

ORIGINAL ARTICLE

Lead exposure, IgE, and the risk of asthma in children

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Lead (Pb) has adverse effects on our nervous system and renal systems. Young children are more vulnerable to Pb exposure. However, the role of low-level Pb exposure in the immune system and allergic diseases in children is not well established. The aims of this study are to investigate the associations between Pb exposure and allergic diseases; between Pb and immunoglobulin E (IgE) as an intervening variable; and gender-based differences. We used multistage stratified random sampling to recruit kindergarten children nationwide in Taiwan. Information about allergic diseases and environmental exposures was collected by questionnaire. We compared children with and without allergic diseases for blood Pb levels measured by inductively coupled plasma mass spectrometry. The association between blood Pb and allergic diseases was assessed by logistic regression and those between Pb and IgE by generalized linear models. We also conducted mediation analysis to evaluate how much risk of allergic diseases related to Pb exposure is explained by IgE. A total of 930 children completed specimen collections. There was a positive association between Pb and asthma. Blood Pb were also positively linked with serum IgE ($\beta = 0.26$ kU/l per In-unit increase Pb concentration; 95% CI 0.009–0.50 kU/l), after adjusting for potential confounders. Analyses stratified by gender revealed that blood Pb correlated with IgE only in boys ($\beta = 0.40$ kU/l; 95% CI 0.03–0.76 kU/l). We estimated that 38% of the total effect of Pb exposure on asthma is mediated by IgE levels. In conclusion, Pb exposure is associated with both blood IgE and asthma in boys. Moreover, the effect of Pb exposure on asthma may be mediated by IgE levels.

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INTRODUCTION

There has been a notable increase in the prevalence of allergic diseases in children worldwide. Besides genetics, environmental factors may play a role in the onset of allergy. Several concepts have been proposed to account for this trend, especially those concerning the impact of environmental toxicants.¹ In the last decades, exposure to lead (Pb) has become more frequent in urban areas of industrialized as well as of developing countries. The prevalence of allergic diseases also has increased in these countries.²

Pb is a heavy metal used in a variety of consumer products, such as toys, paints, construction materials, batteries, and water pipes. Studies have linked higher Pb exposure or body burden to decreased intelligence, reduced fine motor skills, development and behavioral problems, headaches, stomachache, kidney damage, and anemia.³ Moreover, the immune system has been found to be one of the targets most sensitive to Pb toxicity.⁴ Young children are considered to be at greatest risk for health problems attributable to Pb poisoning—their smaller, growing bodies and playing on the floor make them more susceptible to absorbing Pb.⁴ A child's primary route of Pb exposure is oral ingestion of Pb-based paint, Pb-containing dust, or food and water that contains Pb.³ Since Pb is banned from gasoline, paint, and food cans, the levels of Pb in blood of US children have been declining.⁵ Nevertheless, concerns over the extent of old Pb-based water pipes providing water to Taiwanese residents for decades grew in 2015 when water authorities confirmed the existence of

Pb pipes in their jurisdiction.⁶ Moreover, samples of potable water in Hong Kong were found to contain excessive levels of Pb.⁷ The water crisis in Flint has also exposed the danger that Pb could leach into the drinking water of millions of Americans.⁸ Because effects of Pb toxicity may occur at concentration below those currently allowed in legislation, there is an urgent need for assessing the health effects of low-level Pb exposure.⁹

Pb is known to induce immunomodulatory effects resulting in lowered resistance to infectious microorganisms and altered levels of immunoglobulins in humans.¹⁰ However, there is a paucity of data on the role of low-level Pb exposure in allergic diseases such as asthma, allergic rhinitis (AR), and atopic dermatitis (AD) in humans. Moreover, little is known about any gender difference in these associations. Since young children are more vulnerable to toxic chemicals due to the immaturity of their organs and increased dosage per unit body surface area, this study was conducted to evaluate (1) the association between Pb exposure and allergic diseases in children; (2) the association between Pb and IgE as intervening variable explaining the occurrence of allergic diseases; and (3) gender-based differences in exposures and health outcomes.

MATERIALS AND METHODS

Study Population

Based on a stratified random sampling strategy, 930 kindergarten students were recruited from randomly selected townships or city districts

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throughout Taiwan in 2011 after their parents signed a statement of consent.¹¹ All 369 townships/metropolitan districts in Taiwan were primary sampling units. From these, 44 townships/metropolitan districts were randomly selected based on four specified strata of urbanization levels, that is, metropolitan district, local city, town, and village, with population size ratios of approximately 2:2:1:2 to represent the subtotal population composition among these four levels. A total of 106 kindergartens from the 44 districts were invited and 85 agreed to participate. After withdrawals due to scheduling conflicts or weather issues, 80 kindergartens took part in this study. Of those kindergartens, students and their parents were invited to have their children enrolled in the study. A total of 1161 preschoolers were recruited until the total of participants in each selected township/metropolitan district reached 40–80 students or until all kindergartens in the selected district were contacted. Written informed consent was obtained from all parents. The hospital's Institutional Review Board approved the study protocol, which complied with the principles of the Helsinki Declaration.

