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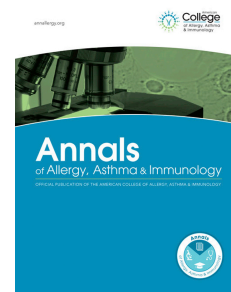
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Key words: PM_{2.5}, vitamin D, atopic dermatitis, prenatal, methylation

Abbreviations

aOR: adjusted odds ratio

AHR: aryl-hydrocarbon receptor

AHRR: aryl-hydrocarbon receptor repressor

AD: atopic dermatitis

BDLIM: Bayesian distributed lag interaction model

COCOA: COhort for Childhood Origin of Asthma and allergic diseases

CI: confidence interval

CB: cord blood

LUR: land use regression

PM_{2.5}: particulate matter with an aerodynamic diameter ranging from 0.1 to 2.5 μm

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Background: The effects of prenatal PM_{2.5} and vitamin D on the atopic dermatitis (AD) phenotypes have not been evaluated. DNA methylation and cord blood (CB) vitamin D could represent a plausible link between prenatal PM_{2.5} exposure and offspring's AD.

Objective: To determine the critical windows of prenatal PM_{2.5} exposure on the AD phenotypes, if vitamin D modulated these effects, and if placental DNA methylation mediated these effects on offspring's AD.

Methods: Mother–child pairs were enrolled from the COCOA birth cohort. PM_{2.5} was estimated by land-use regression models and CB vitamin D was measured by chemiluminescence immunoassay. AD was identified by parental report of a physician's diagnosis. We define four AD phenotypes according to onset age (by age 2) and persistence (by age 3): early onset transient and persistent, late onset, and never. Logistic regression analysis and Bayesian distributed lag interaction model were used. DNA methylation microarray was analyzed using an Infinium Human Methylation EPIC BeadChip in placenta.

Results: PM_{2.5} exposure during the first trimester of pregnancy, especially weeks 6–7 of gestation, was associated with early onset persistent AD. This effect increased in children with low CB vitamin D, especially in those with PM_{2.5} exposure during weeks 3–7 of gestation. AHRR(cg16371648), DPP10(cg19211931), and HLADRB1(cg10632894) were hypomethylated in AD children with high PM_{2.5} and low CB vitamin D.

Conclusion: Higher PM_{2.5} during the first trimester of pregnancy and low CB vitamin D affected early onset persistent AD, and the most sensitive window was weeks 6–7 of gestation. Placental DNA methylation mediated this effect.

1 Introduction

2 The prevalence of atopic dermatitis (AD) has increased in the past two decades. In Korea, the
3 prevalence of AD in children aged 6–7 years, defined as experiencing symptoms in the last 12
4 months, increased from 8.8% in 1995 to 12.7% in 2015.^{1,2} The onset and persistence of AD
5 are heterogeneous.³ Early onset persistent AD is more closely associated with increased risk
6 of asthma and allergic rhinitis at later ages than are late onset or early onset transient AD.^{4,5}
7 Different environmental factors could account for the heterogeneity of AD onset and
8 persistence.^{3,6} Identifying specific environmental factors that affect the onset and persistence
9 of AD could prevent the development of asthma and allergic rhinitis, which often develop at
10 later ages in infants with AD.

11 Because the skin is the primary tissue exposed to air pollutants, air pollutants are
12 considered to be a risk factor for AD.⁷ Prenatal period is considered as critical period for the
13 effects of air pollutants exposures on the development of allergic diseases.⁸⁻¹⁰ The effect of
14 air pollutant exposure on allergic diseases could be dependent on the timing of exposure
15 during fetal development stages.¹¹⁻¹³ Among air pollutants, particulate matter with an
16 aerodynamic diameter ranging from 0.1 to 2.5 μm ($\text{PM}_{2.5}$) is a growing public health concern
17 and is considered to be an important environmental risk factor for allergic disease. Little is
18 known regarding the critical windows of $\text{PM}_{2.5}$ exposure during pregnancy for the onset and
19 persistence of AD. Therefore, additional studies are required, which are critical for the
20 prevention of childhood allergic diseases.

21 Several studies have suggested an association between $\text{PM}_{2.5}$ exposure and vitamin D
22 deficiency. Particulate matter exposure during pregnancy is associated with maternal vitamin
23 D deficiency and lower cord blood (CB) vitamin D levels.^{14,15} Because vitamin D suppresses
24 air pollutant-induced inflammation and oxidative stress,¹⁶⁻¹⁸ vitamin D deficiency increases

the adverse effects of air pollutant exposure.^{19,20} The association between prenatal PM_{2.5} exposure and vitamin D deficiency in relation to offspring's AD is unknown.

