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Research Paper

Association of Air Pollution Exposure and Interleukin-13 Haplotype with the Risk of Aggregate Bronchitic Symptoms in Children

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ABSTRACT

Interleukin-13(IL-13) might play an important role in driving aggregate bronchitic symptoms pathogenesis. However, none of the studies assessed the interaction between air pollutants exposure and IL-13 gene on the risk of aggregate bronchitic symptoms in non-asthma children. To assess the independent and joint effects of the exposure to air pollution and IL-13 haplotypes on the risk of aggregate bronchitic symptoms, we conducted a cross-sectional study and focused on non-asthma children. The study population consisted of 2944 children. The effect of each air pollutant on the risk of aggregate bronchitic symptoms was estimated as odds ratios per interquartile range (IQR) change. In the multiple logistic regressions, adjusted for confounding factors, the risk of chronic phlegm was associated with $PM_{2.5}$ exposure (aOR, 1.59; 95% CI, 1.07–2.37 per 12.51 μg/m3 change), O_3 exposure (aOR, 1.54 95% CI, 1.05–2.27 per 8.28 ppb change) and SO_2 exposure (aOR, 1.19; 95% CI, 1.02–1.39 per 0.98 ppb change). Our study further provides the evidence that gene-environment interactions between IL-13 haplotype and O_3 exposure on chronic phlegm (95% CI for interaction, 1.01–1.38). Identifying children who are more sensitive to air pollution helps us to provide them an efficient prevention to avoid aggregate bronchitic symptoms.

1. Introduction

Aggregate bronchitic symptoms are common respiratory health problems in children, which influence the quality of life and lays socioeconomic burdens (Carroll et al., 2008). Genetic and environmental factors have been recognized associated with this complex disease (Hallberg et al., 2008; Önal and Abbasoglu, 2011). Previous studies suggested that genetic variants are associated with the prevalence of aggregate bronchitic symptoms when the exposure to air pollution triggers an immune response (Lee et al., 2009; Ghosh et al., 2016).

Interleukin-13(IL-13) is a T-helper type 2 (Th2) cytokine that plays a critical role in the regulation of inflammatory immune response. A vivo study has provided the evidence that IL-13 is necessary for the induction of airway hyper -responsiveness (AHR) (Walter et al., 2001). Although the pathogenesis of aggregate bronchitic symptoms remains unclear, the epidemiological studies revealed that IL-13 single-nucleotide polymorphisms (SNPs) were associated with asthma and COPD susceptibility (Beghé et al., 2010; Chen et al., 2013).

Air pollution may be an environmental risk factor of aggregate bronchitic symptoms. Several studies have examined the relationship between air pollution and the prevalence of bronchitis, chronic phlegm and chronic cough in children, but only five studies focused on children with asthma and no remarkably consistent results were provided (Lee et al., 2009; McConnell et al., 1999, 2003; Hwang and Lee, 2010; Berhane et al., 2016). Moreover, assessments of air pollution exposure only relied on air monitoring stations and needs further elaboration.

In the present study, to improve the exposure assessment, home and school addresses were used to calculate the air pollution exposure and children activities are also taken into account. To our knowledge, no epidemiological studies investigated the joint effect of air pollution and IL-13 on the risk of aggregate bronchitic symptoms. Therefore, we conducted a cross-sectional study to investigate the independent effects of air pollution and IL-13 haplotypes on the prevalence of aggregate bronchitic symptoms in children. We further explored the interaction between the exposure to air pollutants and IL-13 to clarify the possible effect modification in the relation between air pollution and aggregate bronchitic symptoms.

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2. Methods

2.1. Study Population

Taiwan Children Health Study (TCHS) focused on children health and outdoor air pollutants, and the details have been described in the previous study (Hwang and Lee, 2010; Tsai et al., 2013). 3837 middle-school children were recruited from public schools in 14 communities and their buccal mucosa DNA were obtained. First, we excluded 429 (11%) children whose home address was missing or incomplete. Then, we excluded 204 (5%) due to incomplete four SNPs of IL-13. Therefore, there were a total of 3204 children with both information of genotyping and air pollution. Since we wanted to clarify the mechanism of air pollution exposure on the risk of aggregate bronchitic symptoms and the sample size of asthmatic children was too small (n = 260), therefore we focused on non-asthma children. Finally, the study population consisted of 2944 children. The study was approved by the Institutional Review Board in National Taiwan University Hospital and complied with the principles outlined in the Declaration of Helsinki. We have informed all the participating children/parents and obtained their written consent forms in this study.

2.2. Health Outcomes

A history of asthma was determined based on the question: "Has your child ever had asthma?" However, our study focused on children without asthma. Aggregate bronchitic symptoms were defined as children ever diagnosed as any of the following symptoms or diseases. Chronic cough was defined as cough in the morning or during other time and persisted at least 3 months in row during the past year. Chronic phlegm was defined as a positive answer to the question "In addition to other colds, does your child usually suffer phlegm stuck in the chest or bring up phlegm? Diagnosed bronchitis was defined based on the question "Has your child ever diagnosed with bronchitis in the past year?" and without any symptoms as the listed above.

