Doctor of Philosophy

Joint association of a polygenic risk score and PM_{10} exposure for asthma and BHR in COCOA study

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Joint association of a polygenic risk score and PM_{10} exposure for asthma and BHR in COCOA study

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ABSTRACT

Background: Each genetic factor and particulate matter with a diameter of less than 10 μm (PM_{10}) exposure contribute to the development of asthma and bronchial hyperresponsiveness (BHR), but joint effect between them is unknown.

Objective: The aims of this study were to assess the joint effect of polygenic risk score (PRS) and PM_{10} exposure on the development of asthma and BHR and identify the critical period of PM_{10} exposure for asthma and BHR.

Methods: We used the integral data composed to genetic data, confounding factors and questionnaires associated with asthma and BHR outcome of 643 children in the Cohort for Childhood Origin of Asthma and allergic diseases (COCOA) study. Asthma was defined as children 1) who has asthma symptom during last 12 months, 2) who has a physician's diagnosis of asthma during life combined with asthma symptom during last 12 months at the age 6 to 10 years. BHR was defined as $PC_{20} \leq 8$ mg/mL by using provocholine provocation test. polygenic risk score (PRS) was calculated using five single-nucleotide polymorphisms, including *IL-13* (rs20541), *NRF2* (rs6726395), *GSTP1* (rs1695), *GSDMB* (rs7216389) and *TSLP* (rs3806933). Multiple logistic regression analysis was performed. To identify the critical window for PM_{10} exposure, Bayesian distributed lag interaction model (BDLIM) was performed.

Results: The weighted PRS showed a significant association with increased asthma symptom (aOR 1.973, 95% CI 1.016-3.832), current asthma (aOR 5.369, 95% CI 1.793-16.078) and BHR (aOR 1.711, 95% CI 1.093-2.680). The risk of BHR increased in both PM_{10} during entire trimester of pregnancy (aOR 2.145, 95% CI 1.338-3.439) and PM_{10} during second trimester of pregnancy (aOR 1.822, 95% CI 1.114-2.980). Children with high PRS/high PM_{10} during entire trimester of pregnancy increased the risk of current asthma (aOR 6.501, 95% CI 1.361-31.044). Children with high PRS/high PM_{10} during first and second trimester of pregnancy increased the risk of current asthma (aOR 6.756, 95% CI 1.422-32.093; aOR 3.822, 95% CI 1.118-13.062, respectively). Children with high PRS/high PM_{10} during second trimester of pregnancy increased the risk of BHR (aOR, 2.603; 95% CI, 1.311-5.166). By BDLIM, critical window of PM_{10} exposure for BHR was 5-11 weeks of gestation in total participants and 6-8 weeks of gestation in males and 6-7 weeks of gestation in females. Depending on the PRS, high PM_{10} exposure during 6-8 weeks of gestation was significantly associated with BHR in low PRS group. In addition, stratifying by PRS and sex, high PM_{10} exposure during 13-14 weeks of gestation was significantly associated with BHR in female with low

PRS.

***Conclusions**:* Our study found that higher PRS and higher PM_{10} exposure during pregnancy increased the risk of current asthma and BHR. In addition, prenatal exposure of PM_{10}, especially during first and second trimesters, has an effect on the development of current asthma and BHR, modified by PRS and sex. The critical window of PM_{10} exposure for BHR was 5-11 weeks of gestation.

Key words: asthma, BHR, polygenic risk score, prenatal, PM_{10}, critical period

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Introduction

Asthma is heterogeneous disease that caused by genetic and environmental factors that occur at critical periods throughout life [1-3]. Twin studies have confirmed the contribution of genetic factors to the risk of asthma [4]. However, asthma-associated genetic variants still only explain collectively account for a small amount of the overall heritability for asthma [5-8]. Previous studies showed that estimates of the heritability of asthma have ranged between 35% and 95% [9], but estimates based on twin studies have been lower [10]. This observation could be due to the existence of additional variants with smaller effect sizes, rare susceptible alleles, and/or some genetic variants only confer risk in individuals exposed to certain environmental factors.

Genetic studies in asthma have been preformed in several approaches. Candidate gene association studies, genome-wide association studies (GWAS), whole-exome sequencing, or whole-genome sequencing. Since most complex diseases are influenced by hundreds of genetic variants each having a small effect on its own, polygenic approaches that deal with the genetic basis em masse often access more of the heritable component of complex traits than is possible by single variant approaches. The most common polygenic approach is the weighted genetic risk score (GRS) approach in which a weighted GRS is calculated from a pre-selected number of genetic variants to define a person's individual genetic risk for disease development [11]. In these applications, the gold standard is to use external weights which is using marginal effects estimated in an independent study population, but in recent publication, GRS with internal weights from the marginal genetic effects of the study itself showed that using these GRS increased the power to detect gene-environment interactions substantially compared to the common single SNPs approach and to the usage of unweighted GRS with a well-controlled type I error [12].

Asthma is characterized by an inflammatory process in the lower respiratory tract. An inflammatory process driven by Th2 cells, which essentially include interleukin (IL)-4, IL-5, and IL-13 plays an important role in the asthma pathogenesis [13]. Risk alleles of IL-13 polymorphism was reported to be associated with asthma susceptibility and bronchial hyperresponsiveness (BHR) in Korean children [14, 15]. In addition, oxidative stress occurs when antioxidant defences, which are regulated by a complex network of genes, are insufficient to maintain the level of reactive oxygen species below a toxic threshold. Outdoor air pollution has been known to adversely affect health by inducing oxidative stress. An individual's susceptibility to the effects of air pollution partly

depends on variation in their antioxidant genes. The evidence from previous studies supported that the interactions between antioxidant genes and outdoor air pollution is strongest for childhood asthma and wheeze, especially for interactions with GSTT1 and GSTM1 and GSTP1 [16, 17].

Air pollution is a global problem and Korea is one of the countries with higher concentration of particulate matter (PM). Among the air pollution factors, PM_{10} is considered to be a major cause of adverse health effects [18]. Throughout the late twentieth century, there has been an increasing trend in both prevalence and exacerbation of asthma, at a time when the issue of air pollution has come to the fore in public and scientific awareness [19]. Many studies have demonstrated that exposure to elevated levels of PM_{10} results in increased asthma medication use, emergency room visits for asthma and prevalence of BHR [20–25]. There is an increasing number of studies showing that maternal exposures during pregnancy to air pollution increased risk of childhood wheeze, asthma and reduced lung function [26-28]. Because prenatal development occurs through a multiple-event process starting in early gestation [29], children's health outcomes could be affected by prenatal exposure to air pollution. [30-32]. There are evidences to suggest sex-specific effects of air pollution exposure during pregnancy [26, 33-34], and we also examined sex stratified associations using Bayesian distributed lag interaction models (BDLIMs). However local specificities regarding the severity of air pollutants and the ancestry of the population will likely result in different pathways to asthma, the interactions between genes and environmental risk factors need to be taken into account to fully understand the impact of PM_{10} on asthma risk.

We hypothesized that children exposed to higher concentration of PM_{10} and with high genetic susceptibility have an increased risk of asthma and BHR in Korean children. The aims of this study were to assess the joint effect of polygenic risk score (PRS) and PM_{10} exposure on asthma and BHR and furthermore, we identified the critical window of PM_{10} exposure.

Materials and Methods

1. Study subject

The COhort for Childhood Origin of Asthma and allergic diseases (COCOA) is a prospective birth cohort study comprising children from 5 hospitals in Seoul, the Republic of Korea constructed to identify various genetic environmental factors for childhood allergic diseases. 3,004 pregnant women were recruited during their pregnancy between 19 November 2007 and 31 December 2015.

Children visited the hospital according to a regular schedule to consult pediatric allergy specialists and to receive physical examinations. The questionnaire, a modified version of the International Study of Asthma and Allergies in Childhood questionnaire, was completed by mothers or care-givers [35]. Additional questionnaires of environmental factors, such as maternal secondhand smoking, and maternal pet ownership during pregnancy were filled in. The study has been described in detail elsewhere [36].

Of 2,846 children who were followed up after delivery, 643 children were analyzed (shown in Fig. 1) in this study. A total of 22 children withdrew consent after delivery and 1,256 children under the age of 6 years were excluded because they were unable to perform provocholine provocation test to evaluate BHR. Children with incomplete questionnaires or missing data and children without gene data were also excluded.