The placenta is the primary interface between the mother and developing fetus.²¹ Environmental exposures, such as air pollution, could interfere with placental DNA methylation during critical periods in fetal development, with long-term health implications.²² Prior studies have reported the association between PM_{2.5} exposure and placental DNA methylation.²³⁻²⁵ However, no prior studies have investigated the contribution of placental DNA methylation to the association between gestational PM_{2.5} exposure, vitamin D deficiency, and offspring's AD risk.

Therefore, the aims of the present study were to determine the critical windows during gestation for the effects of PM_{2.5} exposure on the onset and persistence of offspring's AD, to assess the modifying effect of CB vitamin D on PM_{2.5}-induced AD, and to identify the role of placental methylation changes in modulating the effects of prenatal PM_{2.5} exposure and vitamin D deficiency on offspring's AD.

Methods

Study population

The present study was a cohort study using the COCOA (COhort for Childhood Origin of Asthma and allergic diseases), an ongoing prospective general population-based birth cohort study at five hospitals and eight public health centers in Seoul, Korea. The study protocol has been described previously.²⁶ Children that were followed up for the first 3 years of life and had available PM_{2.5} exposure data from the prenatal period to 3 years of age were included in the analysis (eFigure 1). The study protocol was approved by the Institutional Review Boards of Asan Medical Center (IRB No. 2008-0616), Samsung Medical Center (IRB No. 2009-02-021), Severance Medical Center (IRB No. 4-2008- 0588), CHA Medical Center (IRB No. 2010-010), and Seoul National University Hospital (IRB No. H-1401-086-550). Written informed consent was obtained from the parents of each infant.

Assessment of AD phenotypes

AD in the first 3 years of life was defined as parental report of physician-diagnosed AD at the ages of 6 months, 1, 2, and 3 years of age. Parental report of physician-diagnosed AD was based on a positive answer to the written question: ‘Was your child diagnosed with AD by a doctor in the last 6 months/year?’ AD phenotypes were characterized by age of AD onset and persistence of AD from birth to 3 years of age. We defined four AD phenotypes as follows: (1) early onset transient, with onset within 2 years of age and no further symptoms after 3 years of age; (2) early onset persistent, with onset within 2 years of age and persistence of symptoms after 3 years of age; (3) late onset, with onset of symptoms after 2 years of age; and (4) absence of AD.^{4,5}

Assessment of PM_{2.5} exposure

Estimated environmental PM_{2.5} levels were compiled from air monitoring data that were recorded routinely by 37 monitoring stations in study area (Seoul) operated by the Department of Environment, Republic of Korea. Residential addresses were geocoded and land use regression (LUR) models were applied to predict outdoor PM_{2.5} levels at the subjects' residential addresses during gestation.²⁷ To build a LUR model, measured PM_{2.5} levels at the sampling locations were regressed against the GIS variables.²⁸ Independent variables included traffic intensity, road density, land use, population density, and altitude. If the subjects' address changed during the exposure period, the new address was used for assessment of PM_{2.5} exposure.

Assessment of vitamin D

CB was collected from the umbilical vein at the time of delivery. 25[OH]D concentrations (ng/mL) were measured using a chemiluminescence immunoassay (DiaSorin, Saluggia, Italy). 25[OH]D was dichotomized using the median concentration (14.15 ng/mL) as either "high" or "low" vitamin D level. Dichotomized 25[OH]D values were used in analyses.²⁹

Placental DNA methylation and gene expression microarray

We determined the impact of PM_{2.5} exposure and CB 25[OH]D level on prenatal epigenetics with regard to offspring's AD using differential DNA methylation and gene expression assessments in eight groups (n=6/group, eTable 1).

DNA was extracted from placental tissues using a Gentra Puregene Blood Kit (QIAGEN, Redwood City, CA) in accordance with the manufacturer's protocol. DNA quality was assessed using the picogreen method with synergy HTX. DNA samples that passed quality control were then analyzed using the Infinium Human Methylation EPIC BeadChip (Illumina, San Diego, CA) as described previously.³⁰ Briefly, DNA samples were treated with sodium

bisulfite conversion using the EZ DNA Methylation-Lightning kit (Zymo Research, Irvine, CA).

For statistical significance, CpG sites with $|\Delta\beta| > 0.2$ and $p < 0.05$ were considered to be differentially methylated.

Total placental RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized using a GeneChip Whole Transcript Pico Reagent Kit (Affymetrix, Santa Clara, CA, USA) and hybridized to the Human Gene 2.0 ST GeneChip Array (Affymetrix) in accordance with the manufacturer's protocol. For transcriptome analysis, array data export processing and analysis were performed using Affymetrix® GeneChip Command Console® Software. Data were summarized and normalized with the robust multi-average (RMA) method implemented in Affymetrix® Power Tools. Statistical significance of gene expression was determined using an independent t-test ($p < 0.05$) and relative fold change > 2 .