2.3. Exposure Assessment

Ambient air pollutants data was obtained from 70 Environmental Protection Administration (EPA) air-monitoring stations in Taiwan. The concentrations of air pollutants, including SO_2 (ppb), NO_2 (ppb), O_3 (ppb), CO (ppm), $PM_{2.5}$ (particulate matter <2.5 μm, μg/m^3), were measured continuously and recorded hourly by all stations. The daily average concentration of air pollutants (24-hourly for SO_2, NO_2, CO, $PM_{2.5}$; 8-hourly (10:00–18:00) for O_3) were calculated for subsequent analyses, using the effective data with at least 75% of hourly valid values for the days (EPA, 1999, 2013). If the daily average data of air pollutants from an air-monitoring station was not available, we filled the missing values by using the average value from other monitoring stations of the same type in the same day, the method suggested by Jung et al. (2017).

We performed a geographic information system (ArcGIS version 10; ESRI, Redlands, CA, USA) to identify the locations of the air-monitoring stations and deal with the data of air pollutants. To estimate the daily average concentration of each air pollutant, we applied the inverse distance weighting (IDW) method, that is, the nearest 3 air-monitoring stations within 50 km of each grid cell was used. The spatial resolution is 100 m × 100 m (Stroh et al., 2007). The daily air pollutants data was allocated to individuals corresponding to children's home and school addresses (Fig. 1).

In view of the fact that indoor and outdoor air pollution concentrations were not consistent, we assumed that Taiwan's indoor to outdoor ratio (I/O ratio) was similar. According to the Kirchner's study, I/O ratio was used to convert outdoor air pollution concentrations into indoor ones (Kirchner et al., 2002). Since I/O ratio was different in winter and summer, the days were divided into winter (October–March) and summer (April–September) (Cheng et al., 2016).Then, we used I/O ratio to convert the outdoor concentrations on the basis of children's home addresses into indoor concentrations (Kirchner et al., 2002).

Eventually, the concentrations of air pollutants were calculated as the cumulative mean daily concentration in prior three years taking children's living activities into consideration (Supplement Table 1). Children spent most of their time staying at home except some activities at school. We applied a guideline "the average number of days in the school year is 200" stipulated by The Ministry of Education in Taiwan and average number of hours in the school day for Taiwanese students to define the living activity (TMOE, 2003).

2.4. Genotyping

The genomic DNA experiment was conducted in the National Taiwan University (Tsai et al., 2013). Real-time polymerase chain reaction (RT-PCR) was performed using an ABI 7900HT Sequence Detection System. The genotype was determined by TaqMan Assays. RT-PCR cycling conditions were as follows: initial denaturation for 5 min at 95 °C, 40 cycles at 95 °C for 30 s, and a final extension at 60 °C for 30 s. The results

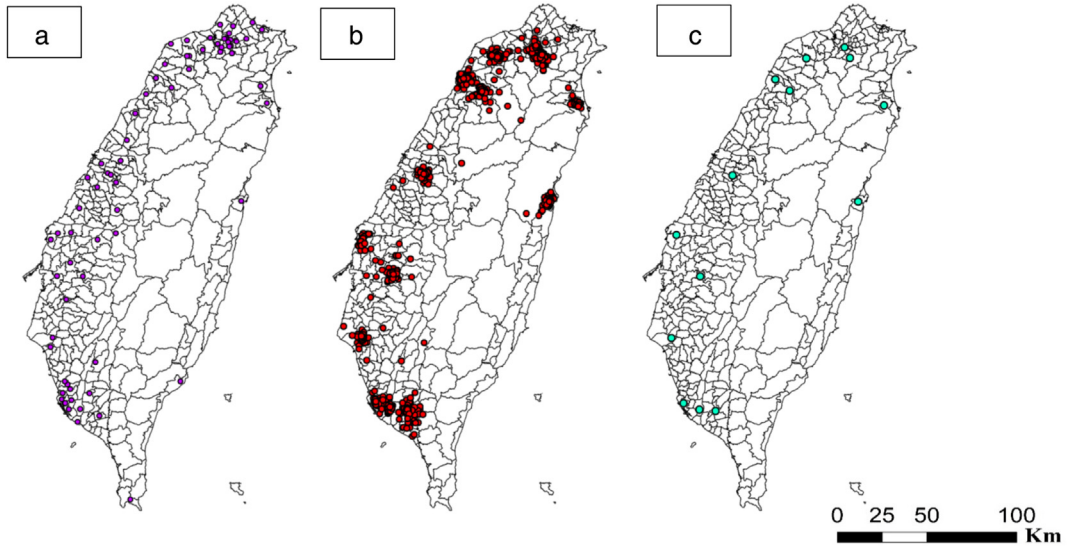

Fig. 1. Distribution of air-monitoring stations (a), children's home (b) and school (c) address.

Table 1
Distribution of demographic and other characteristics among children without asthma.