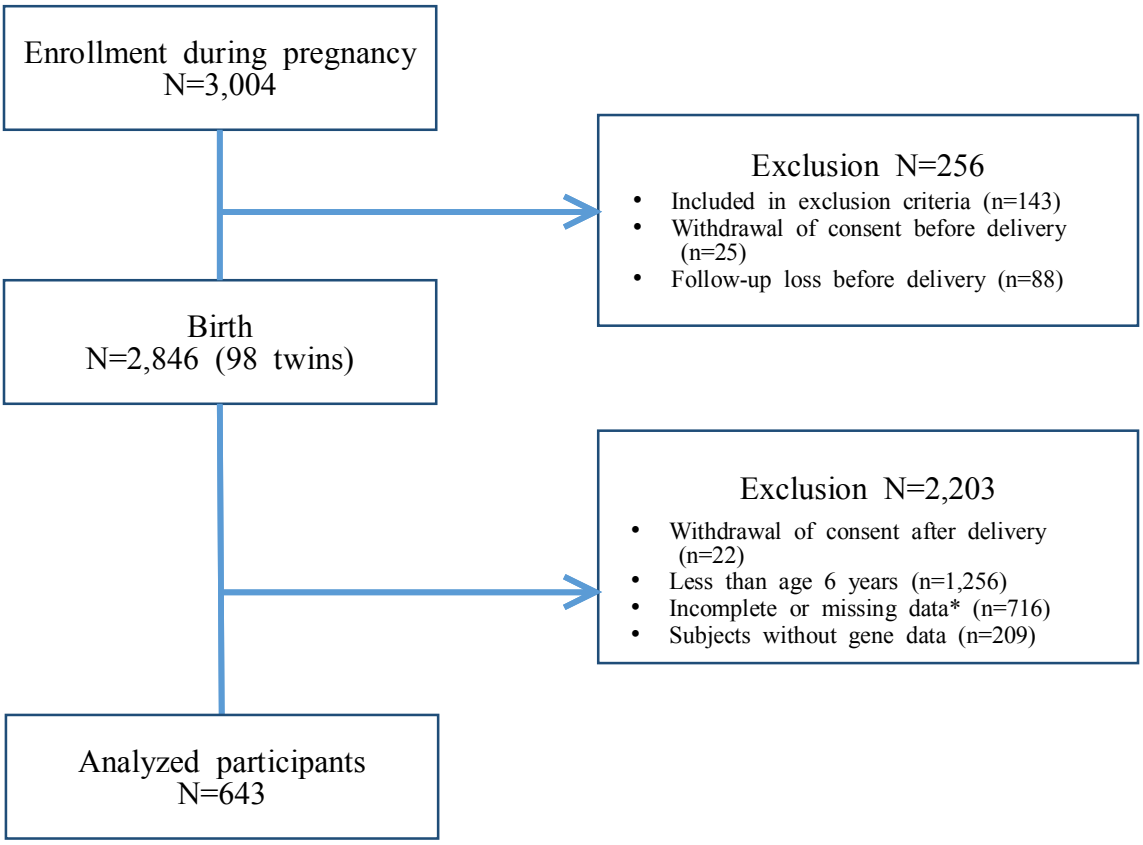


Figure 1. Flow chart of the study subjects

* Incomplete questionnaire or outcome data

2. Definition of asthma and BHR

Children were defined as having asthma symptoms when parents reported that the child had any of the typical asthma symptoms (i.e. nocturnal cough, wheezing, dyspnea, exercise-induced wheezing, or rescue medicine use) in the previous 12 months assessed by questionnaires on the regular visits at age 6 to 10 years. Current asthma was defined as an asthma diagnosis by a physician at any time and the occurrence of the typical asthma symptoms in the previous 12 months assessed by questionnaires on the regular visits at age 6 to 10 years.

Provocholine provocation tests were conducted in children at age 6 to 9 years in accordance with the internationally standardized methacholine guidelines [37]. Fresh solutions of Provocholine® (Methacholine chloride USP, Methapharm Inc., Brantford, ON, Canada) were prepared in buffered saline solution at 5 concentrations (0.625, 0.25, 1, 5, and 16 mg/mL). A KoKo dosimeter (Pulmonary Data Services; Doylestown, PA, USA), triggered by a solenoid valve set to remain open for 0.6 s, was used to generate the aerosol from a DeVilbiss 646 nebulizer (DeVilbiss Health Care, Somerset, PA, USA), with pressurized air at 20 pounds per square inch. Each subject inhaled 5 inspiratory capacity breaths of buffered saline solution and increasing concentrations of provocholine at 5-min intervals, resulting in a mean ± standard deviation output of 0.009 ± 0.0014 mL per inhalation. The FEV1 was measured at 30 and 90 s after the nebulization was completed, and the next dosing schedule proceeded within 5 min. The provocative concentrations of Provocholine® that caused a 20% (PC_{20}) decrease in FEV1 from the baseline were calculated by linear interpolation. BHR was determined as PC_{20} <8 mg/mL.

3. Genotyping

For the evaluation of the association between PRS, PM_{10} exposure and asthma-related outcomes, we obtained DNA extracted from cord blood samples. Cord blood samples obtained from the infants were screened for single nucleotide polymorphisms (SNPs) in *IL-13 (*rs20541), which have been reported to be related with Th2 inflammation, and *nuclear factor erythroid 2-related factor 2* (*NRF2***,** rs6726395), and *GSTP1 (*rs1695), which are related with antioxidant response. SNPs in *Gasdermin-B* (*GSDMB,* rs7216389) and *thymic stromal lymphopoietin* (*TSLP,* rs3806933), which are related with pediatric asthma, were genotyped [38-41].

Genotyping was conducted using a TaqMan assay (ABI, Foster City, CA, USA), and the endpoint fluorescent readings were measured on an ABI 7900 HT Sequence Detection System. Duplicate samples and negative controls were included to ensure the accuracy of genotyping. Distributions of these polymorphisms were in Hardy-Weinberg disequilibrium ($p > 0.1$) [42].

4. Measurement of outdoor PM10 exposure

Exposure to PM_{10} at residential addresses was estimated by land-use regression (LUR) models using a previously described standardized method [43]. We used ambient concentrations of PM_{10} measured at the 37 fixed monitoring stations in the study area (Seoul) operated by the Korean Ministry of Environment (http://www.airkorea.or.kr/web). Each monitoring station measures PM_{10} hourly. We used centrally and locally available geographic variable as potential predictors. Predictor variables, such as traffic indicators, surrounding-land usage, topography, and spatial trends, were computed at each location using ArcGIS version 9.3 (ESRI, Redlands, WA, USA). Multiple linear regression models were built using a supervised forward stepwise procedure. Predictor variables used in the final LUR model for air pollution included lengths of all roads, traffic intensity on nearest roads, total heavy-duty traffic loads of all roads, and a variable representing spatial trends.

The prenatal period was divided into three trimesters; from gestational week 1 to 13 (first), gestational week 14 to 27 (second), and from gestational week 28 to 40 (third) [44]. Period of exposure was categorized as the exposure to PM_{10} during total pregnancy period, each trimester, from birth to 1 year of age and previous 1 year of outcome evaluation. PM_{10} concentrations were estimated using mean concentrations of each period. PM_{10} concentrations were dichotomized using the median concentration as either "high" or "low" exposure. These dichotomous concentrations were used to determine the effect of PM_{10} on asthma outcomes and BHR.

5. Construction of weighted PRS

To estimate the role of the genetic risk for asthma and BHR, we calculated weighted PRS. We constructed a PRS using the five SNPs (*IL-13,* rs20541; *NRF2*, rs6726395; *GSTP1,* rs1695; *GSDMB,* rs7216389; *TSLP,* rs3806933) that were associated with allergic inflammation, oxidative stress and asthma. The estimate was analyzed by performing a

logistic regression of the association between the number of risk alleles and asthma status. The PRS was calculated by multiplying each estimated β-coefficient by the number of risk alleles (0, 1, or 2) of the considered SNPs [45]. For β-coefficient weighting (=ln (OR)), the coefficient estimates for each of the components obtained from the regression model were used to calculate weights for each outcome [46].

$$Weighted\ PRS = \sum_{i=1} Nmber\ of\ risky\ allele\ \in\ SNP_i \times Weight_i$$

$$n\ : \nu mber\ of\ all\ _{jects},\ \ Weight_i\ : the\ weight\ of\ i^{th}$$

6. Bayesian distributed lag interaction models (BDLIM)

In order to identify critical window for the effects of prenatal PM_{10} in relation to asthma and BHR, as well as effect modification by children's sex and PRS, we implemented Bayesian distributed lag interaction models (BDLIMs). BDLIM extends the traditional constrained distributed lag model framework that identifies critical windows [47], and also accounts for within window effects and tests for effect modifications. Critical exposure periods were identified as weeks during pregnancy with a statistically significant association.

