therefore the IL-4 promoter region (chromosome 5q31) seems to be the most likely site for polymorphisms. One of the IL-4 promoter polymorphisms -589C/T (also referred to rs number 2243250) is close to the glucocorticoid response element that positively stimulates IL-4 expression (4). Few studies of genetic epidemiology have assessed the relation between IL-4 promoter and childhood asthma in different populations, but these studies provided inconsistent results (5-8).

In our systematic Medline search, we identified only two previous longitudinal studies (9-10) and a population-based incident case-control study (11) which investigated exposure prior to the onset of asthma in children. All the three studies report that children exposure to dampness problems and molds increase the incidence of asthma (9-11). However, potential modification of the association between exposure to molds and the development of asthma by IL-4 promoter (i.e. gene-environment interaction) has not yet investigated.

We conducted a cohort based, incident case-control study to assess the independent and joint effect of IL-4 promoter and molds exposure on the risk of childhood asthma.

METHODS

Data Collection and Study Design

The source population included 44,000 children born in Taoyuan City between January 1, 1995 and December 31, 2002. Taoyuan is an urban-suburban municipality located across the north-western boarder of Taiwan. In the October 2002 baseline survey, a modified Chinese version of The International Study of Asthma and Allergies in Childhood (ISAAC-C) questionnaire was used to collect information on children's health, environmental exposures, and other relevant factors. The parents or other guardians were asked to provide information on children's personal characteristics, health, details of the environment, and other relevant factors (Table 1). We approached a random sample of 2,253 children aged 1-7 from the source population. The response rate was 85.3%. We excluded 331 children with asthma (n=150), without asthma but with at least one asthmatic symptoms (n=160), or incomplete questionnaire (n=21). The study population included 1,922 children free of asthma and non-asthmatic symptoms at the cohort entry (baseline). The study design has been described in detail previously (12).

In October 2008, we conducted a cohort-based incidence case-control study. We identified 188 new cases who developed asthma during the study period between October 15, 2002 and October 31, 2008. The definition of new asthma subjects was determined by a positive response to the question "Has a physician ever diagnosed your child as having asthma during 2002-2008?" at the follow-up survey. Five questions related to current asthmatic symptoms were also asked at both baseline and follow-up surveys:

1. In the past 12 months, has your child dyspnoea with wheezing in the chest? (wheezing)
2. In the past 12 months, has your child's sleep been disturbed because of wheezing? (night wheezing)
3. In the past 12 months, has whizzing ever been severe enough to limit your child's

speech to only one or two words at a time between breaths? (dyspnoea at rest)

4. In the past 12 months, has your child's sounded wheezy during or after exercise? (exercise wheeze)

5. In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection? (night cough)

Control subjects were selected without asthma or asthmatic symptoms applying one-to-two matching for age and sex from 2002 baseline survey of 1,922 children. The criteria of control subjects was (1) no physician diagnosed asthma or dyspnoea with wheezing in the past; (2) no positive response to any of the five questions concerning current asthmatic symptoms. The final study population constituted 188 case and 376 control subjects. The study protocol was approved by the Institutional Review Board of China Medical University, and it complied with the principles outlined in the Helsinki Declaration.

IL-4 promoter Genotyping

Cotton swabs containing oral mucosa were collected and were immediately maintained at −80°C throughout the transfer and storage. Genomic DNA will be isolated using phenol/chloroform extraction method.

The cotton swabs directly immersed in 300 μL cell lyses buffer (50 mm Tris-HCl, 1 mm EDTA, 0.1 m NaCl, pH 8.0) containing 2% SDS and 20 μg/mL proteinase K in a 1.5 mL micro centrifuge tube. After incubation overnight at 55°C, the swabs were discarded and the DNA in supernatants were purified by phenol/chloroform extraction and then precipitated with ethanol. DNA fragments including the -589C/T variant were amplified a 198 base pair (bp) long by allele-specific polymerase chain reaction (PCR). The forward primer sequence from -683 to -633 nucleotides is 5' TGG GTA AGG ACC TTA TGG ACC 3' whereas the reverse primer sequence from -486 to -505 is 5' GGT GGC ATC TTG GAA ACT GT 3'. The reverse primer