Characteristics	Diagnosed bronchitis (n = 141)			Chronic phlegm (n = 99)			Chronic cough (n = 79)			Aggregate bronchitic symptoms (n = 283)			No aggregate bronchitic symptoms (n = 2661)
	NO.(%)	OR(95%CI)	p	NO.(%)	OR(95%CI)	p	NO.(%)	OR(95%CI)	p	NO.(%)	OR(95%CI)	p	NO. (%)
Age, year	12.63 ± 0.29			12.66 ± 0.31			12.71 ± 0.32			12.65 ± 0.30			12.70 ± 0.35
Sex													
Girl	58(41.13)	1.00(reference)		48(48.48)	1.00(reference)		37(46.84)	1.00(reference)		127(44.88)	1.00(reference)		1389(52.20)
Boy	83(58.87)	1.56(1.11–2.20)	0.01	51(51.52)	1.16(0.78–1.73)	0.47	42(53.16)	1.24(0.79–1.94)	0.35	156(55.12)	1.34(1.05–1.72)	0.02	1272(47.80)
Parental education level (year)[a]													
<9	10(7.19)	1.00(reference)		21(21.21)	1.00(reference)		14(17.95)			43(15.36)	1.00(reference)		425(16.09)
9–11	75(53.96)	2.47(1.27–4.83)	0.03	44(44.44)	0.69(0.41–1.18)	0.29	37(47.44)	0.87(0.47–1.63)	0.75	135(48.21)	1.04(0.72–1.48)	0.96	1289(48.79)
≥12	54(38.85)	2.47(1.25–4.90)	0.04	34(34.34)	0.74(0.43–1.29)	0.61	27(34.62)	0.88(0.46–1.70)	0.83	102(36.43)	1.09(0.75–1.58)	0.64	928(35.12)
Family annual income[a]													
Low	43(32.82)	1.00(reference)		40(43.48)	1.00(reference)		28(37.84)	1.00(reference)		97(36.88)	1.00(reference)		887(36.10)
Median	71(54.20)	1.15(0.78–1.69)	0.82	41(44.57)	0.71(0.46–1.11)	0.29	36(48.65)	1.09(0.51–1.47)	0.54	133(50.57)	0.95(0.72–1.25)	0.64	1278(52.01)
High	17(12.98)	1.20(0.68–2.14)	0.67	11(11.96)	0.84(0.42–1.65)	0.98	10(13.51)	0.89(0.51–1.47)	0.69	33(12.55)	1.03(0.68–1.57)	0.77	292(11.88)
Pet													
No	51(36.17)	1.00(reference)		35(35.35)	1.00(reference)		30(37.97)	1.00(reference)		99(34.98)	1.00(reference)		1135(42.65)
Yes	90(63.83)	1.31(0.92–1.87)	0.13	64(64.65)	1.36(0.89–2.07)	0.15	49(62.03)	1.22(0.77–1.93)	0.41	184(65.02)	1.38(1.07–1.79)	0.01	1526(57.35)
Duration of breastfeeding, month[a]													
0	71(50.71)	1.00(reference)		52(54.17)	1.00(reference)		38(50.00)	1.00(reference)		145(52.35)	1.00(reference)		1372(52.25)
1–2	58(41.43)	1.12(0.79–1.60)	0.35	35(36.46)	0.92(0.60–1.43)	0.92	31(40.79)	1.12(0.69–1.81)	0.59	108(38.99)	1.02(0.79–1.33)	0.59	1000(38.08)
3–5	6(4.29)	0.91(0.39–2.14)	0.93	5(5.21)	1.04(0.41–2.65)	0.80	2(2.63)	0.57(0.14–2.38)	0.33	12(4.33)	0.89(0.48–1.66)	0.80	127(4.84)
≥6	5(3.57)	0.76(0.30–1.92)	0.56	4(4.17)	0.83(0.30–2.34)	0.75	5(6.58)	1.42(0.55–3.68)	0.33	12(4.33)	0.89(0.48–1.66)	0.80	127(4.84)
Parental atopy[b]													
NO	69(48.94)	1.00(reference)		69(69.70)	1.00(reference)		57(72.15)	1.00(reference)		173(61.13)	1.00(reference)		2036(76.51)
Yes	72(51.06)	3.40(2.41–4.79)	<0.01	30(30.30)	1.42(0.91–2.20)	0.12	22(27.85)	1.26(0.76–2.07)	0.37	110(38.87)	2.07(1.61–2.67)	<0.01	625(23.49)
Carpet used[a]													
No	125(89.29)	1.00(reference)		80(80.81)	1.00(reference)		66(84.62)	1.00(reference)		243(86.48)	1.00(reference)		2378(89.74)
Yes	15(10.71)	1.05(0.61–1.81)	0.86	19(19.19)	2.08(1.24–3.48)	<0.01	12(15.38)	1.59(0.85–2.98)	0.15	38(13.52)	1.37(0.95–1.97)	0.09	272(10.26)
Environmental tobacco smoke[a]													
No	67(47.86)	1.00(reference)		52(53.61)	1.00(reference)		38(48.72)	1.00(reference)		140(50.18)	1.00(reference)		1392(52.57)
Yes	73(52.14)	1.21(0.86–1.70)	0.28	45(46.39)	0.96(0.64–1.44)	0.84	40(51.28)	1.17(0.74–1.83)	0.50	139(49.82)	1.10(0.86–1.41)	0.45	1256(47.43)
Maternal smoking during pregnancy[a]													
No	136(96.45)	1.00(reference)		81(89.69)	1.00(reference)		75(94.94)	1.00(reference)		263(93.59)	1.00(reference)		2556(96.42)
Yes	5(3.55)	0.99(0.40–2.47)	0.98	10(10.31)	3.09(1.56–6.14)	<0.01	4(5.06)	1.44(0.51–4.01)	0.49	18(6.41)	1.84(1.10–3.10)	0.02	95(3.58)
Any home dampness, moldy odor and mold													
NO	50(35.46)	1.00(reference)		47(47.47)	1.00(reference)		39(49.37)	1.00(reference)		119(42.05)	1.00(reference)		1303(48.97)
Yes	91(64.54)	1.75(1.23–2.49)	<0.01	52(52.53)	1.06(0.71–1.59)	0.77	40(50.63)	0.98(0.63–1.54)	0.94	164(57.95)	1.32(1.03–1.69)	0.03	1358(51.03)
Cockroaches													
No	8(5.67)	1.00(reference)		5(5.05)	1.00(reference)		4(5.06)	1.00(reference)		15(5.30)	1.00(reference)		309(11.61)
Yes	133(94.33)	2.18(1.06–4.50)	0.03	94(94.95)	2.47(0.99–6.17)	0.05	75(94.94)	2.46(0.90–6.78)	0.08	268(94.70)	2.35(1.38–4.00)	<0.01	2352(88.39)