Statistical analysis

Associations between $PM_{2.5}$ exposure and AD phenotypes were evaluated with a logistic regression model using SPSS 24.0 software (SPSS Inc., Chicago, IL). Separate analyses were conducted to evaluate the effects of $PM_{2.5}$ exposure during the three trimesters of pregnancy. The prenatal period was divided into three trimesters as follows: weeks 1–13 (first), weeks 14–27 (second), and weeks 28–40 (third). $PM_{2.5}$ levels were divided by tertile, and tertile levels were used in logistic regression analysis. We examined the effect modification of the association of AD phenotypes with $PM_{2.5}$ exposure by stratifying the study population according to CB 25[OH]D level. Adjustments included the following: sex, parental history of allergic disease, maternal age at delivery, maternal education level, and maternal exposure to

secondhand smoke during pregnancy. Results are expressed as adjusted odds ratios (aORs) and 95% confidence intervals (CIs). *P*-value < 0.05 considered to be statistically significant.

To identify the critical windows during the prenatal period for the effects of prenatal PM_{2.5} in relation to AD phenotypes, and subsequently to examine the effect modification by vitamin D, we used a Bayesian distributed lag interaction model (BDLIM).³¹ Significant critical windows were identified as weeks during pregnancy with a statistically significant association, as previously described.³² Sex, parental history of allergic disease, maternal age at delivery, maternal education level, and maternal exposure to secondhand smoke during pregnancy were included in the final model. These analyses were conducted using R statistical software (v3.5.1, Vienna, Austria).

Results

Subject characteristics

The prevalence of early onset persistent, early onset transient, and late onset AD were 12.0%, 21.6%, and 3.0%, respectively (Table 1). The mean concentration of PM_{2.5} exposure during the course of the entire pregnancy period was $27.69 \pm 5.80 \mu\text{g}/\text{m}^3$. Average PM_{2.5} exposure levels were similar across trimesters ($28.47 \pm 8.32 \mu\text{g}/\text{m}^3$ during the first trimester, $27.03 \pm 7.62 \mu\text{g}/\text{m}^3$ during the second trimester, and $27.67 \pm 7.86 \mu\text{g}/\text{m}^3$ during the third trimester). Mean CB 25[OH]D level was $18.34 \pm 24.89 \text{ ng}/\text{ml}$ (Table 1).

Association between PM_{2.5} exposure and AD phenotypes

In logistic regression analysis, PM_{2.5} exposure during the first trimester of pregnancy was significantly associated with early onset AD (aOR: 1.41, 95% CI: 1.00–2.00) (Table 2). There were no significant associations between PM_{2.5} exposure during any trimester of pregnancy and other AD phenotypes (Table 2). In BDLIM analysis, weeks 5–8 of gestation were the sensitive PM_{2.5} exposure window for early onset AD, and weeks 6–7 of gestation were the sensitive PM_{2.5} exposure window for early onset persistent AD (Figure 1a and 1c). There were no sensitive PM_{2.5} exposure windows for early onset transient AD (Figure 1b). The sensitive PM_{2.5} exposure window for late onset AD was weeks 24–30 of gestation (Figure 1d).

Modifying effect of vitamin D on PM_{2.5} exposure-induced AD phenotypes

In the logistic regression analysis, CB 25[OH]D level was not associated with any AD phenotypes (Table 2). However, low CB 25[OH]D increased the effect of PM_{2.5} exposure during the first trimester of pregnancy on early onset AD (aOR: 2.37, 95% CI: 1.19–4.71, p value 0.01), especially early onset persistent AD (aOR: 3.53, 95% CI: 1.34–9.33, p value 0.01) (Figure 2-1a and 2-3a). CB 25[OH]D did not modify the effect of PM_{2.5} exposure during any

trimester of pregnancy on early onset transient AD (Figure 2-2).

In the BDLIM, PM_{2.5} exposure during weeks 3–7 of gestation increased the risk of early onset persistent AD in children with low CB 25[OH]D. No such window was detected in children with high CB 25[OH]D (Figure 3c). Significant sensitive windows for other AD phenotypes were not identified (Figure 3a, 3b and 3d).