was designed to contain a base substitution C→T at the fourth last nucleotide from its 3'end. The individual PCR reaction vial contains a final volume of 20 µl solution. Two-hundred nanograms of DNA samples was added to 8 µl of PCR master mix consisting of 2.0 µl of 10X Mg Free PCR Buffer, 2.0 µl of 10 µM forward primer, 2.0 µl of 10 µM reverse primer and 2.0 ml of 10 mM dNTP. An appropriate amount of sterile ultrapure water (which totals up to 20 µl) was added to each of the microfuge tube. One micro liter of 5 units/µl *Taq* DNA polymerase was added to the reaction vial only after 5 minutes of pre-denaturation process prior to performing 'hot start' PCR. The PCR was performed using the Thermal Cycler (Applied Biosystems 9800®) for 35 cycles. The temperature for the initial denaturation of DNA was 94 °C for 1 minute, annealing at 60 °C for 1 minute and extension 72°C for 1 minute and a final extension at 72 °C for 5 minutes following the last cycle. The PCR product was subjected to *BsmF*I digestion for 2 hours at 37°C and electrophoresed on a 2.5% agarose gel with ethidium bromide staining. The IL-4 promoter -589 C/T polymorphisms was visualized as 198 bp fragments for TT genotype, 120 pb and 78 pb fragments for CC genotype and 198 bp, 120 bp, 78 pb fragments for CT genotype. All assays were performed by a laboratory worker unaware of the clinical status of individual subjects, and genotype assignments were based on two consistent experimental results. About 15% of randomly selected samples were directly sequenced, and all of them were concordant with the initial genotyping results.

Environmental Determinants of Interest

We used three indicators of exposure defined from the answers to following structured questions at baseline:

Mold odor. "Have you perceived mold odor in your dwelling during the past 12 months?" (No; Yes, almost daily; Yes, 1-3 days a week; Yes, 1-3 days a month)

Visible mold. "Have you ever had visible mold in your dwelling?"(No; Yes, during the past 12

months; Yes, only earlier.)

Water damage. "Have you ever had water damage in your dwelling?" (No; Yes, during the past 12 months; Yes, only earlier.)

Any exposure indicator. Presence of any of the three exposure indicators.

We decided to focus on exposures documented prior to the study period to ensure a plausible temporal sequence between exposure and the studied outcome for the causal inference.

Covariates

Information on potential confounders was obtained from the baseline questionnaire. The covariates in the present analyses included parental education, duration of breastfeeding, daily activity, furry/feathery pets and environmental tobacco smoke (ETS) (Table 1). The duration of breastfeeding was categorized into i) less than 1 months, ii) 1 to 5 months and iii) 6 months or longer. Daily activity was counted hours per day spent at activity levels corresponding to high (>4 hour/day), medium (2-4 hours/day) and low (<2 hours/day) as the reference category. Parents education was categorized into i) ≤ 9 years, ii) 10-12 years, iii) 13-16 years and iv) ≥ 17 years, and four indicators variables were formed with i) as a reference category. ETS exposures were defined as paternal smoke only, maternal smoke only, or both paternal and maternal smoke exposure (yes) and none (no). Other covariates, such as cockroaches noted monthly, furry/feathery pets were dichotomous. Parental atopy was defined as the father or mother of the index child ever having been diagnosed as having asthma, or allergic rhinitis, or atopic eczema.

Statistical Methods

First, we estimated the incidence rate of asthma during the 6-year study period according to polymorphisms of IL-4 promoter and indicators of exposure to dampness and molds. In the crude analysis, matched odds ratio of the relations between exposure and outcome relations were estimated. We estimated adjusted odds ratios applying conditional logistic regression analysis. The matched odds ratios were adjusted for the aforementioned covariates.