Questionnaire Survey

The International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire was used for collecting data on the children's allergic symptoms. Parents also answered a standardized questionnaire on baseline demographics, birth history, parental history of allergic diseases, parental ages, occupations, education levels, family income, nationality, home environment, use of Chinese herbal medicine, distance from residence to main traffic routes, industrial plants in the vicinity of their residences, incense burning at home, etc.¹¹

Allergic Diseases

At outpatient clinics in the regional hospitals, experienced pediatric allergists performed a standardized history and clinical examination of participants using parental-reported doctor-diagnosed AD, AR, and asthma based on the ISAAC questionnaire. Asthma was determined by pediatric allergists based on at least one of the following three criteria: (i) recurrence of at least two of the three symptoms: cough, wheeze, and shortness of breath within the previous 12 months without having a cold; (ii) doctor's diagnosis of asthma with ongoing treatment; (iii) response to treatment with β_2 -agonists or inhaled corticosteroids.¹²

Laboratory Methods

Pb analyses. A 5-ml blood sample was drawn from each study subject by a nurse visiting the study subject's class. Blood samples were collected in a tube containing heparin and stored at -20°C until further analysis. All samples were first pre-treated with a modifying solution of 5 g/l of 25% ammonia, 0.5 g/l Triton X-100, and 0.5 g/l EDTA in double-deionized water at a sample to solution volume ratio of 1:9.¹¹ After being filtered through 0.45 μm pore size filters, the filtrates were analyzed for Pb concentrations using inductively coupled plasma mass spectrometry (ICP-MS) (7500C; Agilent Technologies, Japan), as described elsewhere.¹³ Standard reference material for the blood Pb sample, 10.2 $\mu\text{g/dl}$ (Seronorm TM Trace Elements Whole Blood L-1; SERO, Norway), was used for quality assurance.

Total IgE analysis. Serum total IgE concentrations were determined using the Pharmacia Uni Cap IgE assay test system according to the manufacturer's protocol (Pharmacia Diagnostics, Uppsala, Sweden). IgE levels of 0.35 kU/l or greater are indicative of allergic sensitization. Concentrations below 0.35 kU/l were defined as absent or undetectable IgE.

Statistical Analysis

Associations between blood Pb and IgE concentrations were analyzed using linear regression. Variables with skewed distributions were log (Ln)-transformed before further analyses. The associations between blood Pb and allergic diseases were analyzed by univariate and multivariate logistic regression analyses. To characterize the associations between allergic diseases with regard to Pb, the Pb concentration was dichotomized as follows: $< 5 \mu\text{g/dl}$ as low Pb exposure (reference category that is defined as the "reference value" by the US Centers for Disease Control and Prevention) and $\geq 5 \mu\text{g/dl}$ as high Pb exposure.^{14,15} Potential confounders from a review of the literature were taken into consideration. Only those potential confounders with a 10% change in point estimate derived from the crude regression model were included in the final model. All

hypothesis testing was two-sided and statistical significance was set at $P < 0.05$. All analyses were performed using the SAS software version 9.1 (SAS Institute, Cary, NC, USA).

To evaluate how much risk of allergic diseases in relation to Pb exposure was explained by changes in IgE, we conducted a mediation analysis.¹⁶ The mediation analyses incorporated the different regression coefficients (exposure: Pb, intervening variable/mediator: IgE, and outcome: asthma). The mediation proportions, the percentage change of the regression coefficients when we included an intermediate variable in the model, were calculated (Supplementary Figure S1).

We also assessed the possible dose–response relationship between Pb exposure and both IgE and asthma by dividing blood Pb level into quintiles and tertiles as well as blood Pb < 5 , 5–10, and $> 10 \mu\text{g/dl}$.^{14,15,17}

RESULTS

A total of 930 children completed specimen collections. The mean age of the children was 5.74 years (SD 0.77). The mean age of their father and mother was 38.70 years (SD 5.57) and 35.08 years (SD 4.79), respectively. AD was found in 9.7% (91/930) of the children, AR in 35.2% (329/930), and asthma in 11.2% (105/930). The geometric mean (GSD) of the IgE and Pb concentrations were 86.93 (2.04) (kU/l) and 1.86 (1.21) ($\mu\text{g/dl}$) (Supplementary Table S1). Geometric means of Pb concentrations in relation to study subjects' baseline characteristics are shown in Table 1. Children from parents with low paternal or maternal education; from fathers or mothers working in agroforestry, fishing, or manufacturing; from mothers with foreign nationality, house wives, and low family income had higher blood Pb levels ($P < 0.05$). Boys had higher Pb concentrations than girls (geometric mean (GSD) boys vs girls: 1.89 (1.22) vs 1.83 (1.20) $\mu\text{g/dl}$).