Placental methylation changes

A total of 195 CpG sites (97 hypermethylated, 98 hypomethylated) exhibited significantly different methylation patterns in subjects with high PM_{2.5} exposure, low CB 25[OH]D, and AD relative to subjects with low PM_{2.5} exposure, high CB 25[OH]D, and without AD (data not shown). A distinctive clustering between the two groups was observed (Figure 4). We then compared differentially methylated CpG sites among the eight study groups (eTable 1) and identified three genes that significantly differed in subjects with high PM_{2.5}, low CB 25[OH]D, and AD. *AHRR* (cg16371648, $\Delta\beta = -0.367$, $p = 0.026$), *DPP10* (cg19211931, $\Delta\beta = -0.263$, $p = 0.013$), and *HLA-DRB1* (cg10632894, $\Delta\beta = -0.318$, $p = 0.026$) were significantly hypomethylated in these subjects (Table 3). We also performed transcriptome analysis to confirm the gene expression level associated with *AHRR*, *DPP10* and *HLA-DRB1*. We observed the differential gene expression of *AHR*. *AHRR* acts as a feedback modulator by repressing *AHR*-dependent gene expression. Placental expression of the *AHR* and the *AHR*-related target genes *OBSCN*, *LRCH1*, *NMRAL1*, *ZNF101*, *PCDHB8*, and *NPR2* were lower in subjects with high PM_{2.5}, low CB 25[OH]D, and AD compared with the four study groups (Table 4). However, gene expression associated with *DPP10* and *HLA-DRB1* did not show any difference.

Discussion

The present study revealed that the critical PM_{2.5} exposure window for early onset persistent AD was 6–7 weeks of gestation, but not in other phenotypes of AD. Low CB vitamin D exacerbated this effect, as the critical exposure windows increased to 3–7 weeks gestation in subjects with PM_{2.5} exposure and low CB vitamin D. Because AD is not fully cured in terms of their treatment, identifying critical windows of PM_{2.5} exposure and the modifying factors regarding phenotypes of AD will be important for effective prevention strategies for AD and other allergic diseases. Placental hypomethylations of the *AHRR*, *DPP10*, and *HLA-DRB1* were observed in subjects with high PM_{2.5}, low CB 25[OH]D, and AD, suggesting that these changes in methylation were potential mechanisms for the association between prenatal PM_{2.5} exposure and vitamin D and offspring's AD. This study shows a possible mechanism explaining the association between prenatal PM_{2.5} exposure and offspring's AD, suggesting the possibility of placental modification of DNA methylation by PM_{2.5} exposure during pregnancy.

Transient eczema during infancy is common but early onset persistent AD is more associated with increased risk of asthma and allergic rhinitis at later ages.⁴⁻⁵ Our findings suggest an association between PM_{2.5} exposure during the first trimester of pregnancy and early onset persistent AD and are generally consistent with prior studies of other air pollutants, such as NO₂, PM₁₀, and CO.¹¹⁻¹³ However, using averaged exposures over relatively broad time windows (trimesters or the entire pregnancy) can potentially limit the precise identification of critical exposure windows. Therefore, to fully evaluate the effect of prenatal PM_{2.5} exposure on offspring's AD and prevent other allergic diseases in addition to AD later in life, analysis of the onset and persistence of AD in a prospective birth cohort study with a more powerful method of identifying critical exposure windows is needed. The present study

not only revealed the association between $PM_{2.5}$ exposure during the first trimester of pregnancy and offspring's AD, but also identified that 6–7 weeks of gestation is the critical windows for the effect of prenatal $PM_{2.5}$ exposure on early onset persistent AD using data from a prospective birth cohort study and a BDLIM model.

In the present study, low CB vitamin D exacerbated the adverse effect of $PM_{2.5}$ exposure during the first trimester of pregnancy on early onset persistent AD. Our prior COCOA study identified that CB vitamin D deficiency increased the risk of offspring's AD.²⁹ Further, prior studies have suggested that prenatal particulate matter exposure is associated with prenatal vitamin D deficiency. Prenatal $PM_{2.5}$ and PM_{10} exposure are associated with decreased maternal plasma and CB vitamin D.^{14,15} Further, vitamin D deficiency makes asthmatic children more susceptible to the adverse effects of air pollution exposure.^{19,20} However, the modifying effect of vitamin D level on $PM_{2.5}$ -induced AD had not previously been evaluated. Our results suggest that low vitamin D during prenatal period may augment the effect of $PM_{2.5}$ exposure during first trimester on the development of AD, leading to the persistency of AD.