[a] Number of subjects does not add up to total number due to the data were missing.
[b] Atopy included asthma, allergic rhinitis and atopic dermatitis.

Table 2
Distribution of air pollutant concentrations between children.

Pollutant	Mean ± SD	Minimum	Maximum	Interquartile Range
$PM_{2.5}$, μg/m^3	29.43 ± 6.66	16.99	40.86	12.51
O_3, ppb	22.56 ± 4.48	15.09	30.07	8.28
CO, ppm	0.58 ± 0.17	0.32	1.61	0.19
NO_2, ppb	19.29 ± 4.98	12.12	38.35	6.89
SO_2, ppb	3.08 ± 1.15	1.45	8.09	0.98

were analyzed by the Sequence Detection Software. The details of primers and probes for IL-13 genetic variants were presented in Supplement Table 2.

2.5. Covariates

The covariates in the study was obtained from the questionnaires including age, sex (boys, girls), parental education level (<9 years, 9–11 years, ≥12 years), family annual income (low, median, high), duration of breastfeeding (0 month, 1–2 months, 3–5 months, ≥6 months), pet ownership (no, yes), carpet used (no, yes), environmental tobacco smoke (no, yes), maternal smoking during pregnancy (no, yes), any home dampness, moldy odor and mold, cockroaches (no, yes) and parental atopy (no, yes). Parental atopy was defined as children's father or mother had been diagnosed as asthma, allergic rhinitis or atopic dermatitis.

2.6. Statistical Analysis

The characteristics and distribution of the study population were analyzed by using Chi-square statistics. Multiple logistic regression models were used to estimate the effect of air pollution on the prevalence of aggregate bronchitic symptoms, adjusted for potential confounding factors, such as age, sex, maternal smoking during pregnancy, pets, ETS, carpets used, cockroaches, any home dampness, moldy odor and mold and parental atopy. The selection of confounders was according to the stepwise selection and the difference between adjusted odds ratio (OR) and crude OR has changed >10%. The effect of each air pollutants on the risk of aggregate bronchitic symptoms was presented as adjusted OR per interquartile changes for $PM_{2.5}$, O_3, CO, NO_2 and SO_2.

To evaluate the association of aggregate bronchitic symptoms with each four IL-13 SNPs (rs1800925, rs2066960, rs20541 and rs848), multiple logistic regressions (codominant and dominant genetic models) were used adjusting the variables described above. We estimated the measurements of linkage disequilibrium (LD) by Haploview software version 4.2.

In the Haplotype-based analysis, we first estimated haplotype frequencies from IL-13 genotypes data using the "partition ligation E-M" algorithm by Haplotype-tagging SNP program (Stram et al., 2003). Then, haplotypes with the frequency >0.05 were used to do further analysis. Adjusted OR were calculated for specific haplotypes, with the group of all other haplotypes as the reference, with the same adjustment variables which were described above.

Table 4
Genotype frequencies of IL-13 SNPs in our study.

SNP	Genotype	N	%
rs1800925	CC	2174	73.85
	CT	690	23.44
	TT	80	2.72
	Minor allele frequency	0.14	
	HWE	0.0054	
rs2066960	CC	1099	37.33
	CA	1343	45.62
	AA	502	17.05
	Minor allele frequency	0.40	
	HWE	0.008	
rs20541	CC	1375	46.71
	CT	1275	43.31
	TT	294	9.99
	Minor allele frequency	0.32	
	HWE	0.95	
rs848	GG	1363	46.30
	GT	1250	42.46
	TT	331	11.24
	Minor allele frequency	0.32	
	HWE	0.08	

HWE: Hardy-Weinberg equilibrium.

The interaction between air pollutants exposure and IL-13 haplotypes was examined by adding an interactive term in the logistic regression models and the results were represented as OR and 95%CI. All statistical analyses were performed by SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), and all tests were two-sided with the significant level of 0.05.