Second, we studied the additive joint effects of IL-4 promoter and the two most relevant exposure indicators, namely 'mold odor' and 'visible mold' on the risk of asthma. Because of small number of CC genotype, we compared the odds ratios (OR) of asthma in four exposure categories: 1) TT genotype and no exposure ($OR_{00,}$ reference category); 2) CT genotype and no exposure (OR_{10}); 3) TT genotype and exposure (OR_{01}); and 4) CT genotype and exposure (OR_{11}). Then their odds (O) in four exposure categories (O_{00}, O_{01}, O_{10} and O_{11}) were derived from the same conditional logistic regression model adjusting for the covariates. On an additive scale, the attributable proportion due to interaction calculated by substituting odds ratios for risk ratios (AP) was suggested by Kalilani and Atashili (13):

$$AP = \left(\frac{1 + O_{11}}{OR_{11}}\right) * \left(\frac{OR_{11}}{1 + O_{11}} - \frac{OR_{10}}{1 + O_{10}} - \frac{OR_{01}}{1 + O_{01}} - \frac{1}{1 + O_{00}}\right)$$

Finally, the Rothman synergy index calculated by substituting odds ratios for risk ratios and its 95% CI were used to assess the joint effect of the two factors (14). The synergy index (S) modified for direct use of odds and odds ratios was employed for more accurate assessment of additive interaction (13). The synergy index was calculated using the following formula:

$$S = \left(\frac{\left(\frac{OR_{11}\,(1 + O_{00})}{1 + O_{11}} - 1\right)}{\left(\frac{OR_{10}(1 + O_{00})}{1 + O_{10}} - 1\right) + \left(\frac{OR_{01}(1 + O_{00})}{1 + O_{01}} - 1\right)}\right)$$

An observed synergy index value that departs substantially from the expected additive null, i.e., synergy index not equal to 1, suggests an additive interaction effect. The IR values and their variance covariance matrix were then used to calculate values for synergy index and 95% CIs (15). SAS version 9.2 was used for all statistical analyses. Furthermore, we studied the multiplicative joint effect of IL-4 promoter and exposure by introducing interaction terms in the model. All tests assumed a two-sided alternative hypothesis and a 0.05 significance level.

RESULTS

Characteristics of Case and Control Subjects

Table 1 compares the demographic and environmental characteristics between the case and control subjects at baseline. The case subjects had lower duration of breastfeeding, more time spent outdoors and higher proportion of parental atopy compared with the control subjects, and were more commonly exposed to cockroaches (94.7 vs. 90.2%) and furry or feathery pets (30.3 vs. 27.1%) in the home.

Independent Effects of IL-4 Promoter and Exposure to Dampness and Mold problems

Hardy-Weinberg equilibrium tests showed non-significance ($p>0.05$) in both case and control groups. IL-4 promoter (CT vs. TT genotype) was not significantly associated with the risk of asthma with an adjusted odds ratio of 1.17 (95% CI 0.76-1.79) as shown in the Table 2.

There was a positive association between visible mold and mold odor (r=0.30). Water damage was not associated with visible mold, but positively associated with mold odor (r=0.22) (Table 3).Table 4 presents the matched odds ratios for asthma according to the three exposure indicators at baseline, as well as odds ratios contrasted to the reference category of no exposure. The risk of asthma was related to any indicator of exposure (adjusted OR 1.43, 95% CI 1.01-2.13), presence of mold odor (adjusted OR 1.61, 95% CI 1.02-2.68), and visible mold (adjusted OR 1.50, 95% CI 1.01-2.31).

Joint Effect of IL-4 Promoter and Visible Mold

Table 4 shows the incidence rates of asthma in four categories representing the reference, independent effects of IL-4 promoter and any exposure indicator, and their additive joint effect. Children carrying CT genotype without visible mold didn't have a significantly increased risk of asthma with an adjusted odds ratio (OR_{10}) of 0.97 (95% CI 0.45 – 2.07)(Table 5). The effect of visible mold exposure on children with TT genotype increased with an OR_{01} of 1.35 (95% CI 0.82-2.22). In children with both CT genotype and visible mold, the adjusted OR_{11} of asthma was 2.14 (95%CI 1.05-4.34), compared with children of the reference category. Thus the attributable proportion due to interaction of CT genotype and visible mold (AP) was 11.4%. Additionally, the Rothman synergy index (s) calculated by substituting odds ratios for risk ratios was 1.41 (95% CI 1.01-3.47) greater than 1. It suggests additive and multiplicative interactions (p for interaction =0.03) between IL-4 promoter and visible mold exposure (Table 5).