Ambient particulates can be translocated directly towards the fetus.³³ Transplacental exposures to $PM_{2.5}$ could directly affect the developing fetus through DNA damage, disruption of DNA transcription and damage to cellular molecules.³⁴ Transplacental exposure to $PM_{2.5}$ could also produce reactive oxygen species, resulting in damage to the skin barrier.⁷ Specifically, $PM_{2.5}$ exposure during first trimester could impair skin barrier function through inhibition of filaggrin expression and reactive oxygen species generation in keratinocytes.^{35,36} Skin barrier dysfunction at 1 year of age and increased $PM_{2.5}$ and PM_{10} exposure during the first trimester affect early onset persistent AD.³⁷ $PM_{2.5}$ exposure also altered CB IgE and lymphocyte distributions, with effects dependent on the exposure time window during

gestation.^{38,39}

Prenatal PM_{2.5} exposure induces oxidative stress and systemic inflammation in the placenta, which could result in placental insufficiency with decreased transport of nutrients and oxygen to the developing fetus.⁴⁰ Recent evidences indicated that PM_{2.5} exposure during first trimester associated with placental methylation changes.^{23,24} Placental DNA methylation changes induce abnormal fetal development, resulting in persistent changes in organ function, leading to development of disease.²⁵ Placental DNA methylation changes could be possible mechanisms underlying the skin barrier dysfunction and immunologic changes caused by PM_{2.5} exposure.⁴¹ The first trimester is critical in the development of dermal systems, as the fetal skin structures develop rapidly.¹² Therefore, PM_{2.5} exposure during first trimester could result in increased risk of AD later in life.

Meanwhile, vitamin D contributes to maintenance of skin barrier function by regulating keratinocyte proliferation and differentiation, and synthesizing skin protein.^{42,43} Vitamin D also have immunoregulatory activity, which may influence of allergic diseases.^{44,45} And vitamin D modify the adverse effects of air pollutant-induced inflammation and oxidative stress.¹⁶⁻¹⁸ Thus, low vitamin D during prenatal period may augment adverse effect of PM_{2.5} exposure during first trimester leading to the persistency of AD.

In the present study, we found that methylation changes in the placental *AHRR*, *DPP10*, and *HLA-DRB1* were associated with prenatal PM_{2.5} exposure, CB vitamin D, and offspring's AD. Aryl-hydrocarbon receptor repressor (AHRR) is an inhibitor of aryl-hydrocarbon receptor (AHR).⁴⁶ AHR is a transcription factor that responds to synthetic and naturally occurring chemicals by regulating expression of several genes with toxic or protective effects.⁴⁶ Importantly, AHR regulates the responses to hydrocarbons and xenobiotic metabolism.⁴⁶ Polycyclic aromatic hydrocarbons, which are associated with the toxic effects of PM_{2.5},

activate AHR.⁴⁷ AHR activation upregulates expression of AHRR.⁴⁶ AHR is important not only for xenobiotic metabolism, but also regulates genes involved in cell development and the immune response. AHR activation skews the Th1/Th2 balance toward Th1 dominance, boosts Treg cell differentiation, and induces Th17 cell polarization.^{46,48} AHR is expressed in Langerhans cells, and AHR-deficient Langerhans cells do not mature or function properly.^{49,50} The present study suggested that placental *AHRR* gene was hypomethylated in subjects with high PM_{2.5} exposure, low CB vitamin D, and AD compared with subjects with low PM_{2.5} exposure, high CB vitamin D, and without AD. *AHR* gene expression decreased in subjects with high PM_{2.5} exposure, low CB vitamin D, and AD. Thus, placental *AHRR* hypomethylation could suppress expression of AHR, decreasing AHR signaling and disrupting the immune response and skin development, which could modulate the increased risk of offspring's AD.

DPP10 encodes a dipeptidyl peptidase that cleaves terminal dipeptides from cytokines and chemokines. *DPP10* potentially modulates inflammation by regulating the activities of cytokines and chemokines in this manner.⁵¹ *DPP10* is associated with serum IgE, eosinophils, asthma, and allergies.^{51,52} *HLA-DR*, part of the *HLA* class II, is associated with total serum IgE and increased susceptibility to allergies.⁵³ *HLA-DRB1* polymorphisms are associated with AD in Korean children.^{54,55} In the Pediatric Eczema Elective Registry (PEER) study, *HLA-DRB1* was associated with increased prevalence and persistence of AD.⁵⁶ While we report hypomethylation in *AHRR*, *DPP10*, and *HLA-DRB1*, the expected change in protein expression was limited to AHR associated with *AHRR*. Moreover, there is no overlap between differentially methylated genes and differentially expressed genes. Because of the presence of variability for individual genes, these differences may be an effect by genomic location, time points, tissue and environmental conditions.^{57,58} Therefore, how placental

AHRR, *DPP10*, and *HLA-DRB1* hypomethylation, which occur as a consequence of prenatal PM_{2.5} exposure and vitamin D deficiency, influence the development and persistence of AD in the offspring remains incompletely understood. Further studies are required to better elucidate the association between placental *AHRR*, *DPP10*, and *HLA-DRB1* methylation, AD, and PM_{2.5} exposure.