3. Results

3.1. Study Population and Prevalence of Aggregate Bronchitic Symptoms

A total of 3204 children with both information on genotyping and air pollution was included in our study. Of the 2944 non-asthmatic children, 141 (4.79%) had been diagnosed as bronchitis, and 283(9.61%) as aggregate bronchitic symptoms. The distribution of demographic characteristics of children with aggregate bronchitic symptoms was shown in Table 1. The mean and standard deviation of ages with and without aggregate bronchitic symptoms were 12.70 ± 0.35 and 12.65 ± 0.30, respectively. Boys had a higher risk of having aggregate bronchitic symptoms (odds ratio (OR), 1.34; 95% CI, 1.05–1.72) than girls. Children whose parents had atopic disease (OR, 2.07; 95%CI, 1.60–2.67), smoking during pregnancy (OR, 1.84; 95%CI: 1.10–3.10), pets (OR, 1.38; 95% CI, 1.07–1.79), and carpets used (OR, 1.37; 95% CI, 0.95–1.97) tend to suffer aggregate bronchitic symptoms. We also found positive associations between aggregate bronchitic symptoms and, the presence of cockroaches (OR, 2.35; 95% CI, 1.38–4.00), and any home dampness, moldy odor or mold at home (OR, 1.32; 95% CI, 1.03–1.69). Positive associations between diagnosed bronchitis and sex (OR, 1.56; 95% CI, 1.11–2.20), parental education level over 12 years (OR, 2.47; 95% CI, 1.25–4.90), parental atopy (OR, 3.40; 95% CI, 2.41–4.79), the presence of cockroaches (OR, 2.18; 95% CI, 1.06–

Table 3
Adjusted odds ratio and 95% CI of aggregate bronchitic symptoms among children without asthma in single air pollutant models.

Pollutant	Diagnosed bronchitis		Chronic phlegm		Chronic cough		Aggregate bronchitic symptoms	
	aOR(95%CI)	p	aOR(95%CI)	p	aOR(95%CI)	p	aOR(95%CI)	p
$PM_{2.5}$ (12.51 μg/m^3)	0.74(0.54–1.03)	0.07	1.59(1.07–2.37)	0.02	1.28(0.83–1.97)	0.26	1.01(0.80–1.28)	0.94
O_3(8.28 ppb)	0.84(0.61–1.16)	0.30	1.54(1.05–2.27)	0.03	1.30(0.85–1.97)	0.23	1.08(0.85–1.36)	0.53
CO(0.19 ppm)	1.02(0.83–1.24)	0.86	1.00(0.78–1.27)	0.99	0.92(0.70–1.21)	0.55	1.00(0.86–1.16)	0.01
NO_2(6.89 ppb)	0.99(0.78–1.27)	0.94	1.06(0.79–1.41)	0.70	0.98(0.71–1.35)	0.90	1.03(0.86–1.23)	0.76
SO_2(0.98 ppb)	0.92(0.78–1.08)	0.30	1.19(1.02–1.39)	0.03	1.07(0.89–1.29)	0.46	1.04(0.93–1.15)	0.50

Model adjusting for age, sex, maternal smoking during pregnancy, pet, ETS, carpet used, cockroaches, any home dampness, moldy odor and mold and parental atopy.

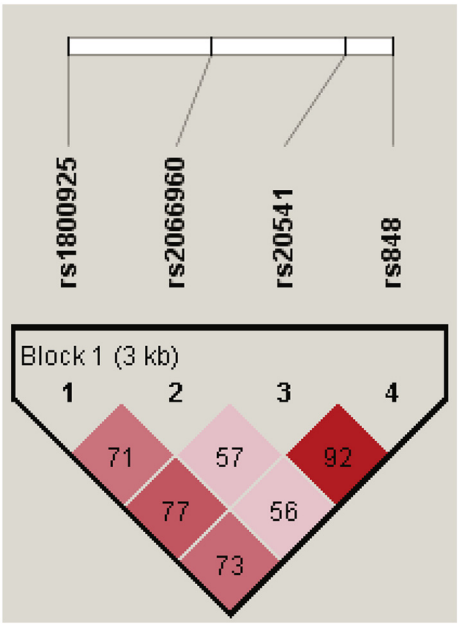

Fig. 2. Location and LD of four IL-13 SNP.

4.50) and any dampness problems at home (OR, 1.75; 95% CI, 1.23–2.49) were found. In addition, the chronic phlegm was also associated with carpets used (OR, 2.08; 95% CI, 1.24–3.48), and maternal smoking during pregnancy (OR, 3.09; 95% CI, 1.56–6.14) (Table 1).

3.2. Air Pollution and Aggregate Bronchitic Symptoms

The distributions of the air pollutants concentrations were presented in Table 2. There were significantly positive associations between chronic phlegm without asthma and exposure to $PM_{2.5}$ (aOR, 1.59; 95% CI, 1.07–2.37 per 12.51 μg/m3 change), O_3 (aOR, 1.54 95% CI, 1.05–2.27 per 8.28 ppb change) and SO_2 (aOR, 1.19; 95% CI, 1.02–1.39 per 0.98 ppb change). Although the OR of exposure to $PM_{2.5}$, O_3 and SO_2 was elevated, we did not identify any significant associations between air pollutant exposure and prevalence of chronic cough and aggregate bronchitic symptoms in children without asthma (Table 3).

3.3. IL-13 SNPs and Aggregate Bronchitic Symptoms

Frequencies of IL-13 SNPs (SNP1 rs1800925, SNP2 rs2066960, SNP3 rs20541 and SNP4 rs848) polymorphisms were presented in Table 4. In the distribution of our study population, the frequencies of the four IL-13 SNPs were in accordance with the Hardy-Weinberg equilibrium which was at significance level 10^{-4} (Stram et al., 2003). All four IL-13 SNPs were part of one haplotype block and a strong LD was found in SNP3 rs20541 and SNP4 rs848 (Fig. 2). We found that SNP4 rs848 was associated with the increased risk of chronic phlegm under the homozygous codominant model (adjusted odds ratio (aOR), 2.10; 95% CI, 1.17–3.77) and dominant model (aOR, 1.89; 95% CI, 1.11–3.22). Children with homozygous of SNP3 rs20541 (minor allele T) and SNP4 rs848 (minor allele T) had a significantly higher risk of aggregate bronchitic symptoms (Table 5).