Geometric means of Pb concentrations in relation to environmental factors of the study population are shown in Table 2. Study children with more number of siblings, dehumidifier at home, incensing at home, and residence that used groundwater tended to have higher blood Pb levels ($P < 0.05$) (Table 2 and Supplementary Table S2). New paintings on walls or on furniture within the last year tended to be associated with higher blood Pb concentrations ($P = 0.06$) (Table 2).

The Association of Blood Pb Concentrations and Serum Total IgE One ln-unit (or 2.72 $\mu\text{g/dl}$) increase of the blood Pb concentration was positively associated with 0.26 ln-unit (or 1.30 kU/l) of serum IgE (regression coefficient $\beta = 0.26$ kU/l; 95% CI 0.009–0.50 kU/l) (Table 3). Analyses stratified by gender further revealed that the statistically significant association between Pb and IgE concentration existed only in boys ($\beta = 0.40$ kU/l; 95% CI 0.03–0.76 kU/l).

The Association of Pb Levels and Allergic Diseases

Pb levels ($\geq 5 \mu\text{g/dl}$) were positively associated with asthma (adjusted OR: 5.50, 95% CI 1.69–17.94) (Table 4). Pb levels ($\geq 5 \mu\text{g/dl}$) were also positively associated with AD (adjusted OR: 2.60, 95% CI 0.71–9.49) and AR (adjusted OR: 1.39, 95% CI 0.48–4.04), though failed to reach statistical significance. Analyses stratified by gender further revealed a significantly positive association between Pb and asthma only in boys (adjusted OR: 6.40, 95% CI 1.49–27.42) (Table 4).

Mediation Analysis

To estimate whether IgE is an intervening variable between Pb and asthma, we conducted a mediation analysis. The mediation analyses incorporated the different regression coefficients (Pb and IgE; Supplementary Figure S1). In the present study, we estimated that 38% of the total effect of Pb concentration on asthma was mediated by IgE.

Table 1. Characteristic of the study population according to blood Pb levels ($N = 930$).

	Blood Pb levels ($\mu\text{g/dl}$)			
	n^a	%	GM (GSD)	P-value
Parents				
Paternal education				< 0.001*
High school and below	641	68.9	1.95 (1.21)	
College and above	237	25.5	1.57 (1.19)	
Missing	52	5.6	2.24 (1.19)	
Paternal occupation				< 0.001*
Agroforestry, fishing, manufacturing	325	34.9	2.07 (1.21)	
Wholesale and retail trade	181	19.5	1.80 (1.22)	
Service	370	39.8	1.68 (1.20)	
Others	3	0.3	1.92 (1.18)	
Missing	51	5.5	2.21 (1.22)	
Maternal education				< 0.001*
High school and below	639	68.7	1.92 (1.21)	
College and above	241	25.9	1.62 (1.20)	
Missing	50	5.4	2.35 (1.19)	
Maternal occupation				0.014*
Agroforestry, fishing, manufacturing	99	10.6	1.90 (1.23)	
Wholesale and retail trade	77	8.3	1.71 (1.18)	
Service	343	36.9	1.78 (1.20)	
Others	409	44.0	1.95 (1.22)	
Missing	2	0.2	2.28 (1.07)	
Maternal working status				0.019*
Non-house wives	519	55.8	1.79 (1.20)	
House wives	387	41.6	1.93 (1.22)	
Missing	24	2.6	2.20 (1.15)	
Maternal nationality				0.009*
Taiwan	791	85.0	1.83 (1.21)	
Foreign countries	139	15.0	2.00 (1.23)	
Family income per year (NT dollars)				< 0.001*
< 30,000	204	21.9	2.10 (1.23)	
30,000–50,000	235	25.3	1.92 (1.20)	
> 50,000	430	46.2	1.68 (1.20)	
Missing	61	6.6	2.21 (1.20)	
Child				
Gender				0.328
Male	469	50.4	1.89 (1.22)	
Girl	461	49.6	1.83 (1.20)	
Birth weight (< 2500 g)				0.498
Yes	58	6.2	1.88 (1.21)	
No	752	80.9	1.84 (1.21)	
Missing	120	12.9	1.98 (1.21)	
Premature birth (< 37weeks)				0.795
Yes	63	6.8	1.82 (1.22)	
No	774	83.2	1.84 (1.21)	
Missing	93	10.0	2.08 (1.20)	
Duration of breast feeding ≥ 6 months				0.240
Yes	244	26.2	1.89 (1.21)	
No	628	67.5	1.82 (1.21)	
Missing	58	6.3	2.17 (1.20)	
Chinese herbal medicine consumption (BabaoSan)				0.746
Yes	69	7.4	1.90 (1.23)	
No	861	92.6	1.86 (1.21)	

Abbreviations: GM, geometric mean; GSD, geometric mean standard deviation. * $P < 0.05$. ^a n is equal to total N because of the missing value.