A major strength of the study is its design as a general population-based, prospective birth cohort study. We assessed AD at multiple time points throughout early childhood, providing more robust results than a single time point assessment, and allowing analysis of AD phenotypes. In addition to logistic regression analysis of PM_{2.5} exposure during each trimester, we used BDLIM and obtained similar findings from each statistical approach. This allowed more precise identification of critical windows of PM_{2.5} exposure during pregnancy. Furthermore, we evaluated the association between prenatal PM_{2.5} exposure and vitamin D deficiency and placental methylation associated with offspring's AD. Identifying the specific functions of methylation in the human placenta and understanding the effects of PM_{2.5} exposure and vitamin D deficiency could contribute to understanding the mechanisms of PM_{2.5} exposure and vitamin D deficiency-induced AD development and persistence of AD.

The study has several limitations that should be acknowledged to avoid its overinterpretation. First, we were unable to use individual and continuous direct PM_{2.5} monitoring in this large general population. And our exposure modeling used only residential address to estimate levels of PM_{2.5}. Although exposure modeling is widely used to estimate exposure levels in epidemiologic studies, some misclassification of PM_{2.5} exposure is possible with this system. Considering exposure from other places (e.g. employment address and transportation) or measurement of exposure biomarker could make the estimate exposure levels of PM_{2.5} more precise.

Second, we did not evaluate the effects of other environmental factors, such as indoor pollutants, pollens, fungal spores, and meteorological conditions, which can confound or modify the effects of PM_{2.5} levels. Third, because CB 25[OH]D is an indicator of prenatal vitamin D status in late pregnancy,⁴⁴ a single measurement of CB 25[OH]D might not represent vitamin D status throughout pregnancy. However, several epidemiologic studies have indicated that CB 25[OH]D is the best indicator of vitamin D status in newborns, allowing evaluation of its immune modulatory properties and infant allergic outcomes.⁵⁹ Further longitudinal studies with repetitive measurements of 25(OH)D levels at different stages of pregnancy are required. Fourth limitation is about methylation results and its interpretation. DNA methylation is tissue specific. Thus, it is unclear whether the observed placental DNA methylation changes occur in CB or fetal tissues.⁶⁰ Further, the present study had a small sample size that might affect the statistical power for detection of DNA methylation changes. Therefore, it is difficult to identify the specific mechanisms for PM_{2.5} and vitamin D to regulate DNA methylation and ultimately cause AD. Further mechanistic experimental studies are needed to confirm the associated mechanism. Finally, because follow-up period was not enough, the numbers of children with late onset AD were relatively small. Therefore, the present study's findings regarding late onset AD should be interpreted with caution. Future studies with longer follow-up periods are required to fully evaluate the effects of PM_{2.5} exposure on AD, particularly late onset AD.

In conclusion, increased PM_{2.5} exposure during the first trimester of pregnancy (6–7 weeks gestation) could increase susceptibility to early onset persistent AD. The persistence of PM_{2.5} exposure-associated AD during the first trimester of pregnancy (3–7 weeks gestation) increased in children with low CB vitamin D. In addition, placental *AHRR*, *DPP10*, and *HLA-DRB1* hypomethylation are a potential mechanism underlying the association between

310 prenatal PM_{2.5} exposure and vitamin deficiency D and offspring's AD. Avoiding PM_{2.5}
311 exposure and using interventions to increase vitamin D during the first trimester of pregnancy
312 could decrease the risk of early onset persistent AD in the offspring. Further, prevention of
313 early onset persistent AD could mitigate the risk of asthma and allergic rhinitis at later ages.
314 Future methylation and longitudinal cohort studies will provide additional insights into the
315 mechanisms underlying the association between prenatal PM_{2.5} exposure and vitamin D
316 deficiency and offspring's AD.

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Figure 1. Association between weekly PM_{2.5} exposure during gestation and atopic dermatitis phenotypes

BDLIM was used to estimate the week-specific effects of PM_{2.5} exposure during pregnancy on atopic dermatitis (AD) phenotypes, including a) early onset, b) early onset transient, c) early onset persistent, and d) late onset. Models were adjusted for sex, parental history of allergic disease, maternal age at delivery, maternal education level, and maternal exposure to secondhand smoke during pregnancy. The y-axis represents the odds ratio (OR) of AD in relation to PM_{2.5} exposure. The x-axis represents gestational age in weeks. The black solid line represents predicted OR, and the gray area indicates the 95% confidence interval (CI). A sensitive window is considered to be present when the estimated pointwise 95% CI does not include zero.

Figure 2. Effect of PM_{2.5} exposure on atopic dermatitis phenotypes modulated by cord blood vitamin D

Adjusted odds ratios (aOR) and 95% CIs obtained from logistic regression analysis adjusted for sex, parental history of allergic disease, maternal age at delivery, maternal education level, and maternal exposure to secondhand smoke during pregnancy. Tertile PM_{2.5} levels were used in logistic regression analysis, where T1 was the lowest tertile and T3 was the highest tertile.