Joint Effect of IL-4 Promoter and Exposure to Mold Odor

One the basis of the table 5, the joint effect for CT genotype and exposure to mold odor (adjusted OR_{11} 1.99 95% CI 1.03-4.41), corresponded with the independent effects for CT genotype (adjusted OR_{01} 0.99 95% CI 0.45-2.22) and exposure to mold odor (adjusted OR_{10} 1.46 95% CI 0.83-2.59). Thus there was an apparent additive interaction (AP=8.9%) between IL-4 promoter and mold odor exposure (s=1.30 95% CI 1.03-2.35). In addition, a multiplicative interaction between IL-4 promoter and mold odor exposure (p for interaction =0.04) was also found (Table 6).

.

DISCUSSION

The results of present study, we found approximately 43%, 61%, and 50% increased the risk of development of asthma for children living in homes with any mold problem, mold odor, or visible mold respectively. Although the IL-4 promoter did not predict asthma, the results indicate that the joint effect of IL-4 promoter, representing genetic constitution, and exposure to visible mold and mold odor was stronger than expected on the basis of their independent effects in additive and multiplicative scales.

Validity of Results

A cohort-based incident case-control study offers an appropriate approach to assess the role of mold problems on the development of asthma. The prospective study design minimizes selection bias and information bias. A selection bias will be eliminated if the parents of children with asthma are more likely to change housing conditions after the first symptoms and signs of asthma compared with parents of healthy children. Information bias will not introduce if the parents of the symptomatic children report or recall similar exposure indicators differently from the parents of healthy children.

The exposure assessment was based parental reporting at baseline rather than objective measurements, which is a limitation of the present study. Objective measurements were not yet used in any of the epidemiologic studies conducted at the time of the data collection. Visual observation by a trained person would also have improved the exposure assessment (11, 16). However, our exposure information was collected before the onset of the asthma and therefore any bias due to awareness of the disease or exposure to molds was avoided. Another limitation was that it's not possible to address the changes in children's living environment during the 6-year period based on two surveys (baseline and follow-up).

We were able to take into account most of the known potential confounders related to individual characteristics, other environmental exposure in the condition logistic regression

analysis. However, dampness problems may also be related to other indoor environmental factors, such as dust mites. Dampness problems may also imply low ventilation rate and consequently increase the levels of indoor pollutants. We cannot rule out these indoor environmental factors will influence our results.

Synthesis with Previous Knowledge

We identified only three previous prospective cohort or incident case-control studies with incident asthma in children as the outcome of interest (9-11). Wickman and colleagues conducted a population-based birth cohort study of 4089 children in Stockholm, where they reported an increased risk of asthma among children in damp home environment during the first two years of life compared with unexposed with an adjusted odds ratio of 1.75 (95% CI 1.26-2.43). The exposure was defined as smell and visible signs of mold, water damage inside construction, and persistent windowpane condensation in dwellings with double-glazing (9). Jaakkola and colleagues conducted a 6-year cohort study of 1916 children in Finland using self-reported exposure indicated an association between the risk of asthma and mold odor with an adjusted incidence rate ratio of 2.44 (95% CI 1.07-5.60), but not visible mold, and water damage (10). Pekkane and colleagues conducted an incident case-control study in Finland and found the presence of visible mold and moisture damage in main living parts increase the risk of asthma (11). The present study strengths the evidence that home dampness due to any problem of mold, mold odor, or visible mold increase the risk of development of asthma in childhood.

An important rationale for presenting the interaction on the additive scale is that it fits with the sufficient-component concept of causality (17). It has been suggested that the additive scale is more appropriate to assess "biologic interaction" which is implied by terms such as synergism or antagonism (18). We further performed test interaction on multiplicative scale as used in most genetic epidemiology literature. Our study shows apparent joint effects between IL-4 promoter and molds exposure on both additive and multiplicative scales.

The specific causal agents of asthma related to indoor dampness and mold problems are not well understood, and several potential causes have been suggested including molds, bacteria, house dust mites, and enhanced emission of chemicals from surface materials. Our results suggest that mold odor, and visible mold are important indicators of relevant exposure. Several biological mechanisms by which indoor molds, particular concerning Penicillium, Aspergillus, Cladosporium and Alternaria could induce asthma have been suggested including immunoglobulin E (IgE) or immunoglobulin G (IgG)-mediated hypersensitivity reactions, toxic reactions caused by mycotoxins, and nonspecific inflammatory reactions caused by irrigative volatile organic compounds produced by microbes (MVOCs) or cell wall components, such as 1, 3-ß-D-glucan and ergosterol (20-26).