3.4. IL-13 Haplotypes and Aggregate Bronchitic Symptoms

IL-13 haplotype frequencies were shown in Table 6. Children with the haplotype h0011 (SNP1: common allele C; SNP2: common allele C; SNP3: minor allele T; SNP4: minor allele T) had an increased risk for the prevalence of diagnosed bronchitis (aOR, 1.09; 95% CI, 0.74–1.60), chronic phlegm (aOR, 1.33; 95% CI, 0.86–2.07), chronic cough (aOR, 1.48; 95% CI, 0.92–2.38) and aggregate bronchitic symptoms (aOR, 1.23; 95% CI, 0.94–1.62) without asthma in comparison with all other haplotypes as the reference, but not statistically significant (Table 7).

3.5. Association of Air Pollution and IL-13 Haplotypes with Aggregate Bronchitic Symptoms

Because the effects of h0011 were positive in aggregate bronchitic symptoms (Table 7), we further evaluated the joint effects between this haplotype and air pollutants exposure on the prevalence of aggregate bronchitic symptoms without asthma. In children without asthma, we found that children carrying the haplotype h0011 had an increased risk of chronic phlegm exposure to $PM_{2.5}$ (aOR, 2.31; 95% CI, 1.11–4.79), O_3 (aOR, 2.86; 95% CI, 1.38–5.94), and SO_2 (aOR, 1.22; 95% CI, 1.01–1.46). However, only a significant joint effect between haplotype h0011 and O_3 exposure (aOR, 1.11; 95% CI, 1.01–1.24) on the risk of chronic phlegm in children without asthma was found (Table 8).

Table 5
Association of 4 tagging SNPs in IL-13 with aggregate bronchitic symptoms.

IL-13 genotype	Diagnosed bronchitis		Chronic phlegm		Chronic cough		Aggregate bronchitic symptoms	
	aOR(95%CI)	p	aOR(95%CI)	p	aOR(95%CI)	p	aOR(95%CI)	p
rs1800925								
CC	1.00(reference)		1.00(reference)		1.00(reference)		1.00(reference)	
CT	1.15(0.77–1.71)	0.69	0.87(0.53–1.45)	0.58	0.73(0.40–1.31)	0.23	0.93(0.69–1.26)	0.18
TT	1.66(0.69–4.02)	0.33	1.16(0.35–3.80)	0.72	1.41(0.43–4.62)	0.41	1.56(0.80–3.03)	0.16
CT/TT	1.20(0.82–1.76)	0.34	0.90(0.56–1.46)	0.67	0.80(0.46–1.38)	0.41	0.99(0.75–1.32)	0.96
rs2066960								
CC	1.00(reference)		1.00(reference)		1.00(reference)		1.00(reference)	
CA	0.81(0.56–1.19)	0.45	1.37(0.86–2.18)	0.13	0.48(0.29–0.80)	0.10	0.80(0.61–1.05)	0.51
AA	0.87(0.53–1.44)	0.89	0.97(0.51–1.85)	0.52	0.56(0.28–1.10)	0.51	0.77(0.53–1.11)	0.37
CA/AA	0.83(0.58–1.18)	0.30	1.26(0.81–1.96)	0.31	0.50(0.32–0.79)	<0.01	0.79(0.61–1.02)	0.07
rs20541								
CC	1.00(reference)		1.00(reference)		1.00(reference)		1.00(reference)	
CT	1.04(0.72–1.51)	0.41	1.06(0.68–1.67)	0.32	1.06(0.65–1.73)	0.50	1.00(0.76–1.31)	0.11
TT	1.49(0.87–2.57)	0.14	1.77(0.96–3.27)	0.06	1.58(0.79–3.18)	0.20	1.57(1.06–2.33)	0.02
CT/TT	1.46(0.88–2.44)	0.14	1.72(0.97–3.04)	0.06	1.54(0.80–2.95)	0.20	1.57(1.09–2.27)	0.02
rs848								
GG	1.00(reference)		1.00(reference)		1.00(reference)		1.00(reference)	
GT	1.08(0.74–1.56)	1.57	1.24(0.78–1.95)	0.47	1.12(0.68–1.83)	0.65	1.06(0.81–1.39)	0.28
TT	1.29(0.75–2.22)	2.22	2.10(1.17–3.77)	0.02	1.56(0.79–3.07)	0.22	1.51(1.03–2.21)	0.03
GT/TT	1.24(0.75–2.07)	0.40	1.89(1.11–3.22)	0.02	1.48(0.79–2.77)	0.22	1.47(1.03–2.10)	0.03

Model adjusting for age, sex, maternal smoking during pregnancy, pet, ETS, carpet used, cockroaches, any home dampness, moldy odor and mold and parental atopy.

Table 6
Haplotype frequencies of IL-13 in this study.