Dose–Response Relationship between Blood Pb and Serum IgE Levels and Asthma

When we divided Pb levels into quintiles or tertiles, all results between blood Pb and serum IgE levels were statistically

insignificant, probably due to the relatively small sample size in each quintile or tertile (data not shown). We further divided blood Pb level into three categories according to their clinical significance (i.e., < 5 , $5\text{--}9.9$, $\geq 10 \mu\text{g/dl}$). Serum IgE levels (median and range) between the three groups (< 5 , $5\text{--}9.9$, $\geq 10 \mu\text{g/dl}$) were 104.80 (1–2500), 412.10 (5–2500), and 2500 (2500) kU/l in boys, which showed a statistically significant dose–response relationship ($P = 0.048$) (Supplementary Table S3). There was also a dose–response relationship between Pb levels and asthma ($P = 0.011$) (Supplementary Table S4).

DISCUSSION

This study contributes to the literature on the potential association between background Pb blood concentration and asthma in children. Pb concentration was significantly associated with total serum IgE, only in boys. Moreover, Pb exposure was associated with IgE and might increase the risk of asthma in children. To our knowledge, this is a novel study in which the associations between Pb and asthma were assessed by mediation analysis. These findings may contribute to a better understanding of the mechanism of asthma in Pb-exposed children.

Consistent with our study, inhaled Pb has been demonstrated to increase serum IgE and histamine levels in sensitized Guinea pigs.¹⁸ Heo *et al.*¹⁰ reported that adults with increased occupational exposure to Pb were significantly more likely to have increased IgE concentrations.¹⁰ A positive dose–response relationship between blood Pb levels and total IgE concentration was also noted in US adults.¹⁹ In a German Study of 334 children, the effect of Pb on total serum IgE was significant as what was reported in this study.²⁰ In addition to a positive association between blood Pb level and serum IgE concentration, we also found that Pb concentration was associated with the prevalence of asthma. A study of children residing in low-income households in USA showed that asthmatic children are over five times more likely to have elevated Pb concentration than non-asthmatics.²¹ A case–control study in Egypt further reported that blood Pb levels were correlated with asthma severity in children.²² Significant linear trends across quintiles were also found for the association between whole blood Pb with total IgE and asthma in a Korean study.²³

In contrast to our study, in an inner-city case–control study in the US ($n = 202$), there was no increased prevalence of asthma or its symptoms among young children with Pb poisoning.²⁴ In 2005–2006 NHANES, exposure to Pb was demonstrated to be associated with increased odds of sensitization to food allergens in adults but not in children.²⁵ Moreover, Well *et al.*²⁶ reported a lack of association between blood Pb concentration and asthma, atopic asthma, or general atopy in children, but suggested an underlying modulation in the immune system may induce allergic diseases. Variation in the upper range of Pb blood concentration, different geographic areas, lifestyles, dietary habits, genetic variations, exposure routes, duration, concentrations, laboratory methods, and rates of metabolism may account for the above-mentioned inconsistencies. Further increases in sample size to address the variations of Pb exposures, and exploration of the complex interrelationships between gender, race, genetic susceptibilities, and environmental exposures of Pb, are warranted to better understand the role of Pb in causing or precipitating asthma in children.

The underlying mechanism for the association between Pb exposures and increased asthma risk remains an open question. Given that susceptibility to allergic responses is directly associated with IgE production, exposure to Pb might be responsible for the increased risk in asthma.²⁷ In this study, we estimated that 38% of the total effect of Pb concentration on asthma is mediated by IgE concentration. Joseph *et al.*²⁸ reported that Pb exposure may be associated with excessive production of IgE, possibly increasing

Table 2. Environmental factors of the study population according to blood Pb levels (N = 930).