We found IL-4 promoter might not be a determinant of developing asthma in childhood, which was inconsistent with a recent meta-analysis of 14 studies, representing a pooled total of 2,476 asthmatic cases and 2,339 controls (8). The differences in direction of effects with IL-4 promoter genotypes may be due to chance, insufficient power, different populations or ethnic origins, variations of study design and different phenotypes studies. This effect and these results warrant further investigation in larger studies. The results showed that the joint effect of IL-4 promoter, representing genetic constitution, and exposure to visible mold or mold odor was stronger than expected on the basis of their independent effects in additive scale. An apparent gene-environment interaction between IL-4 promoter and exposure to molds was found. It is possible that IgE antibody responses to ubiquitous allergens, such as molds. IL-4 plays an important role in IgE synthesis by activating the pre-T helper cells that trigger isotype switching from IgM/IgG to IgE in B cells by promoting Th2 cell development. Children with both IL-4 promoter (CT genotype) and molds exposure increase expression of IL-4 and make them more likely to develop asthma.

Concluding Remarks

Our results are consistent with the hypothesis that molds play an important role of childhood asthma. The results also provide further evidence that IL-4 promoter may influence sensitivity to molds on the development of asthma.

Funding

This study was supported by the National Science Council (NSC# 99-2314-B-039-021-MY3) and China Medical University (CMU#99-S-25).

Acknowledgments

We thank all the field workers who supported data collection, the school administrators and teachers, and especially the parents and children who participated in this study.

Conflict of interest

We have no conflicts to disclose.

REFERENCES

1. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjuntivitis, and atopic eczema: ISSAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Lancet 1998; 351: 1225-32.
2. Global Initiative for Asthma, Updated from. NHLBI/WHO Workshop Report: Global Strategy for Asthma Management and Prevention Issued January 1995. NIH Publication No. 02-3659. 2002.
3. Holloway JW, Neghe B, Holgate ST. The genetic basis of atopic asthma. Clin Exp Allergy 1999; 29: 1023-32.
4. Rosenwasser LJ. Promoter polymorphism in the candidate genes, IL4, IL9, TGF-β1 for atopy and asthma. Int Arch Allergy Immunol 1999; 118: 268-270.
5. CuiT, Hu L, Pan S, et al. Association between interleukin-4 and interleukin-4 receptor gene polymorphism and atopic asthma in children. Chin J pathophysiol 2005; 21: 125-8.
6. Lee SG, Kim BS, Kim JH, et al. Gene-gene interaction between interleukin-4 and interleuin-4 receptor alpha in Korean children with asthma. Clin Exp Allergy 2004; 34: 1202-8.
7. Takabayashi A, Ihara K, Sasaki Y, et al. Children atopic asthma: positive association with a polymorphism of IL-4 receptor alpha gene but not with that of IL-4 promoter or Fc epsilon receptor I beta gene. Exp Clin Immunogenet 2000; 17: 63-70.
8. Li Y, Guo B, Zhang L, et al. Association between C-589T polymprophisms of interleukin-4 gene promoter and asthma: A meta-analysis. Respir Med 2008; 102: 984-992.
9. Wickman M, Melen E, Berglind N, et al. Strategies for preventing wheezing and asthma in small children. Allergy 2003; 58: 742-747.