7. Statistical analysis

An independent t-test was used to compare continuous variables between two groups, a chi-square test and fisher's exact test was used to compare categorical variables between to groups, ANCOVA was used to adjust for confounding factors. Continuous variables were divided into "low" and "high" categories based on each concentration's median values, and points (0 or 1) were given. A multivariable logistic regression analysis was performed incorporating both asthma outcomes and BHR as well as the PM_{10} exposure and PRS, adjusting for child's sex, parental history of allergy, maternal educational level, maternal environmental tobacco smoke exposure during pregnancy, maternal pet ownership during pregnancy, BMI of the child at asthma diagnosis and outdoor NO_2 exposure. Effect estimates were calculated for crude and adjusted models and are presented as odds ratios (OR) with 95% confidence intervals (95% CI) and P-value of <0.05 considered statistically significant. All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Result

1. Characteristics of participants

Table 1 shows the characteristics of the participants. A total of 643 in COCOA study had both genetic and environmental data with asthma outcome and BHR. Gender proportion (male: 53.7% vs 53.0%), parental history of allergic diseases (48.4% vs 52.1%), maternal pet ownership during pregnancy (5.1% vs 7.0%), BMI of children (15.96 ± 2.26 vs 20.50 ± 62.21), and prevalence of asthma symptoms (7.3% vs 7.5%), current asthma (3.9% vs 4.2%), BHR (34.6% vs 30.9%) were not different between analyzed group and excluded group, except for maternal education level ($P = 0.015$) and maternal exposure to environmental tobacco smoke during pregnancy (61.9% vs 53.1%; $P < 0.001$).

Table 1. Characteristics of the participants between analyzed and excluded groups

	Analyzed group	Excluded group	P-value
Gender (Male), n (%)	345/643 (53.7%)	1,184/2,236 (53.0%)	0.753
Maternal education level, n (%)			
≤Graduated high school	**45/643 (7.0%)**	**91/2,099 (4.3%)**	**0.015**
Graduated college	**451/643 (70.1%)**	**1,554/2,099 (74.0%)**	
≥Graduated postgraduate school	**147/643 (22.9%)**	**454/2,099 (21.6%)**	
Parental history of allergic diseases, n (%)	311/643 (48.4%)	1,151/2,208 (52.1%)	0.093
Maternal exposure to environmental tobacco smoke during pregnancy, n (%)	**398/643 (61.9%)**	**1,097/2,068 (53.1%)**	**<0.001**
Maternal pet ownership during pregnancy, n (%)	33/643 (5.1%)	146/2,086 (7.0%)	0.095
BMI of children at current asthma, Mean ± SD	15.96 ± 2.26	16.07 ± 1.84	0.517
Prevalence of asthma symptoms, n (%)	47/643 (7.3%)	18/239 (7.5%)	0.911
Prevalence of current asthma, n (%)	25/643 (3.9%)	10/239 (4.2%)	0.841
BHR (PC_{20} <8 mg/mL), n (%)	147/425 (34.6%)	46/149 (30.9%)	0.409

Values are presented as mean ± standard deviation (SD) or n (%).

2. Characteristics of participants according to the exposure levels of PM_{10} during pregnancy and PRS

There were no significant differences according to the exposure levels of PM_{10} during pregnancy, except for maternal exposure to environmental tobacco smoke during pregnancy (58.1% vs 65.7%; P = 0.045) (Table 2).

Current asthma was more prevalent in the high PRS group compared to the low PRS group (6.1% vs 1.6%; P = 0.002), but the other characteristic variables were not statistically different according to PRS (Table 2).

Table 2. Characteristics of participants according to the exposure level of PM_{10} during pregnancy and PRS

	PM_{10} during pregnancy			Weighted (according to current asthma) PRS		
	Low	High	P-value	Low	High	P-value
Gender (Male), n (%)	174/322 (54.0%)	171/321 (53.3%)	0.846	173/316 (54.8%)	172/327 (52.6%)	0.585
Maternal education level, n (%)						
≤Graduated high school	24/322 (7.5%)	21/321 (6.5%)	0.651	19/316 (6.0%)	26/327 (8.0%)	0.535
Graduated college	229/322 (71.1%)	222/321 (69.2%)		221/316 (69.9%)	230/327 (70.3%)	
≥Graduated postgraduate school	69/322 (21.4%)	78/321 (24.3%)		76/316 (24.1%)	71/327 (21.7%)	
Parental history of allergic diseases, n (%)	166/322 (51.6%)	145/321 (45.2%)	0.105	157/316 (49.7%)	154/327 (47.1%)	0.511
Maternal exposure to environmental tobacco smoke during pregnancy, n (%)	**187/322 (58.1%)**	**211/321 (65.7%)**	**0.045**	193/316 (61.1%)	205/327 (62.7%)	0.673
Maternal pet ownership during pregnancy, n (%)	13/322 (4.0%)	20/321 (6.2%)	0.206	20/316 (6.3%)	13/327 (4.0%)	0.176
BMI of children at current asthma, Mean ± SD	16.05 ± 2.63	15.85 ± 1.74	0.319	15.93 ± 1.76	16.00± 2.68	0.906
Prevalence of asthma symptoms, n (%)	26/322 (8.1%)	21/321 (6.5%)	0.455	18/316 (5.7%)	29/327 (8.9%)	0.121
Prevalence of current asthma, n (%)	12/322 (3.7%)	13/321 (4.1%)	0.832	**5/316 (1.6%)**	**20/327 (6.1%)**	**0.002**
Prevalence of BHR, n (%)	**52/184 (28.3%)**	**95/241 (39.4%)**	**0.017**	65/204 (31.9%)	82/221 (37.1%)	0.256

*SD, standard deviation

3. Construction of the weighted PRS

Table 3 reports associations between each of the five SNPs and asthma symptom, current asthma and BHR. Only *NRF2* showed a marginal effect on BHR (aOR 0.748, 95% CI 0.559-1.000). PRS was constructed using beta value of five SNPs (converted by beta=log(OR)). The units of the derived risk score are per risk-allele weighted by effect size.

4. Associations between PRS and the risk of asthma and BHR in children

The counted PRS showed a marginal association with increased asthma symptom (aOR 1.952, 95% CI 0.997-3.822) and no association with current asthma and BHR. But, the weighted PRS showed a positive association with increased asthma symptom (aOR 1.973, 95% CI 1.016-3.832), current asthma (aOR 5.369, 95% CI 1.793-16.078) and BHR (aOR 1.711, 95% CI 1.093-2.680) (Table 4).

Table 3. Associations between five SNPs and childhood asthma and BHR

SNPs	risk allele	Asthma symptom OR (95% CI)	current asthma OR (95% CI)	BHR (PC_{20} ≤8 mg/mL) OR (95% CI)
IL-13	*[A]*	1.385 (0.905-2.12)	1.587 (0.905-2.784)	0.797 (0.591-1.074)
NRF2	*[G]*	1.045 (0.671-1.626)	0.819 (0.456-1.469)	0.748 (0.559-1.000)
GSTP1	*[G]*	0.949 (0.554-1.625)	0.876 (0.417-1.842)	1.106 (0.774-1.581)
GSDMB	*[T]*	1.174 (0.710-1.94)	1.862 (0.865-4.008)	1.306 (0.930-1.835)
TSLP	*[T]*	1.141 (0.738-1.765)	1.478 (0.835-2.618)	1.143 (0.850-1.537)

OR, odds ratio; 95% CI, 95% confidence interval

Table 4. Associations between polygenic risk score and the risk of childhood asthma and BHR

		Asthma symptom			Current asthma			BHR (PC_{20} ≤8 mg/mL)		
		No	Yes	aOR* (95% CI)	No	Yes	aOR* (95% CI)	No	Yes	aOR* (95% CI)
Counted PRS	Low	228	14	1	235	7	1	103	58	1
	High	243	29	1.952 (0.997-3.822)	255	17	2.195 (0.885-5.444)	123	69	1.025 (0.658-1.597)
Weighted PRS	Low	242	15	1	255	4	1	130	57	1
	High	229	28	**1.973 (1.016-3.832)**	235	20	**5.369 (1.793-16.078)**	96	70	**1.711 (1.093-2.680)**

aOR, adjusted odds ratio; 95% CI, 95% confidence interval

*Adjusted for sex, parental history of allergy, maternal educational level, maternal environmental tobacco smoke exposure during pregnancy, maternal pet ownership during pregnancy, and BMI of the child at asthma diagnosis

5. Associations between PM_{10} exposure and the risk of childhood asthma and BHR

High level of exposure to PM_{10} during total pregnancy period was associated with BHR compared to low level of exposure to PM_{10} during pregnancy (aOR 2.145, 95% CI 1.338-3.439) (Table 5). In addition, the risk of BHR was increased with high exposure level to PM_{10} during 2nd trimester of pregnancy compared to low level of exposure to PM_{10} during 2nd trimester of pregnancy (aOR 1.822, 95% CI 1.114-2.980). Development of BHR was not significantly associated with PM_{10} exposure levels during first and third trimester of pregnancy and after birth. High levels of prenatal and postnatal PM_{10} exposure were neither significantly associated with asthma symptom nor current asthma.