Figure 3. Association between weekly PM_{2.5} levels during pregnancy and atopic dermatitis phenotypes according to cord blood vitamin D

Association between weekly PM_{2.5} levels during gestation and AD phenotypes according to cord blood (CB) vitamin D.

BDLIM was used to estimate week-specific effects for the association between $PM_{2.5}$ exposure over pregnancy and AD phenotypes according to cord blood vitamin D for a) early onset, b) early onset transient, c) early onset persistent, and d) late onset. Models were adjusted for sex, parental history of allergic disease, maternal age at delivery, maternal education level, and maternal exposure to secondhand smoke during pregnancy. The y-axis represents the OR of AD in relation to $PM_{2.5}$ exposure. The x-axis represents gestational age in weeks. The black solid line represents predicted OR, and the gray area indicates 95% CI. A sensitive window was identified when the estimated pointwise 95% CI did not include zero.

(b) Increased prenatal $PM_{2.5}$ exposure did not change the prevalence of early onset transient AD between infants with low and high cord blood vitamin D.

Figure 4. Heat map of 195 genes differentially methylated between the two groups (high $PM_{2.5}$, low cord blood vitamin D, and with atopic dermatitis vs. low $PM_{2.5}$, high cord blood vitamin D, and without atopic dermatitis)

Table 1. Study population

Variable	Total	Number or mean $\pm$ SD	%
Male	1180	610	51.7
Family history of allergic diseases	1163	563	48.4
Maternal age at delivery (years)	1180	32.80 $\pm$ 3.42	
Maternal educational state	1167		
$\leq$ High school		68	5.8
University		851	72.9
$\geq$ Graduate school		248	21.0
Maternal secondhand smoke exposure during pregnancy	1109	675	60.9
Atopic dermatitis phenotypes	971		
Never		616	63.4
Early transient		209	21.5
Early persistent		117	12.0
Late onset		29	3.0
PM _{2.5} during entire pregnancy ($\mu\text{g}/\text{m}^3$)	1180	27.69 $\pm$ 5.80	
Low	362	21.78 $\pm$ 1.76	
Middle	385	26.40 $\pm$ 1.24	
High	433	33.77 $\pm$ 4.48	
PM _{2.5} during 1 st trimester of pregnancy ($\mu\text{g}/\text{m}^3$)	1180	28.47 $\pm$ 8.32	
Low	344	18.93 $\pm$ 3.04	
Middle	406	27.44 $\pm$ 1.98	
High	430	37.07 $\pm$ 5.56	
PM _{2.5} during 2 nd trimester of pregnancy ($\mu\text{g}/\text{m}^3$)	1180	27.03 $\pm$ 7.62	
Low	375	18.71 $\pm$ 2.54	
Middle	390	26.22 $\pm$ 2.11	
High	415	35.32 $\pm$ 4.81	
PM _{2.5} during 3 rd trimester of pregnancy ($\mu\text{g}/\text{m}^3$)	1180	27.67 $\pm$ 7.86	
Low	358	18.76 $\pm$ 2.78	
Middle	404	26.82 $\pm$ 1.88	
High	418	36.12 $\pm$ 4.92	
Cord blood 25 (OH) vitamin D (ng/ml)	671	18.37 $\pm$ 24.93	

Table 2. Effect of PM_{2.5} exposure and cord blood vitamin D on atopic dermatitis phenotypes

Early Onset (Transient + Persistent)				
	aOR*	95% CI		p-value
First trimester T1	1.00			
First trimester T2	1.09	0.76	1.55	0.64
First trimester T3	1.41	1.00	2.00	0.05
Second trimester T1	1.00			
Second trimester T2	1.06	0.75	1.49	0.76
Second trimester T3	1.19	0.84	1.67	0.32
Third trimester T1	1.00			
Third trimester T2	1.17	0.83	1.65	0.38
Third trimester T3	1.09	0.77	1.55	0.62
Low cord blood vitamin D (median)	1.04	0.72	1.50	0.84
Early Onset Transient				
	aOR*	95% CI		p-value
First trimester T1	1.00			
First trimester T2	1.16	0.76	1.75	0.49
First trimester T3	1.35	0.89	2.04	0.16
Second trimester T1	1.00			
Second trimester T2	1.08	0.72	1.64	0.70
Second trimester T3	1.45	0.97	2.16	0.07
Third trimester T1	1.00			
Third trimester T2	1.24	0.83	1.85	0.30
Third trimester T3	1.05	0.69	1.59	0.82
Low cord blood vitamin D (median)	0.93	0.61	1.44	0.75
Early Onset Persistent				
	aOR*	95% CI		p-value
First trimester T1	1.00			
First trimester T2	0.96	0.56	1.64	0.87
First trimester T3	1.53	0.92	2.55	0.10
Second trimester T1	1.00			
Second trimester T2	1.01	0.62	1.63	0.98
Second trimester T3	0.82	0.49	1.37	0.45
Third trimester T1	1.00			
Third trimester T2	1.05	0.63	1.75	0.85
Third trimester T3	1.17	0.70	1.94	0.55
Low cord blood vitamin D (median)	1.28	0.75	2.19	0.36