	Blood Pb levels (µg/dl)			
	n ^a	%	GM (GSD)	P-value
<i>Number of siblings</i>				< 0.001*
≥ 2	190	20.4	2.04 (1.21)	
< 2	720	77.4	1.81 (1.21)	
Missing	20	2.2	1.92 (1.18)	
<i>Furry pets at home</i>				0.192
Yes	210	22.6	1.93 (1.21)	
No	647	69.6	1.81 (1.21)	
Missing	73	7.8	2.15 (1.23)	
<i>Carpets at home</i>				0.424
Yes	57	6.1	1.88 (1.18)	
No	800	86.1	1.83 (1.21)	
Missing	73	7.8	2.21 (1.22)	
<i>Cockroaches at home</i>				0.250
Yes	807	86.8	1.85 (1.21)	
No	50	5.4	1.74 (1.18)	
Missing	73	7.8	2.10 (1.22)	
<i>Fungi on the house wall</i>				0.206
Yes	466	50.1	1.80 (1.21)	
No	375	40.3	1.88 (1.21)	
Missing	89	9.6	2.12 (1.22)	
<i>Dehumidifier at home</i>				< 0.001*
Yes	394	42.4	1.70 (1.20)	
No	460	49.5	2.00 (1.21)	
Missing	76	8.1	2.14 (1.23)	
<i>Pesticides use at home</i>				0.302
Yes	513	55.2	1.87 (1.21)	
No	323	34.7	1.79 (1.22)	
Missing	94	10.1	2.08 (1.22)	
<i>Incensing at home</i>				< 0.001*
Yes	562	60.4	1.92 (1.21)	
No	295	31.7	1.70 (1.20)	
Missing	73	7.9	2.10 (1.22)	
<i>Environmental tobacco smoke at home</i>				0.915
Yes	513	55.2	1.85 (1.22)	
No	263	28.3	1.82 (1.19)	
Missing	154	16.5	1.94 (1.22)	
<i>New paintings over walls or furniture within one year</i>				0.060
Yes	382	41.1	1.81 (1.21)	
No	548	58.9	1.90 (1.21)	
<i>Pesticide factory close to the place of residence</i>				0.245
Yes	6	0.6	1.92 (1.60)	
No	708	76.1	1.84 (1.21)	
Missing	217	23.3	1.89 (1.19)	
<i>Iron and copper factory close to the place of residence</i>				0.254
Yes	61	6.6	1.99 (1.27)	
No	665	71.5	1.83 (1.21)	
Missing	204	21.9	1.91 (1.19)	
<i>Chemical plant close to the place of residence</i>				0.542
Yes	35	3.8	1.96 (1.26)	
No	683	73.4	1.84 (1.21)	
Missing	212	22.8	1.88 (1.20)	
<i>Incinerator close to the place of residence</i>				0.657
Yes	95	10.2	1.85 (1.20)	
No	642	69.0	1.84 (1.22)	
Missing	193	20.8	1.90 (1.20)	

Table 2. (Continued)

	Blood Pb levels (µg/dl)			
	n ^a	%	GM (GSD)	P-value
<i>Distance from the residence to the main traffic routes</i>				0.241
< 1 km	80	8.6	1.88 (1.21)	
> 1 km	711	76.5	1.80 (1.21)	
Missing	139	14.9	2.16 (1.21)	
<i>Residence with old lead water pipes</i>				0.173
Yes	735	79.0	1.84 (1.22)	
No	195	21.0	1.92 (1.19)	
<i>Residence with ground water use</i>				< 0.001*
Yes	164	17.6	2.09 (1.25)	
No	766	82.4	1.81 (1.20)	

Abbreviations: GM, geometric mean; GSD, geometric mean standard deviation. * $P < 0.05$. ^a n is equal to total N because of the missing value.

Table 3. Regression coefficients β of blood Pb levels (µg/dl) for serum total IgE levels (kU/l), stratified by gender.

	Ln-Pb	P-value
<i>Total (n = 930), Ln-IgE</i>		
Crude β (95% CI)	0.27 (0.03 to 0.51)	0.028*
Adjusted β (95% CI) ^a	0.26 (0.009 to 0.50)	0.042*
<i>Girls (n = 461), Ln-IgE</i>		
Crude β (95% CI)	0.12 (-0.22 to 0.46)	0.499
Adjusted β (95% CI) ^a	0.02 (-0.35 to 0.40)	0.901
<i>Boys (n = 469), Ln-IgE</i>		
Crude β (95% CI)	0.37 (0.04 to 0.70)	0.029*
Adjusted β (95% CI) ^a	0.40 (0.03 to 0.76)	0.036*

$P < 0.05$. ^aAdjusted for age, maternal age, maternal history of atopy, maternal nationality, parental education and occupation, family income, number of siblings, breast feeding, environmental tobacco smoke, incensing at home, dehumidifier at home, new paintings of houses and residence using groundwater.

the asthma risk among children 1–3 years of age screened for Pb. An elevation of blood Pb level was also reported to be related to the increase of bronchial responsiveness following elevated IgE.⁴ It has been proposed that Pb exposure results in type I hypersensitivity mediated by IgE.¹ Pb may modulate the immune reaction by activation of type-2 helper T cells and inhibition of type-1 T-cell activation.²⁰ This effect is dependent on the enhanced production of cytokines and interleukins (IL), thereby promoting isotype switching to IgE.²⁷ Exposure to Pb seems to be associated with atopic sensitization and modulation of several cytokines (e.g., IL-12, IL-10, interferon (IFN)- γ , and IL-4 production) and with T-cell dysregulation.²⁵ Pb exposure during allergic sensitization may induce increased tracheal responsiveness, eosinophil counts, and IL-4 concentration. Pb exposure has also been suggested to result in a decrease in lymphocyte count, IFN γ concentration, and IFN γ /IL-4 ratio.²⁹ Aside from the mechanism of IgE, DNA methylation represents a promising new frontier, and likely one of several explanatory factors for the effect of Pb on asthma.³⁰ Our study implies that Pb exposures may increase IgE levels and may thereby confer a higher susceptibility for asthma. Whether other pathways also play a role in the association between Pb exposures and asthma require further investigations.