Table 5. Effect of PM_{10} exposure on the development of childhood asthma and BHR

PM_{10}		Asthma symptom			Current asthma			BHR (PC_{20} ≤8 mg/mL)		
		No	Yes	aOR* (95% CI)	No	Yes	aOR* (95% CI)	No	Yes	aOR* (95% CI)
Total pregnancy	Low	255	23	1.000	267	11	1.000	113	44	1.000
	High	216	20	0.989 (0.507-1.929)	223	13	1.302 (0.534-3.174)	113	83	**2.145 (1.338-3.439)**
1st trimester	Low	240	17	1.000	249	8	1.000	100	58	1.000
	High	231	26	1.427 (0.701-2.903)	241	16	1.674 (0.641-4.373)	126	69	1.025 (0.627-1.674)
2nd trimester	Low	254	24	1.000	266	12	1.000	120	56	1.000
	High	217	19	0.933 (0.455-1.911)	224	12	1.183 (0.462-3.031)	106	71	**1.822 (1.114-2.980)**
3rd trimester	Low	243	25	1.000	253	15	1.000	108	65	1.000
	High	228	18	0.663 (0.320-1.372)	237	9	0.459 (0.170-1.237)	118	62	1.247 (0.759-2.047)
From birth to 1 year	Low	219	21	1.000	229	11	1.000	87	47	1.000
	High	251	22	0.894 (0.459-1.740)	260	13	0.905 (0.371-2.21)	138	80	1.252 (0.776-2.020)
The latest 12 months	Low	176	21	1.000	185	12	1.000	85	44	1.000
	High	223	19	0.699 (0.359-1.360)	231	11	0.702 (0.297-1.661)	123	78	1.209 (0.755-1.935)

BHR, bronchial hyperresponsiveness; PM, particulate matter; aOR, adjusted odds ratio; 95% CI, 95% confidence interval

*Adjusted for sex, parental history of allergy, maternal educational level, maternal environmental tobacco smoke exposure during pregnancy, maternal pet ownership during pregnancy, BMI of the child at asthma diagnosis, and NO_2 exposure

6. Joint effects of PM_{10} exposure and PRS on the risk of childhood asthma and BHR

Participants were stratified by PRS and level of PM_{10} exposure and evaluated for the association with the development of childhood asthma and BHR (Table 6). Asthma symptom was not associated with the stratified group by PRS and PM_{10} exposure according to the exposure period. Children with both high PRS and high PM_{10} exposure during total pregnancy, 1st trimester and 2nd trimester were in higher risk to develop current asthma compared to low PRS and low PM_{10} exposure during each period (aOR 6.501, 95% CI 1.361-31.044; aOR 6.756, 95% CI 1.422-32.093; aOR 3.822, 95% CI 1.118-13.062), but children with high PRS and high PM_{10} exposure during 3rd trimester, from birth to 1 year of age, and the latest 12 months were not associated with the risk of current asthma. BHR was more frequently developed in the groups of high PRS and high PM_{10} exposure during total pregnancy and 2nd trimester (aOR 2.751, 95% CI 1.378-5.492; aOR 2.603, 95% CI 1.311-5.166). BHR prevalence was not different among the stratified group according to PRS and PM_{10} exposure during 1st trimester, from birth to 1 year of age, and the latest 12 months.

Table 6. Association of polygenic risk score (PRS) and PM_{10} exposure on the development of childhood asthma and BHR

PRS	PM_{10}	Asthma symptom			Current asthma			BHR (PC_{20} ≤8 mg/mL)		
		No	Yes	aOR* (95% CI)	No	Yes	aOR* (95% CI)	No	Yes	aOR* (95% CI)
PRS	Total pregnancy									
Low	Low	131	11	1.000	137	2	1.000	61	18	1.000
Low	High	111	4	0.415 (0.124-1.383)	118	2	0.994 (0.134-7.373)	68	38	**1.979 (1.012-3.869)**
High	Low	124	12	1.172 (0.486-2.807)	130	9	4.490 (0.935-21.546)	49	26	1.708 (0.829-3.520)
High	High	105	16	1.739 (0.748-4.043)	105	11	**6.501 (1.361-31.044)**	54	38	**2.751 (1.378-5.492)**
PRS	1st trimester									
Low	Low	129	8	1.000	135	2	1.000	60	25	1.000
Low	High	113	7	0.809 (0.270-2.427)	120	2	0.836 (0.110-6.345)	69	31	1.143 (0.590-2.215)
High	Low	114	10	1.297 (0.486-3.457)	118	6	3.221 (0.628-16.528)	43	31	1.787 (0.918-3.478)
High	High	115	18	2.263 (0.891-5.743)	117	14	**6.756 (1.422-32.093)**	60	33	1.420 (0.722-2.794)
PRS	2nd trimester									
Low	Low	134	12	1.000	146	4	1.000	68	24	1.000
Low	High	108	3	0.311 (0.083-1.172)	109	0	-	61	32	1.771 (0.912-3.438)
High	Low	122	13	1.195 (0.514-2.779)	123	8	2.286 (0.655-7.980)	56	30	1.515 (0.787-2.917)
High	High	107	15	1.571 (0.659-3.749)	112	12	**3.822 (1.118-13.062)**	47	34	**2.603 (1.311-5.166)**
PRS	3rd trimester									
Low	Low	122	8	1.000	129	2	1.000	61	29	1.000
Low	High	120	7	0.872 (0.283-2.683)	126	2	0.891 (0.116-6.868)	68	27	1.073 (0.554-2.078)
High	Low	116	16	2.424 (0.980-5.997)	119	12	**7.608 (1.627-35.569)**	44	33	1.534 (0.798-2.948)
High	High	113	12	1.412 (0.522-3.817)	116	8	3.368 (0.650-17.457)	59	31	1.559 (0.897-3.044)
PRS	From birth to 1 year									
Low	Low	102	8	1.000	105	2	1.000	51	18	1.000
Low	High	139	7	0.640 (0.217-1.890)	149	2	0.606 (0.081-4.547)	77	38	1.509 (0.762-2.988)
High	Low	111	12	1.438 (0.555-3.724)	118	8	3.593 (0.736-17.530)	34	27	2.104 (0.994-4.454)
High	High	118	16	1.692 (0.674-4.248)	117	12	4.724 (0.994-22.460)	69	37	1.770 (0.882-3.553)
PRS	The latest 12 months									
Low	Low	80	7	1.000	81	2	1.000	44	16	1.000
Low	High	124	8	0.700 (0.241-2.037)	134	2	0.570 (0.078-4.168)	72	35	1.364 (0.669-2.781)
High	Low	88	13	1.559 (0.582-4.176)	96	9	3.549 (0.735-17.139)	36	26	2.150 (0.987-4.682)
High	High	107	12	1.206 (0.446-3.261)	105	10	3.663 (0.765-17.524)	62	38	1.781 (0.869-3.648)

aOR, adjusted odds ratio; 95% CI, 95% confidence interval

*Adjusted for sex, parental history of allergy, maternal educational level, maternal environmental tobacco smoke exposure during pregnancy, maternal pet ownership during pregnancy, BMI of the child at asthma diagnosis, and NO_2 exposure

7. Critical windows of prenatal PM_{10} exposure on current asthma and BHR modified by children's sex and PRS

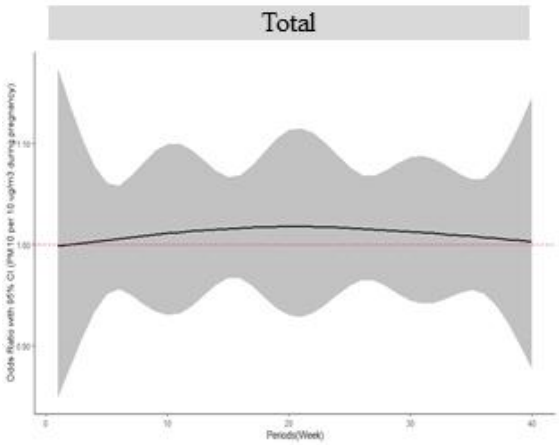
There were no statistically significant PM_{10} exposure period for the development of current asthma, but high PM_{10} exposure at 5-11 weeks of gestation and 35-36 weeks of gestation increased the risk of BHR (Figure 2). When examining the modification effect according to sex, the critical window of PM_{10} exposure was not significantly associated with current asthma in both males and females (Figure 3a), whereas BHR was more prevalent when exposed PM_{10} was high at 6-8 weeks of gestation in males and at 6-7 weeks of gestation in females (Figure 3).