Haplotype[a]	Frequency
h0100	0.3311
h0000	0.3086
h0011	0.1373
h1011	0.1106
h0111[b]	0.0470
h0001[b]	0.0183
h1000[b]	0.0124
h1100[b]	0.0086
h0010[b]	0.0079
h1111[b]	0.0061
h1010[b]	0.0046
h0101[b]	0.0032
h0110[b]	0.0016
h1001[b]	0.0012
h1101[b]	0.0010
h1110[b]	0.0005

[a] 0: Common allele and 1: minor allele, by the order of SNP1(rs1800925): C/T; SNP2(rs2066960): C/A; SNP3 (rs20541): C/T; SNP4(rs848): G/T.
[b] Haplotypes were collapsed into a single combined category in the haplotype analysis.

4. Discussion

To our best knowledge, this study investigated the independent and joint effects of air pollutants exposure and IL-13 haplotypes in the relation to aggregate bronchitic symptoms in non-asthma children. Our findings strengthen the prevalence of chronic phlegm was elevated with an increase of $PM_{2.5}$, O_3 and SO_2 exposure. We also found that children having the haplotype h0011 are more likely to suffer aggregate bronchitic symptoms. We further confirmed that IL-13 haplotype may modify the effect of O_3 exposure and chronic phlegm.

4.1. Validation of Air Pollutants Exposure Assessment

In our study, we used IDW method to estimate air pollution concentration on the basis of school and home addresses relying on daily air pollution monitoring data. We used I/O ratio to convert outdoor air pollution concentrations into indoor ones and took children's living activities into account, which would make our exposure assessment closer to individual level of exposure. The previous study was only used 3-year averaged concentration of air pollution from air monitoring stations as children exposed to air pollution (Hwang and Lee, 2010). In order to determine if our exposure assessment was more appropriate than previous method, we compared the effects of air pollution exposure between the previous method and ours. Although the ORs did not show substantial differences, the confidence intervals of OR for O_3 and NO_2 exposure in our study were narrower than previous ones (Supplement Table 3). It shows the precision of our exposure assessment is better than previous ones, especially in O_3 and NO_2. (Supplement Table 3).

4.2. Potential Limitations

Our study has some potential limitations. The potential misclassification of respiratory symptoms is a limitation to this study. Questionnaires of self-respiratory symptoms are commonly used in the epidemiological studies and have been proved to be valid and repeatable (Burney et al., 1989). Aggregate bronchitic symptoms including chronic phlegm and chronic cough, similar to the symptoms of asthmatic attack, may consequently lead to the risk overestimation. However, this seems improbable because we primarily focused on children without asthma. Another possible limitation is the difference in the misclassification of IL-13 genetic variants. Since aggregate bronchitic symptoms are defined without knowing the genotypes, it may not lead to the bias. Given 48 associations (4 outcomes × 3 air pollutants × 4 Haplotypes) reported here, we might expect at least two of the associations to be significant due to chance (if $\alpha = 0.05$). Our findings suggest the IL-13 haplotype may modify the effects of O3 exposure on chronic phlegm, which are consistent with our knowledge about biological mechanisms of aggregate bronchitic symptoms development, but we cannot rule out the possibility of chance alone.

Children's living activities might be a potential limitation for exposure assessment. Most of students in Taiwan might go to cram school and go on vacation in summer or winter, but we did not investigate this question in the questionnaire. Therefore, the information on children living activities may not be complete, and the concentration of air pollutants may be underestimated. Another limitation for exposure assessment was the method of Indoor and outdoor concentration conversion. Our study used I/O ratio which was measured in Paris might cause bias (Kirchner et al., 2002). Since our study covered the whole Taiwan, where climate and urban types were different, the concentration of air pollutants at some place, such as Yilan and Hualian may be overestimated.

4.3. Synthesis With Previous Knowledge

Several studies explored the relationship between air pollution and aggregate bronchitic symptoms (Braun-Fahrländer et al., 1997; Jedrychowski and Flak, 1998; Gao et al., 2014), but these studies did not confirm that asthma was associated with air pollution exposure. Therefore, we focused on non-asthma children to clarify the association of air pollution exposure with aggregate bronchitic symptoms. Furthermore, we also elaborated the effects of air pollution and IL-13 gene on aggregate bronchitic symptoms focusing on non-asthma children.

Our study showed that the exposure to $PM_{2.5}$, O_3, NO_2 and SO_2 were negatively but not significantly associated with diagnosed bronchitis in non-asthma children, which is consistent with the Southern California study (McConnell et al., 1999). They reported a weak and negative association between diagnosed bronchitis and $PM_{2.5}$ and NO_2 in children without asthma. Moreover, we found that the exposure to a 12.51 μg/m3 had an increase in $PM_{2.5}$, 8.28 ppb increase in O_3 and 0.98 ppb increase in SO_2, and the prevalence of chronic phlegm was elevated with 59%, 54% and 19% respectively.

The possible mechanism of $PM_{2.5}$ is that inhalation of fine suspended particles stimulates expression of a number of inflammation-related

Table 7
Association of IL-13 haplotypes and aggregate bronchitic symptoms among children without asthma.