Table 4. The association of asthma and blood Pb levels stratified by gender.

	Blood Pb (µg/dl)		P-value
	< 5 (N = 916)	≥ 5 (N = 14)	
Total (n = 930)			
Asthma crude OR (95% CI)	1.00	4.59 (1.51 to 13.95)	0.007*
Asthma-adjusted OR (95% CI) ^a	1.00	5.50 (1.69 to 17.94)	0.005*
Girls (n = 461)			
Asthma crude OR (95% CI)	1.00	2.67 (0.29 to 24.51)	0.384
Asthma-adjusted OR (95% CI) ^a	1.00	4.73 (0.44 to 50.60)	0.199
Boys (n = 469)			
Asthma crude OR (95% CI)	1.00	5.33(1.39 to 20.42)	0.015*
Asthma-adjusted OR (95% CI) ^a	1.00	6.40(1.49 to 27.42)	0.012*

* $P < 0.05$. ^aAdjusted for age, maternal age, maternal history of atopy, maternal nationality, parental education and occupation, family income, number of siblings, breast feeding, environmental tobacco smoke, incensing at home, dehumidifier at home, new paintings of houses and residence using groundwater.

Analyses stratified by gender in this study revealed that Pb levels positively correlated with serum IgE, only in boys. Prior studies on gender-related influence on Pb exposure and atopy are limited. In contrast to our study, Sun *et al.*³¹ demonstrated that the effect of Pb on IgG, IgM, and IgE was stronger in women than in men. However, as in our study, in a German study boys also had higher Pb concentrations than girls.²⁰ In addition, men tended to have higher Pb levels than women in all age groups.^{32,33} IgE levels and asthma prevalence were higher in boys than in girls before puberty, with an opposite association thereafter. It is possible that Pb acts as an endocrine disruptor of adrenal and ovarian steroidogenesis. However, whether Pb acts as a biological estrogen disruptor and thus is related to asthma remains to be elucidated.

Compared with children aged 6–11 years in the United States (NHANES 2005–2006) (geometric mean, 1.13 $\mu\text{g/dl}$) and children aged 3–5 years in Canada (CHMS 2009–2011) (geometric mean, 0.93 $\mu\text{g/dl}$), children in the present study from Taiwan had higher Pb levels (1.86 $\mu\text{g/dl}$).^{26,32} More than 10 years have passed since the complete cessation of leaded gasoline use in Taiwan in February 2000. However, there are other sources of Pb exposures. The most common Pb exposure is incense burning or Chinese herbal medicine, which has been sporadically reported to be associated with Pb-poisoning in children.³⁴ However, residence in area with higher Pb in ground water showed the strongest difference in the geometric mean (Table 2) in our study. Old lead water pipes also need to be assessed as exposures, since it is of great concern in terms of future Pb exposure prevention.^{6–8}

Several potential limitations of the study deserve to be discussed. First, the major limitation concerns the fact that Pb was measured in blood spot samples. Blood Pb concentrations may not be an accurate reflection of the lifetime cumulative toxicant burden compared to bones. However, it is an adequate biomarker of recent exposure and the half-life of Pb in human blood has been estimated to be from 28 to 36 days.^{35,36} The use of blood biomarkers offered a more precise evaluation of individual exposure compared with an integrated estimation based of ingestion, inhalation, and dermal absorption. Moreover, the measurement of Pb by ICP-MS with high validity is a strength of this study. Second, the cross-sectional design of this study does not allow us to distinguish the temporal sequence between exposures and effects. However, it allows us to evaluate regional effects and difference. Third, we cannot completely exclude the possibility of residual confounding due to unmeasured confounders in this study. However, we have tried our best to control for as many confounders as we can. Potential confounders from a

review of the literature, such as age, maternal age, maternal history of atopy, maternal nationality, parental education and occupation, family income, number of siblings, breast feeding, environmental tobacco smoke, incensing at home, dehumidifier at home, new paintings of houses, distance from the residence to the main traffic routes, and residence using groundwater, were all taken into consideration. Therefore, the magnitude of such confounding effects is likely to be limited.

Fourth, due to relatively small sample size in children with a blood Pb level of 5–9.9 $\mu\text{g/dl}$ and ≥ 10 $\mu\text{g/dl}$, the potential dose–response relationship between blood Pb level and serum IgE and asthma should be validated in future larger studies. Moreover, the statistically significant association between blood Pb level and asthma and serum IgE should be interpreted with great caution.

Finally, the finding that subjects with missing value in Tables 1 and 2 tended to have a higher blood Pb level may raise the concern of the presence of differential information bias. However, subjects with missing value in Tables 1 and 2 did not always had a higher blood Pb level. Moreover, when we compared the baseline characteristics between those with and those without missing value, we did not find any statistically significant differences between the two groups. Therefore, the above-mentioned finding may simply be a chance phenomenon.