Current asthma was not associated with PM_{10} exposure according to PRS (Figure 4a), but BHR was associated with high PM_{10} exposure at 37 weeks of gestation in high PRS group, whereas high PM_{10} exposure at 6-8 weeks of gestation was significantly associated with BHR in low PRS group (Figure 4b).

Prenatal PM_{10} exposure was not associated with current asthma according to the stratified groups by PRS and sex (Figure 5a). When examining the modification effect according to sex and PRS, PM_{10} exposure at 13-14 weeks of gestation had an effect on BHR in females with lower PRS (Figure 5b).

Figure 2. Associations between prenatal PM_{10} exposure and current asthma and BHR

a) Current asthma



b) BHR

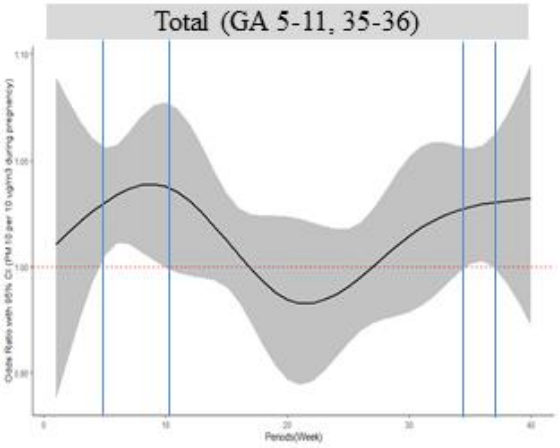
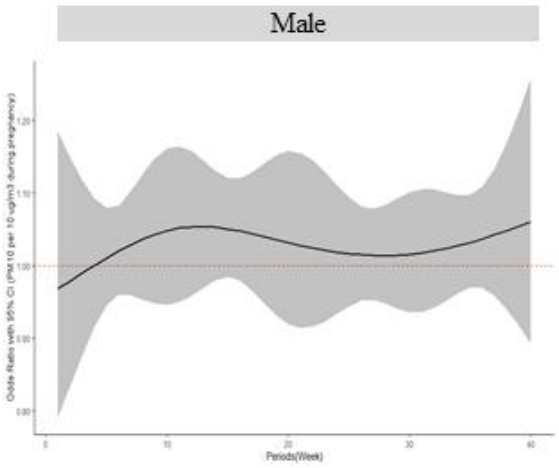
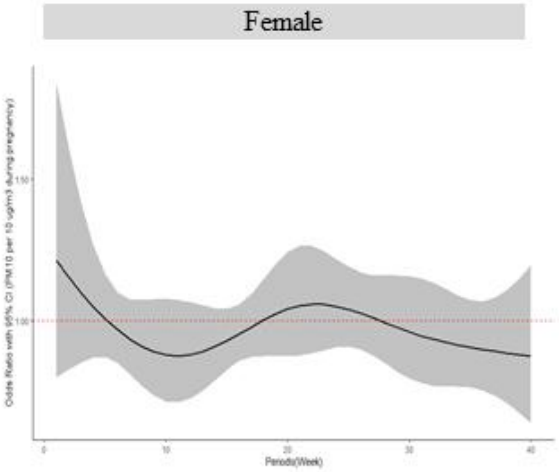


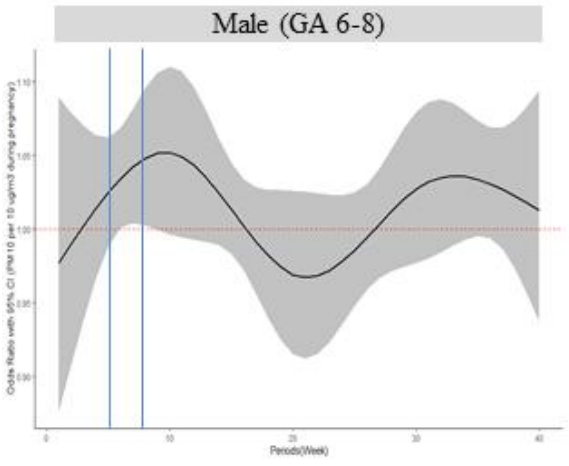
Figure 3. Associations between prenatal PM_{10} exposure and current asthma and BHR according to children's sex

a) Current asthma





b) BHR



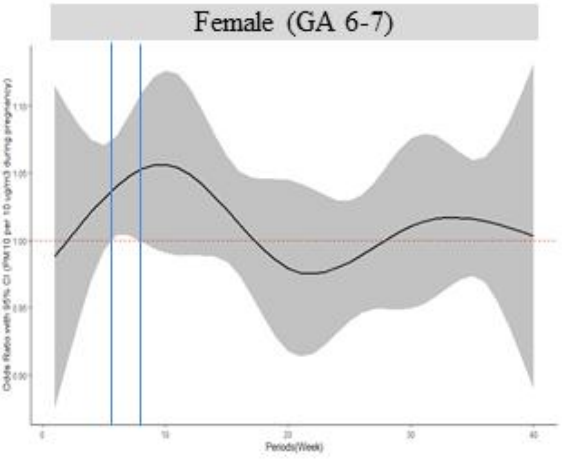
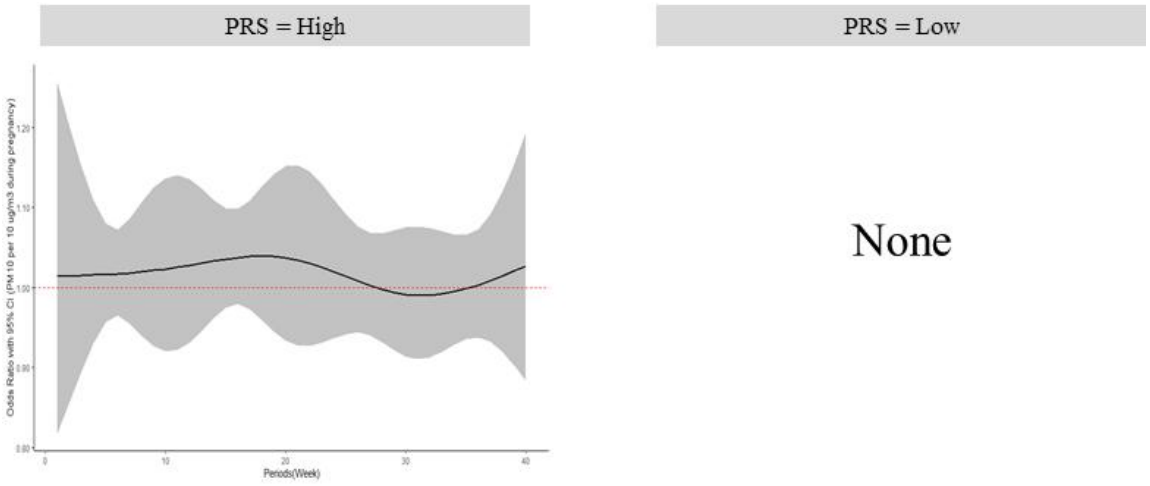