10. Jaakkola JJ, Hwang BF, Jaakkola N. Home dampness and molds, parental atopy and asthma in children: a six year population-based cohort study. Envrion Health Perspect 2005; 113: 357-361.
11. Pekkanen J, Hyvarinen A, Haverinen-Shaughnessy U, Korppi M, Putus T, Nevalainen A. Moisture damage and childhood asthma: a population-based incident case-control study. Eur Respir J 2007; 209: 509-515.
12. Hwang BF, Liu I-Ping, Huang TP. Molds, Parental Atopy and Pediatric Incident Asthma. Indoor Air 2011 in press.
13. Kalilani L, Atashili J. Measuring additive interaction using odds ratios. Epidemiol Perspect Innov 2006; 3: 5.
14. Rothman KJ. The estimation of synergy or antagonism. Am J Epidemiol 1976; 103:506-11.
15. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992; 3:452-456.
16. Nafstad P, Øie L, Mehl R et al. Residential dampness problems and symptoms and signs of bronchial obstruction in young Norwegian children. Am J Respir Crit Care Med 1998; 157: 410-14.
17. Knol MJ, Egger M, Scott P, Greerlings MI, Vandenbroucke JP. When one depends on the other: reporting of interaction in case-control and cohort studies. Epidemiology 2009; 20: 161-166.
18. Kaufman JS. Interaction reaction. Epidemiology 2009; 20: 159-160.
19. VanderWeele TJ, Robins JM. The identification of synergism in the sufficient-component-cause framework. Epidemiology 2007; 18, 329-339.
20. Norbäck D, Björnsson E, Janson C, Palmgren U, Boman G. Current asthma and biochemical signs of inflammation in relation to building dampness in dwellings. Int J Tuberc Lung Dis 1999; 3: 368-76.

21. Husman T. Health effects of indoor-air microorganisms. Scand J Work Environ Health1996; 22: 5-13.

22. Thorn J, Rylander R. Airway's inflammation and glucan in a row house area. Am J Respir Crit Care Med 1989; 157: 1798-1803.

23. Johanning E, Landsbergis P, Gareis M, Yang CS, Olmsted E. Clinical experience and results of a sentinel health investigation related to indoor fungal exposure. Environ Health Perspect 1999; 107 (Suppl 3): 489-94.

24. Etzel RA. How environmental exposures influence the development and exacerbation of asthma. Pediatric 2003; 112: 233-239.

25. Mutius E, Martinez FD, Fritzsch C, Nicolai T, Roell G, Thiemann HH. Prevalence of asthma and atopy in two areas of West and East Germany. Am J Respir Crit Care Med 1994; 149: 358-364.

26. Laitinen T, Räsänen M, Kaprio J, Koskenvuo M, Laitinen LA. Importance of genetic factors in adolescent asthma: a population-based twin-family study. Am J Respir Crit Care Med 1998; 157: 1073-1078.

Table 1. Demographic and environmental characteristics of case and control subjects.

Categories	Case subjects n=188 (%)	Control subjects n=376 (%)	Matched odds ratio (95% CI)
Highest level of parental education (years)			
≤9	13 (6.9)	23 (6.2)	Reference
10-12	76 (40.4)	173 (46.4)	0.88 (0.43-1.80)
13-16	87 (46.3)	158 (42.4)	1.10 (0.54-2.25)
≥17	12 (6.4)	19 (5.1)	1.26 (0.47-3.37)
Daily activity (hours per day)			
<2	21 (11.2)	34 (9.0)	Reference
2-4	107 (56.9)	202 (53.7)	0.86 (0.47-1.55)
>4	60 (31.9)	140 (37.2)	0.69 (0.37-1.29)
Duration of Breastfeeding (months)			
< 1	147 (78.2)	264 (70.2)	Reference
1-5	29 (15.4)	81 (21.5)	0.66 (0.41-1.04)
≥ 6	12 (6.4)	31 (8.3)	0.69 (0.34-1.40)
Cockroaches noted monthly			
No	10(5.3)	37(9.8)	Reference
Yes	178(94.7)	339(90.2)	1.94(0.94-4.00)
Furry/feathery pets			
No	131(69.7)	274(72.9)	Reference
Yes	57(30.3)	102(27.1)	1.17(0.80-1.72)
Environmental Tobacco Smoke			
No	76(40.4)	152(40.4)	Reference
Yes	112(59.6)	224(59.6)	1.00 (0.70-1.43)
Parental atopy			
No	84(44.7)	268(71.3)	Reference
Yes	104(55.3)	108(28.7)	3.30 (2.24-4.90)

Table 2. Genotype of IL-4 promoter in the relation to asthma.

.