Haplotypes	Diagnosed bronchitis		Chronic phlegm		Chronic cough		Aggregate bronchitic symptoms	
	aOR(95%CI)	p	aOR(95%CI)	p	aOR(95%CI)	p	aOR(95%CI)	p
h0000 vs. others[a]	0.82(0.58–1.17)	0.27	0.68(0.44–1.03)	0.07	1.08(0.68–1.69)	0.75	0.84(0.65–1.08)	0.17
h0011 vs. others[a]	1.09(0.74–1.60)	0.67	1.33(0.86–2.07)	0.20	1.48(0.92–2.38)	0.11	1.23(0.94–1.62)	0.13
h1011 vs. others[a]	1.17(0.78–1.75)	0.45	0.94(0.57–1.56)	0.81	0.72(0.40–1.32)	0.29	0.98(0.72–1.33)	0.88

Model adjusting for age, sex, maternal smoking during pregnancy, pet, ETS, carpet used, cockroaches, any home dampness, moldy odor and mold and parental atopy.
[a] 0: Common allele and 1: minor allele, by the order of SNP1(rs1800925): C/T; SNP2(rs2066960): C/A; SNP3(rs20541): C/T; SNP4(rs848): G/T.

Table 8
Joint effects of air pollution exposure and IL-13 haplotype h0011 on aggregate bronchitic symptoms among children without asthma.

	h0011[a]	Diagnosed bronchitis		Chronic phlegm		Chronic cough		Aggregate bronchitic symptoms	
		aOR(95%CI)	p	aOR(95%CI)	p	aOR(95%CI)	p	aOR(95%CI)	p
$PM_{2.5}$ (12.51 μg/m^3)	No	0.77(0.53–1.13)	0.18	1.34(0.83–2.17)	0.23	1.26(0.74–2.15)	0.40	0.97(0.73–1.29)	0.84
	Yes	0.68(0.37–1.24)	0.66	2.31(1.11–4.79)	0.03	1.34(0.66–2.76)	0.84	1.11(0.73–1.70)	0.86
	Interaction term	0.99(0.93–1.05)	0.72	1.04(0.97–1.12)	0.22	1.01(0.94–1.08)	0.88	1.01(0.97–1.05)	0.60
O_3 (8.28 ppb)	No	0.89(0.61–1.30)	0.55	1.18(0.74–1.87)	0.50	1.37(0.82–2.31)	0.23	1.02(0.77–1.35)	0.90
	Yes	0.73(0.40–1.34)	0.53	2.86(1.38–5.94)	<0.01	1.17(0.57–2.41)	0.51	1.22(0.80–1.88)	0.70
	Interaction term	0.98(0.90–1.06)	0.58	1.11(1.01–1.24)	0.04	0.98(0.88–1.09)	0.73	1.02(0.96–1.09)	0.49
SO_2 (0.98 ppb)	No	0.93(0.77–1.12)	0.45	1.15(0.87–1.53)	0.43	1.12(0.90–1.40)	0.31	1.06(0.94–1.20)	0.36
	Yes	0.89(0.65–1.23)	0.73	1.22(1.01–1.46)	0.04	0.99(0.71–1.38)	0.25	0.99(0.81–1.22)	0.30
	Interaction term	0.96(0.66–1.40)	0.82	0.95(0.67–1.34)	0.76	0.88(0.59–1.32)	0.54	0.94(0.73–1.19)	0.59

Model adjusting for age, sex, maternal smoking during pregnancy, pet, ETS, carpet used, cockroaches, any home dampness, moldy odor and mold and parental atopy.
[a] 0: Common allele and 1: minor allele, by the order of SNP1(rs1800925): C/T; SNP2(rs2066960): C/A; SNP3(rs20541): C/T; SNP4(rs848): G/T.

cytokine genes, which induce neutrophils, eosinophils and T cells to release more inflammatory cytokines and chemokines. However, the excessive response of inflammatory cytokines causes inflammatory damage causing the risk of human respiratory system (Xing et al., 2016). Inhalation of $PM_{2.5}$, O_3, NO_2 and SO_2, which are known to form reactive oxygen species (ROS), might cause airway inflammation and impaired immune function, activated the inflammatory cells.

IL-13 is located on chromosome 5q31, which is generated by stimulated Th2 cells and has been involved in the pathogenesis of asthma. The mechanism for the effects of IL-13 on respiratory symptoms may involve pathways that affect airway inflammatory. IL-13 enhances activation of eosinophils and induces B cells to produce immunoglobulin E (IgE), thereby activating mast cells, which produce excessive immune responses (Brightling et al., 2010).

Our findings also found that IL-13 haplotype may modify the effects of ozone exposure on chronic phlegm in children without asthma. Although the biological mechanism is still unclear, it is possible that the exposure to ozone may cause overexpression of IL-13 gene, inducing mucus cell hyperplasia, thereby excessive production of airway mucus. An animal study also provides the evidence that IL-13 overexpression exacerbated ozone induced airway hyper-responsiveness and inflammatory response (Williams et al., 2008). Further studies are still needed to confirm the IL-13 effect modification with O_3 exposure on chronic phlegm and elaborated potential biological mechanisms.

5. Conclusion

Our results revealed that genetic variants in IL-13, especially h0011 haplotype increased the risk of the prevalence of aggregate bronchitic symptoms in non-asthmatic children. It may also modify the effects of exposure to air pollutants on the prevalence of aggregate bronchitic symptoms. Thus, IL-13 may be a suitable gene to identify the children susceptible to the exposition to air pollution.

Conflicts of Interest

The authors have no competing interest to declare.

Authors' contributions

Conception and design: YL, BF; Analysis and interpretation: JH, CM, BF; Drafting the manuscript for important intellectual content: JH, CH, BF. JH contributed equally as YL.

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The founders have no role in the study design, data collection, analysis or interpretation; or writing the paper.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.02.008.

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