Figure 4. Associations between prenatal PM_{10} exposure and current asthma and BHR according to PRS

a) Current asthma



b) BHR

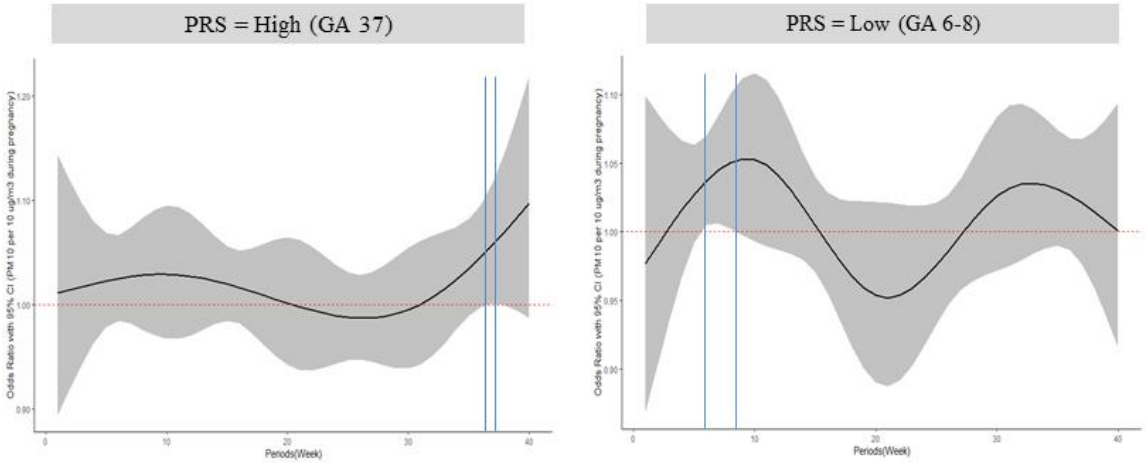
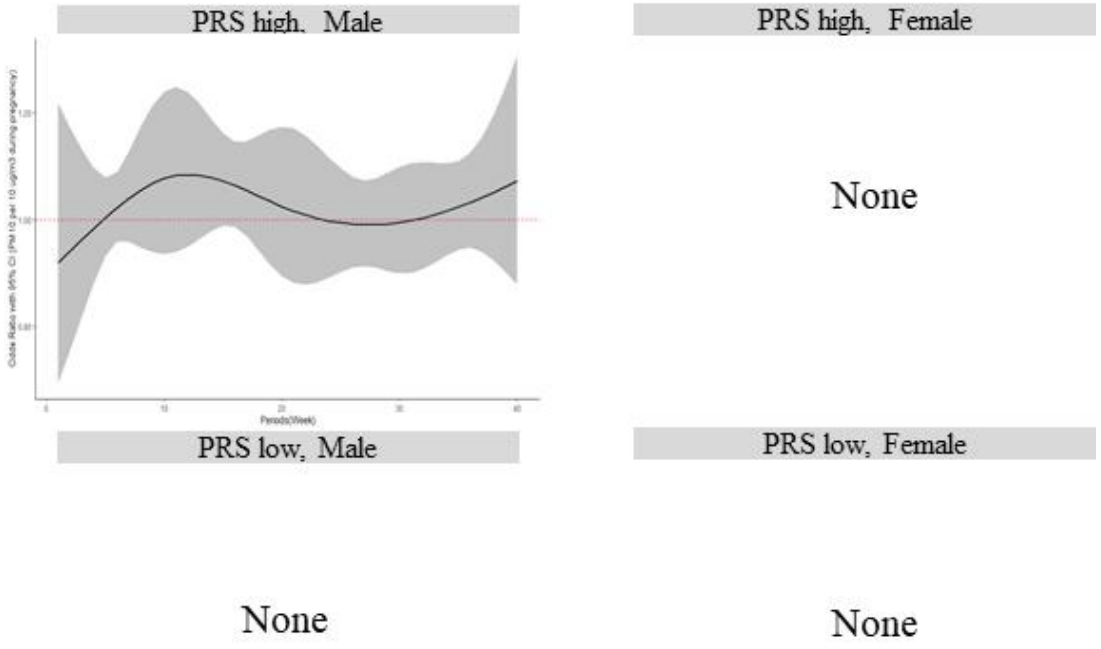
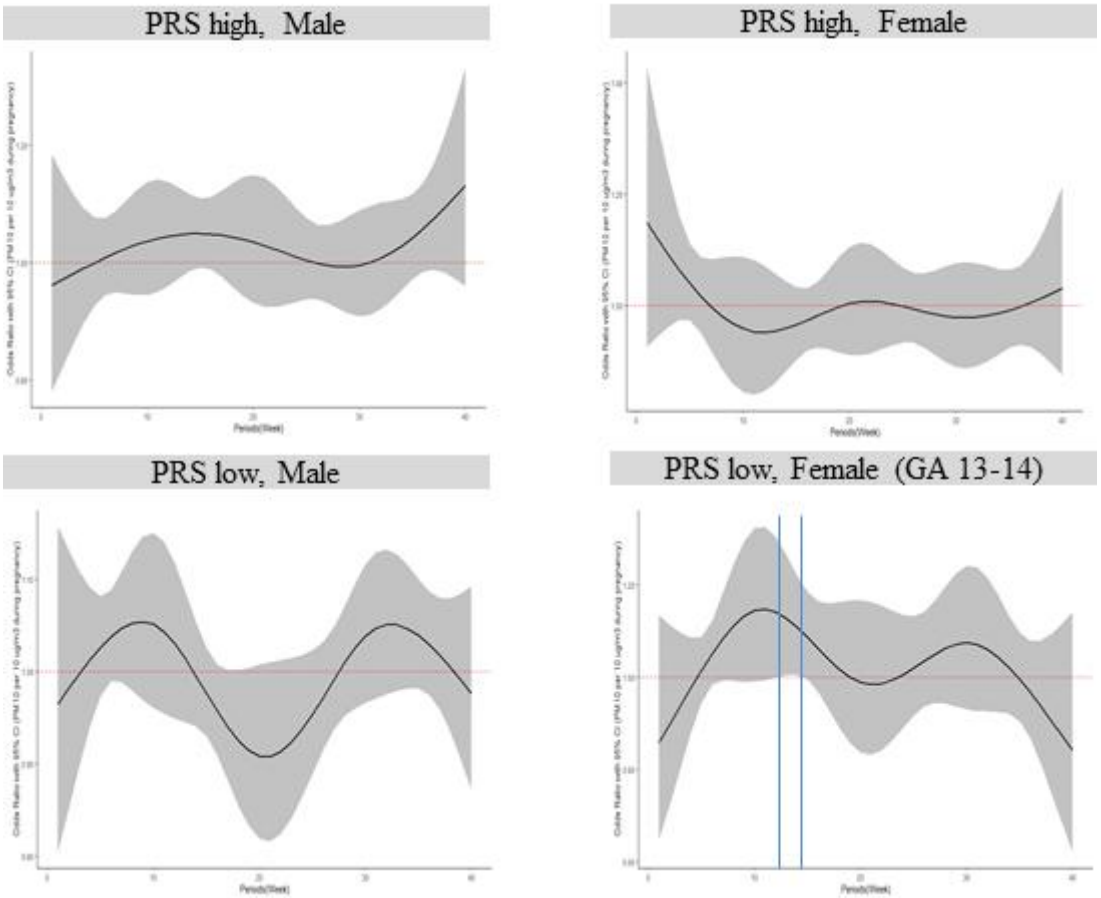


Figure 5. Associations between prenatal PM_{10} exposure and current asthma and BHR according to children's sex and PRS

a) Current asthma



b) BHR



8. Lung function at age 7 years stratified by PRS and PM_{10} exposure according on the exposure period

Table 7 showed the levels of pulmonary function performed at age 7 years in the groups stratified by PRS and PM_{10} exposure according to the exposure period. FVC (%)pred was the lowest in the high PRS/high PM_{10} during the total pregnancy period and 1st and 2nd trimester compared to the low PRS/low PM_{10} during each exposed period (P = 0.001, P= 0.003, P = 0.002, respectively). FEV_1 (%)pred, FEV1/FVC ratio, and $FEF_{25-75\%}$ (%)pred were not different by the stratified groups according to PRS and PM_{10} exposure depending on the period.