One of the strengths of this study is the inclusion of a nationwide and sociodemographically diverse population of children. We believe that this is helpful in leading to unbiased assessments of the associations between blood Pb level and total serum IgE concentration and asthma. Because of the large sample size, we could adjust for a variety of confounders (e.g., family income, occupation, incensing at home, residence) in statistical analyses. In addition, the diagnosis of asthma was confirmed by physicians via standardized diagnostic criteria in addition to maternal self-report information from ISAAC questionnaires.

CONCLUSION

In conclusion, the present study provides evidence of a positive relationship between blood Pb and total serum IgE concentrations only in boys. Moreover, Pb may be related to the IgE production and may thus constitute an important step in asthma. Although allowable concentrations of Pb are now regulated, the health risk posed by a low level of Pb exposure is still of concern and suggests a continuing need for their control through environmental management. Preventive measures by reducing potential sources of Pb, such as eliminating Pb from incense and decreasing

ground water levels of Pb, should be introduced as early as possible to prevent health hazards in young children.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conception and design, data analysis and interpretation, and manuscript writing: IJW. Interpretation and revisions of the manuscript: WJJK and CCY. All authors read and approved the final manuscript.

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Supplementary Information accompanies the paper on the Journal of Exposure Science and Environmental Epidemiology website (<http://www.nature.com/jes>)

Supplemental Material

Table S1. The distribution of Pb and IgE levels

	Minimum	25th	Medium	75th	Maximum	GM (GSD)
Pb (µg/dL)	0.42	1.40	1.84	2.43	15.50	1.86(1.21)
IgE (KU/L)	0.20	27.45	82.45	299.85	2500.00	86.93(2.04)

GM, Geometric mean; GSD, geometric standard deviation.

Table S2. The distribution of Pb levels in different residence area

	Blood Pb levels (µg/dL)	P-value
	GM(GSD)	
Changhua County #	2.54(1.10)	<0.001
Yunlin County #	2.20(1.17)	
Pingtung County #	2.40(1.18)	
Penghu County #	2.45(1.19)	
Chiayi County#	1.80(1.31)	
Taipei City *	1.49(1.15)	
Taipei County *	1.83(1.16)	
Hsinchu City *	1.32(1.17)	
Miaoli County *	2.02(1.11)	
Yilan County *	1.78(1.17)	
Hualien County *	1.66(1.14)	
Taoyuan County	1.28(1.21)	
Taichung City	1.78(1.23)	
Taichung County	2.29(1.16)	
Nantou County	2.46(1.23)	
Tainan City	1.76(1.22)	
Tainan County	2.10(1.17)	
Kaohsiung City	1.84(1.16)	
Kaohsiung County	2.22(1.16)	
Taitung County *	2.48(1.16)	
Kinmen County *	2.79(1.14)	
Lienchiang County *	2.51(1.22)	

* Residence with old lead water pipes

Residence with ground water

P-value was calculated by npar1way

Reference from

(1) Lead water pipeline

area <http://www.appledaily.com.tw/realtimenews/article/life/20151019/714183/>
(accessed Jul 2016)

(2) Use of groundwater resources and water quality status in Taiwan

http://www.cv.nctu.edu.tw/chinese/teacher/Ppt-pdf/teacher13_shan/gwater_doc.pdf
(accessed Jul 2016)

Table S3. The association between blood lead levels and serum total IgE concentrations, stratified by gender

	Blood lead levels (µg/dL)			
	<5	5-9.9	≥ 10	
Total (n=930)	n = 916	n = 12	n = 2	p=0.124
IgE (KU/l)				
median (range)	80.40(0 <u>to</u> -2500)	270.10(5- <u>to</u> 2500)	1257.4(15 <u>to</u> -2500)	
Girls (n = 461)	n = 456	n = 4	n = 1	p=0.325
IgE (KU/l)				
median (range)	64.65(0 <u>to</u> -2500)	64.30(15- <u>to</u> 862)	14.8(15- <u>to</u> 15)	
Boys (n = 469)	n = 460	n = 8	n = 1	p=0.048*
IgE (KU/l)				
median (range)	104.80(1 <u>to</u> -2500)	412.10(5- <u>to</u> 2500)	2500(2500- <u>to</u> 2500)	

* p< 0.05

Table S4. The association between asthma and blood lead levels, stratified by gender