Genotype	**Size of the group**	**No of new asthma cases**	**Crude matched odds ratio (95% CI)**	**Adjusted* matched odds ratio (95% CI)**
TT	441	136	1.00	1.00
CT	140	51	1.16 (0.78-1.73)	1.17 (0.76-1.79)
CC	13	1	0.17 (0.02-1.31)	0.20 (0.03-1.66)

*Conditional logistic regression controlling for parents' highest education, parental atopy, duration of breastfeeding, daily activity, presence of hairy or feathery pets at home and exposure to environmental tobacco smoke (ETS).

Table 3. Correlations of visible mold, mold odor and water damage.

	Visible mold	Mold odor	Water damage
Visible mold	1.00	0.30*	0.07
Mold odor		1.00	0.22*
Water damage			1.00

* Spearman correlation coefficient is significant at the 0.05 level.

Table 4. Matched odds ratios of asthma calculated contrasting the reference category and adjusted for confounding in conditional logistic regression analysis.

Exposure at baseline	Size of the group	No of new asthma cases	Crude matched odds ratio (95% CI)	Adjusted*matched odds ratio (95% CI)
Total	564	188	-	-
No exposure (reference)	244	63	1.00	1.00
Any exposure indicator	320	125	1.78 (1.25-2.57)	1.43 (1.01-2.13)
Mold odor	200	83	2.20 (1.41-3.43)	1.61 (1.02-2.68)
Visible mold	137	54	1.82 (1.25-2.64)	1.50 (1.01-2.31)
Water damage	20	10	2.57 (0.65-10.1)	2.28 (0.40-13.1)

*Conditional logistic regression controlling for parents' highest education, parental atopy, duration of breastfeeding, daily activity, presence of hairy or feathery pets at home and exposure to environmental tobacco smoke (ETS).

Table 5. Independent and joint effects of IL-4 promoter and exposure visible mold on the development of asthma.

Exposure category	**Size of the group**	**No of new asthma cases**	**Crude matched odds ratio (95% CI)**	**Adjusted* matched odds ratio (95% CI)**
TT, No exposure	175	46	1.00	1.00
CT, No exposure	69	17	1.01 (0.55-2.21)	0.97 (0.45-2.07)
TT, Exposure	191	76	1.68 (1.10-2.56)	1.35 (0.82-2.22)
CT, Exposure	72	31	2.01 (1.16-3.51)	2.14 (1.05-4.34)
Attributable proportion due to interaction (AP)				0.114
Rothman synergy index				1.41 (1.01-3.47)
P for interaction #				0.03

* Conditional logistic regression controlling for parents' highest education, parental atopy, duration of breastfeeding, daily activity, presence of hairy or feathery pets at home and exposure to environmental tobacco smoke (ETS).

p-value for an interaction term between IL-4 promoter (CT vs. TT) and exposure to visible mold exposure (yes vs. no any exposure indicator)

Table 6. Independent and joint effects of IL-4 promoter and exposure to mold odor on the development of asthma.

Exposure category	**Size of the group**	**No of new asthma cases**	**Crude matched odds ratio (95% CI)**	**Adjusted* matched odds ratio (95% CI)**
TT, No exposure	175	46	1.00	1.00
CC+CT, No exposure	69	17	0.99 (0.51-1.93)	0.99 (0.45-2.22)
TT, Mold odor	149	61	1.97 (1.22-3.18)	1.46 (0.83-2.59)
CC+CT, Mold odor	51	22	2.41 (1.22-4.74)	1.99 (1.03-4.44)
Attributable proportion due to interaction (AP)				0.089
Rothman synergy index				1.30 (1.03-2.35)
P for interaction[#]				0.04

[*] Conditional logistic regression controlling for parents' highest education, parental atopy, duration of breastfeeding, daily activity, presence of hairy or feathery pets at home and exposure to environmental tobacco smoke (ETS).

[#] p-value for interaction term between IL-4 promoter (CT vs. TT) and exposure to mold odor (yes vs. no any exposure indicator)

Conflict of Interest and Authorship Conformation Form

Please check the following as appropriate:

X
- All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

X
- This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

X
- The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

- The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript:

Author's name	Affiliation
Bing-Fang Hwang	China Medical University
I-Ping Liu	China Medical University
Tzu-Pi Huang	National Chung Hsing University