Table 7. Pulmonary function of children at age 7 years according to polygenic risk score (PRS) based on the weight of current asthma and prenatal PM_{10} exposure

PRS	PM_{10}	FVC (%)pred				FEV_1 (%)pred				FEV1/FVC (%)				$FEF_{25-75\%}$ (%)pred			
		N	Mean	SD	P-value*	N	Mean	SD	P-value*	N	Mean	SD	P-value*	N	Mean	SD	P-value*
PRS	Total pregnancy																
Low	Low	41	99.195 d	10.987	0.001	41	97.293	12.260	0.148	27	103.296	5.960	0.280	40	95.500	22.804	0.165
Low	High	59	95.441	10.694		59	95.610	10.806		38	105.395	6.879		51	103.157	26.776	
High	Low	43	96.581	11.925		43	96.047	12.596		37	102.351	18.312		42	97.214	21.824	
High	High	59	94.678 a	10.867		59	93.237	11.996		48	103.625	8.587		48	93.208	25.605	
PRS	1st trimester																
Low	Low	48	96.979	10.209	0.003	48	96.958	11.396	0.224	29	105.552	6.957	0.184	43	103.186	28.157	0.208
Low	High	52	96.981	11.634		52	95.692	11.470		36	103.694	6.173		48	96.750	22.219	
High	Low	45	98.400 d	10.903		45	96.022	11.645		36	100.278	19.239		37	93.892	25.465	
High	High	57	93.175 c	11.176		57	93.158	12.701		49	105.122	6.660		53	95.906	22.899	
PRS	2nd trimester																
Low	Low	42	99.143 d	10.821	0.002	42	97.071	11.286	0.230	28	102.536	6.872	0.142	36	95.750	22.662	0.270
Low	High	58	95.414	10.811		58	95.741	11.537		37	106.027	5.951		55	102.436	26.699	
High	Low	47	97.383	11.569		47	95.702	12.632		38	100.579	17.974		44	95.136	22.212	
High	High	55	93.855 a	10.920		55	93.327	11.960		47	105.085	8.330		46	95.022	25.597	
PRS	3rd trimester																
Low	Low	46	97.978	11.973	0.005	46	97.457	12.343	0.240	34	104.206	5.426	0.349	46	99.913	22.564	0.318
Low	High	54	96.130	9.968		54	95.315	10.536		31	104.871	7.671		45	99.667	28.013	
High	Low	44	94.341	11.947		44	94.727	12.719		41	103.220	17.721		43	96.279	17.599	
High	High	58	96.345	10.820		58	94.190	12.025		44	102.932	8.365		47	93.979	28.580	

SD, standard deviation

*Adjustment for sex, parental history of allergy, maternal educational level, maternal environmental tobacco smoke exposure during pregnancy, maternal pet ownership during pregnancy, BMI of the child at asthma diagnosis and outdoor NO_2 exposure

a : $p < 0.05$ compared with controlgroup (PRS low and PM_{10} low)

b : $p < 0.05$ compared with PRS low and PM_{10} high group

c : $p < 0.05$ compared with PRS high and PM_{10} low group

d : $p < 0.05$ compared with PRS high and PM_{10} high group

Discussion

In this study, we estimated the combined effect of genetic and environmental factors on the development of asthma and BHR. PRS and PM_{10} exposure were the factors considered important and each showed some effects on asthma and BHR, but the effects was more pronounced when the both factors were combined. As a result of the effort to search the vulnerable time window during pregnancy, it was found that PM_{10} exposure to the 1st and 2nd trimesters of pregnancy affects current asthma and BHR according to the method of analyzing the effect of PM_{10} exposure modified by gender and PRS. The modification effect according to sex and PRS by BDLIM, first trimester of pregnancy was a critical period on BHR. Furthermore, children with higher PRS and higher PM_{10} exposure during pregnancy, especially first and second trimesters showed the lowest FVC pred%. These results suggest that prenatal PM_{10} exposure in children with genetic susceptibility may affect on the lung growth and develop asthma and BHR in schoolchildren.

Asthma is the most prevalent chronic airway disease in children that is highly heritable. Genome-wide association studies (GWASs) of asthma have reported many risk loci, which have provided an understanding of the genetic association involved in asthma [7]. However, most GWASs and large meta-analyses of asthma have involved subjects of European-ancestry, inferences regarding the genetic architecture of asthma are based on observations in this population. Much less is known about the populations of Asian ancestry. As populations vary with respect to allele frequencies, patterns of linkage disequilibrium, and effect sizes of variants that underlie disease risk, inferences based on Europeans may have limited utility in other groups. Our group has been previously studied the genetic effects on asthma and we herein investigated the genetic effect on asthma using PRS, which could be one of the method. The most common polygenic approach is the weighted PRS approach in which a weighted PRS is calculated from a pre-selected number of genetic variants to define a person's individual genetic risk for disease development [11]. PRS allows for patient stratification and sub-phenotyping [48]. Moreover, PRS were successfully used in interaction analyses to examine the genetic susceptibility to air pollution-induced airway inflammation [49]. The use of PRS increases the power to detect GxE interactions in comparison to the common univariate single-variant approaches [50, 51]. PRS have been employed to summarize genetic effects among an ensemble of markers that do not individually achieve significance and to estimate the variance explained by a marker panel [11]. Furthermore, by combing SNPs of a certain biological pathway, PRS can be used as a simple statistical approach for the complex biological pathways through which environment-induced diseases might be caused [49]. PRS aggregate measured genetic effects and therefore increase the power to detect gene-environment interactions [12]. In this study, each gene, which we selected by the proven association with asthma, did not show an association with asthma or BHR, but PRS from these genes were significantly associated with asthma symptom, current asthma and BHR. As previous

reports have shown marginal effect of SNPs on asthma, each SNP may not show an association with asthma because of the small number of the asthma patients in this study. Multiple genes may affect on the development of asthma, therefore PRS would be a better way to evaluate the genetic effect on asthma. In addition, in cases where the expression of genes can vary depending on the environment, the method using PRS is considered to be effective because environmental factors thought to be related to disease development and the influence of various genes must be considered together. Therefore, we selected genes that have been reported to be associated with allergic inflammation and oxidative stress, which is involved in the mechanism of the effect of air pollution.

According to the particle size, pollutants can be categorized as gaseous and PM and the pollutants with the greatest impact on humans health are PM, which are commonly used as a measure of air quality [52]. Air pollution can be defined as the presence in the air of substances harmful to humans and is associated with a high risk for various cardiac and pulmonary diseases [53, 54]. Asthma is a chronic respiratory disease characterized by variable airflow obstruction, BHR, and airway inflammation and evidence suggests that air pollution has a negative impact on asthma outcomes in pediatric populations. A large number of reports of the association between exposure to traffic-related air pollution (TRAP) and subsequent development of childhood asthma have been published. A meta-analysis examining 41 studies involving the association between TRAP exposure and their risk of asthma incidence or lifetime prevalence in children and adolecsents showed significant associations for PM_{10}, $PM_{2.5}$, and NO_2 exposures and risk of asthma development [55]. In this study, the occurrence of BHR was also increased when PM_{10} exposure was high, particularly when it was exposed during pregnancy, especially second trimester. Previous studies have suggested that there must be a critical window of PM_{10} exposure and pregnancy will be one, that is associated with an increased risk of developing asthma [56]. Regarding the developmental origins of health and disease (DOHaD) hypothesis that noncommunicable disease originate from maternal exposure to unfavorable environment and have long-term effects influencing offspring susceptibility to disease later in adulthood [57], childhood asthma originate in fetal life and are triggered by air pollution in sensitive trimesters. PM is an important matter that has been known to have an harmful effect on the development of asthma. With the help of evolving analytic methods, a birth cohort study in Taiwan reported both prenatal and postnatal exposures to $PM_{2.5}$ were associated with later development of asthma by using a cox proportional hazard model combined with a distributed lag nonlinear model [58]. Whereas recent studies are reporting the effect of fine PM, we could only evaluate PM_{10} effect because $PM_{2.5}$ was measured from 2015 in Korea. Herein we evaluated PM_{10} effect according to the previous studies [55].

Genetic and environmental factors may jointly contribute to susceptibility clarifying the gene-environment interactions, which can be defined as a different effect of environmental exposure in disease risk in persons with different genotypes [59]. Gene-environment

interactions have been found to play an important role in the development of asthma in the genome-wide interaction analysis of air pollution exposure and childhood asthma [60]. Especially genes belonging to the oxidative stress, such as glutathione S-transferase (GST) family, are of particular interest, which is a potential pathway for air pollution effects [17]. There are evidences that have found that GSTP1 genotypes may constitute a susceptible population at increased risk of asthma associated with air pollution [61, 62].

Although there is a widespread knowledge that asthma and allergy results from multiple factors including gene–environment interactions, the vast majority of all studies on allergic diseases have not taken such interactions into consideration. Particularly challenging therein is how to model interactions and how to take high-order interactions (interaction between several factors) into consideration. Secondly, assessment of interactions demands large data sets and statistical power frequently poses a limitation. Nonetheless, studying the effects of various air pollutants on respiratory health, in relation to an individual's genetic setup, is attractive given the emerging evidence across epidemiological and experimental research. Evidence from gene-expression analyses suggests that children may be particularly susceptible to adverse effects of exposure, which underlines the potential for new policies designed to protect such vulnerable populations [63-65].