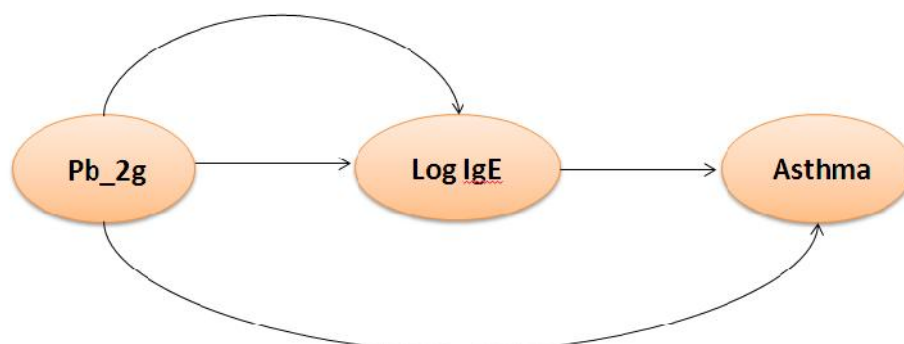
	Blood lead levels (µg/dL)			Trend test
	Y5 (N=916)	5-9.9 (N=12)	≥ 10 (N=2)	
Asthma				0.011*
Crude OR (95% CI)	1.00	4.13(1.22- <u>to</u> 13.95)*	8.25(0.51- <u>to</u> 132.975)	
Adjusted OR (95% CI) [#]	1.00	5.11(1.40- <u>to</u> 18.65)*	7.96(0.48- <u>to</u> 131.73)	
Girls (n = 461)				-
Crude OR (95% CI)	1.00	3.56(0.36- <u>to</u> 35.08)	-	
Adjusted OR (95% CI) [#]	1.00	5.10(0.44- <u>to</u> 59.68)	-	
Boys (n = 469)				-
Crude OR (95% CI)	1.00	4.00(0.93- <u>to</u> 17.17)	-	
Adjusted OR (95% CI) [#]	1.00	5.05(1.07- <u>to</u> 23.78)*	-	

Abbreviations: SD, standard deviation; OR, odds ratio; CI, confidence interval

* p< 0.05

[#]Adjusted for age, gender, maternal age, maternal history of atopy, maternal education and occupation, family income, breast feeding, environmental tobacco smoke, incensing at home at home, new paintings of houses, and factory near house

Figure S1. Steps of mediation causal analysis showing the effect of Pb exposure on asthma was mediated by IgE.



Step 1 Show that the independent variable (X) is correlated with the mediator (M) (Model 1)

Step 2 Show that the independent variable (X) is correlated with the outcome (Y) (Model 2)

Step 3 Show that the mediator affects the outcome variable (Model 3)

Variables in our article are as follows:

Y: asthma (1 = No; 2=Yes)

X: Pb_2g (Pb_2g)

M: log IgE

@ Pb_2g : **Pb** levels were divided into 2 categories by <5 ng/ml standing for low Pb exposure and ≥ 5 ng/ml standing for high Pb exposure

Step 1 Mediator Model (Pb_2g $\rightarrow$ logige)

Call:

```
lm(formula = logige ~ lead2g, data = Lead)
```

Residuals:

Min	1Q	Median	3Q	Max
-2.6257	-0.5003	-0.0319	0.5281	1.4712

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	1.92677	0.02443	78.871	<2e-16 ***
lead2g	0.40169	0.20693	1.941	0.0526 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.7118 on 859 degrees of freedom

(4 observations deleted due to missingness)

Multiple R-squared: 0.004368, Adjusted R-squared: 0.003208

F-statistic: 3.768 on 1 and 859 DF, p-value: 0.05256

Step 2 Outcome Model (Pb_2g → Asthma)

```
Call:
glm(formula = ac7 ~ lead2g + logige, family = binomial, data = Lead)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-0.7322 -0.5050 -0.4453 -0.3951  2.3908

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -3.0581     0.3572  -8.561 < 2e-16 ***
lead2g         0.3592     0.7930   0.453  0.65054
logige        0.4471     0.1613   2.772  0.00557 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for binomial family taken to be 1)

    Null deviance: 576.74  on 860  degrees of freedom
Residual deviance: 568.41  on 858  degrees of freedom
(4 observations deleted due to missingness)
AIC: 574.41

Number of Fisher Scoring iterations: 5
```

Step 3 Mediation Analysis

```
Causal Mediation Analysis

Nonparametric Bootstrap Confidence Intervals with the Percentile Method

      Estimate 95% CI Lower 95% CI Upper p-value
ACME (control)    0.01767   -0.00520    0.05709   0.13
ACME (treated)    0.02289   -0.00909    0.06770   0.13
ADE (control)     0.03786   -0.11161    0.29902   0.75
ADE (treated)     0.04308   -0.14315    0.31111   0.75
Total Effect      0.06075   -0.11161    0.32492   0.58
Prop. Mediated (control) 0.29091   -2.64686    2.57262   0.69
Prop. Mediated (treated) 0.37675   -1.91978    2.26729   0.69
ACME (average)    0.02028   -0.00760    0.05852   0.13
ADE (average)     0.04047   -0.12806    0.30465   0.75
Prop. Mediated (average) 0.33383   -2.21900    2.42094   0.69

Sample Size Used: 861

Simulations: 1000
```

The direct mediation effect is 62%.

38% risk of asthma in relation to Pb_2g is mediated by changes in log IgE.

Hence, other pathways are likely to contribute to the mediation as well.

If P_M is to be interpreted as a proportion, then log IgE mediates 38% of the total effect of Pb_2g on asthma.