Exposure to air pollution during pregnancy affects respiratory health in different ways. The plausible biological mechanism of the effect of air pollution during pregnancy period could be explained by air pollutants inhaled by the pregnant mother penetrating the alveoli and diffusing into the placental barrier to act directly on the fetus or disturbed development of the immune system, which influences fetal lung function [34]. For the pregnancy period, our results revealed that the vulnerable time windows for the development of BHR caused by PM_{10} exposure coincided with pseudoglandular, and saccular stages of lung development. During the pseudoglandular stage (5–17 weeks), the conducting airways, including the terminal bronchioles, and initial acinar framework develop [67, 68]. The saccular stage (24 weeks-birth) allows the differentiation of alveolar epithelial cells into type I and type II pneumocytes [29, 69]. Air pollution exposures can lead to a disturbed alveolarisation and thus impairment of lung development and function after birth [70, 71]. In addition, repair mechanisms of the developing lung tissue are not as efficient as those of the mature lung and therefore, the immature lung is more vulnerable to respiratory insults [29].

Another aspect that has to be taken into account when investigating a leading mechanism underlying the link between prenatal air pollution exposure and childhood asthma is the role of the immune system including oxidative stress pathways and proinflammatory cytokine production [72-75]. The developing fetus is particularly vulnerable to oxidative stress because fetal antioxidant capabilities do not increase until the third trimester. Environmental exposure in early life can influence immune maturation and immune responses [76, 77]. A sub-study of the BILD cohort found an association between an attenuated expression of the cytokines IL-10 and 1L-1β in cord blood of unselected infants

and higher prenatal exposure towards PM_{10} [75]. Although further studies are needed, these results suggest that prenatal air pollution can play a role in the development of the immune system of the fetus. To evaluate the immune status of the participants according to PRS and PM_{10} exposure levels, measured total IgE, IL-13 and IFN-γ levels in cord blood were not different between the groups stratified by PRS and PM_{10} levels during pregnancy (data not shown).

The strength of this study could be that the results of this study can be generalized because it is a general population based prospective birth cohort study. To evaluate the factors that could be associated with the development of asthma, questionnaire and objective measurements were investigated. We conducted provocholine provocation test, which is an objective tool for the BHR, a characteristic of asthma. In addition, factors that have been found to be associated with asthma previously were investigated and considered as confounding factors for the adjustment to analyze the effect of genetic factors and PM_{10} on asthma and BHR. Furthermore, this study confirmed not only the independent effect of PRS and PM_{10} exposure on asthma and BHR, but also the joint effect of PRS and PM_{10}. In order to evaluate the result as the effect of PM_{10} in particular among air pollution, out door NO_2 exposure levels were simultaneously adjusted when analyzing.

In this study, we sought to find out the critical window of PM10 exposure that affects the development of asthma. As a way to find critical window of PM10 exposure, first we used averaged value over relatively broad time windows, such as total pregnancy period and trimesters. In an additional way, BDLIM model was used to identify the week that shows the association with asthma and BHR.

However, there are some limitations in this study. First, COCOA study enrolled a large population, but the analysis to investigate the PRS and PM_{10} effect on asthma and BHR was only performed in a small subgroup. We, herein, could find that PM_{10} exposure during pregnancy, especially early period, may have an effect on asthma and BHR, but the critical period of PM_{10} exposure was not consistent depending on the statistical method. Further large population study or replication study, and more advanced statistical methods are needed. Second, we evaluated PM_{10} effect on asthma and BHR, whereas recent studies found that fine PM has further harmful effect on respiratory health. Measures of $PM_{2.5}$ from national monitoring stations were performed from 2015 in Korea, therefore only PM_{10} levels were gathered in this study by LUR models. Third, to investigate the genetic effect on asthma, five SNPs were used for PRS in this study, which may cause selection bias. However these five SNPs were verified by our previous studies of the association with allergic disease. For the next step, it is necessary to accompany GWAS study in the future. Forth, further mechanism studies using placenta or cord blood that could show the effect of PM may be proceeded because the association has been shown in this study.

Conclusion

The joint effect found between PRS and PM_{10} exposure implies that individuals with both high PRS and higher concentration of prenatal PM10 are particularly at risk for developing current asthma and BHR from a general population based birth cohort. Critical PM10 exposure window on BHR was the first trimester (gestational period 5-11 weeks) using BDLIM model. It is generally agreed that the first trimester of pregnancy is the critical period for the development of the fetus, because it is the time when the organogenesis occurs. Therefore, PM10 exposure during the first trimester could result in increased risk of asthma or BHR.

Future research are needed to confirm these effects and to understand the mechanisms underlying the joint effects of prenatal PM_{10} exposure and genetic susceptibility.

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국문요약

연구목적: 유전적 요인과 PM_{10}은 천식 및 기관지과민성 발생에 영향을 미치지만, 이것들의 공동효과에 관한 연구는 부족하다. 따라서 본 연구에서는 천식 및 기관지과민성에 대한 다유전자위험점수와 PM_{10}의 공동효과를 평가하고, PM_{10}이 질환 위험에 가장 영향을 미치는 중요한 시기를 찾고자 하였다.

대상 및 방법: 소아 호흡기·알레르기질환 장기추적 코호트 (COCOA)에 등록된 영유아 중 연구 조건을 만족하는 총 643명의 영유아가 본 연구에 포함되었다. 6세부터 10세까지 1) 천식 증상; 최근 12개월 동안 천식 증상이 있는 아동, 2) 현증 천식; 최근 12개월 동안 천식 증상이 있고, 태어나서 한 번이라도 의사에게 천식 진단을 받은 아동을 천식으로 정의하였으며, 프로보콜린 자극 검사의 PC_{20}값이 8 mg/mL 이하인 경우를 기관지과민성으로 정의하였다. 다유전자위험점수는 *IL-13* (rs20541), *NRF2* (rs6726395), *GSTP1* (rs1695), *GSDMB* (rs7216389) and *TSLP* (rs3806933) 로 계산하였다. 다중 로지스틱 회귀분석이 질환발생 위험 확인에 사용되었으며, Bayesian distributed lag interaction model (BDLIM)을 사용하여 PM_{10} 노출에 대한 중요한 시기를 확인하였다.

결과: 가중 PRS는 천식 증상(aOR 1.973, 95% CI 1.016-3.832), 현증 천식(aOR 5.369, 95% CI 1.793-16.078) 및 기관지과민성(OR 1.711, 95% CI 1.093-2.806)에 유의한 연관성을 보였다. 기관지과민성은 임신 중 PM_{10} 노출이 높을 때(aOR 2.145, 95% CI 1.338-3.439)와 임신 중기 PM_{10} 노출이 높을 때(aOR 1.822, 95% CI 1.114-2.980) 각각 위험이 증가하였다. 다유전자위험점수가 높고, 산모가 임신 중 PM_{10} 노출이 높으면 영유아의 현증천식 위험을 높이는 것으로 나타났다(aOR 6.501, 95% CI 1.361-31.044). 다유전자위험점수가 높고, 산모가 임신 초기 및 중기 때 PM_{10} 노출이 높으면 영유아의 현증천식 위험이 증가하였으며(각각 aOR 6.756, 95% CI 1.422-32.093; aOR 3.822, 95% CI 1.118-13.062), 다유전자위험점수가 높고, 산모가 임신 중기 때 PM_{10} 노출이 높으면 영유아의 기관지과민성 위험이 증가하였다(aOR, 2.603, 95% CI, 1.311-5.166). BDLIM 결과에 따르면, PM_{10} 노출이 질환에 영향을 미치는 중요한 시기는 임신 5-11주였고, 남아의 경우 임신 6-8주, 여아의 경우 임신 6-7주로 나타났다. 다유전자위험점수가 낮은 군에서 PM_{10} 노출이 기관지과민성에 영향을 미치는 중요한 시기는 임신 6-8주로 나타났고, 성별로 층화하여 분석하였을 때, 여아에서는 13-14주가 중요한 시기로 나타났다.

결론: 높은 다유전자위험점수와 임신 동안의 높은 PM_{10} 노출이 현증천식과 기관지과민성 위험을 증가시켰다. 특히, 임신 초기와 중기의 높은 PM_{10} 노출이 다유전자위험점수와 성별을 고려하였을 때에도 현증천식과 기관지과민성에 영향을 미쳤고, 기관지과민성에 대한 중요한 시기는 5-11주였다. 일반적으로 임신 초기는 태아의 발달에 중요한 시기이며, 이 시기 동안의 PM_{10} 노출이 천식과 기관지과민성에 영향을 미치는 것을 확인할 수 있었다.

중심단어: 천식, 기관지과민성, 다유전자위험점수, 임신, PM_{10}, Bayesian distributed lag interaction model