Late Onset				
	aOR*	95% CI		p-value
First trimester T1	1.00			
First trimester T2	1.99	0.79	5.01	0.15
First trimester T3	0.24	0.05	1.20	0.08
Second trimester T1	1.00			
Second trimester T2	0.91	0.30	2.78	0.87
Second trimester T3	1.92	0.73	5.04	0.19
Third trimester T1	1.00			
Third trimester T2	0.64	0.20	2.06	0.45
Third trimester T3	1.89	0.72	4.90	0.19
Low cord blood vitamin D (median)	2.09	0.51	8.62	0.31

*Adjusted for sex, parental history of allergic disease, maternal age at delivery, maternal education level, and maternal secondhand smoke exposure during pregnancy.

Tertile PM_{2.5} levels were used in logistic regression analysis, where T1 was the lowest tertile and T3 was the highest tertile.

Table 3. Three hypomethylated sites showing significantly affected in high PM_{2.5}, low cord blood vitamin D, and atopic dermatitis subjects

CpG site	$\Delta\beta$	p-value	Chr	Gene
cg16371648	-0.367	0.026	5	<i>AHRR</i>
cg19211931	-0.263	0.013	2	<i>DPP10</i>
cg10632894	-0.318	0.026	6	<i>HLA-DRB1</i>

Table 4. Placental *AHR* and *AHR* related genes differentially expressed in high PM_{2.5}, low cord blood vitamin D, and atopic dermatitis subjects

Gene name	Low CB* vitamin D & AD† (+)		High PM _{2.5} & AD (+)		High PM _{2.5} & Low CB vitamin D		High PM _{2.5} , low CB vitamin D, AD (+)	
	PM _{2.5} High vs. Low		CB vitamin D Low vs. High		AD (+) vs. AD (-)		vs. Low PM _{2.5} , high CB vitamin D, AD (-)	
	Fold change	p-value	Fold change	p-value	Fold change	p-value	Fold change	p-value
<i>AHR</i>	-1.465	0.229	-1.895	0.088	-1.900	0.073	-1.132	0.759
<i>OBSCN</i>	-1.245	0.093	-1.343	0.034	-1.164	0.146	-1.044	0.634
<i>LRCH1</i>	-1.321	0.124	-1.295	0.044	-1.529	0.003	-1.149	0.373
<i>NMRAL1</i>	-1.280	0.015	-1.023	0.827	-1.016	0.817	-1.547	0.014
<i>ZNF101</i>	-1.219	0.124	-1.209	0.197	-1.077	0.487	-1.376	0.042
<i>PCDHB8</i>	-1.321	0.024	-1.046	0.490	-1.009	0.912	-1.033	0.748
<i>NPR2</i>	-1.043	0.787	-1.030	0.841	-1.084	0.667	-1.922	0.007

*CB: cord blood, †AD: atopic dermatitis

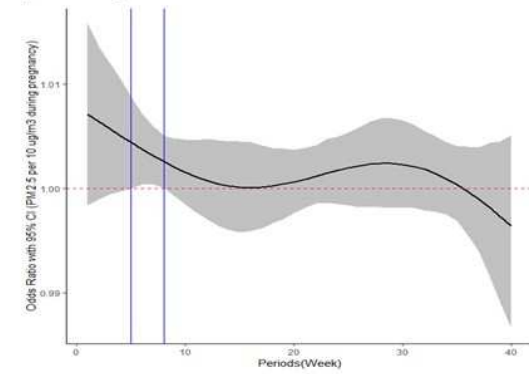
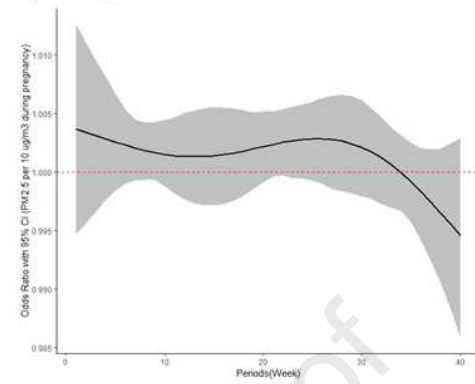
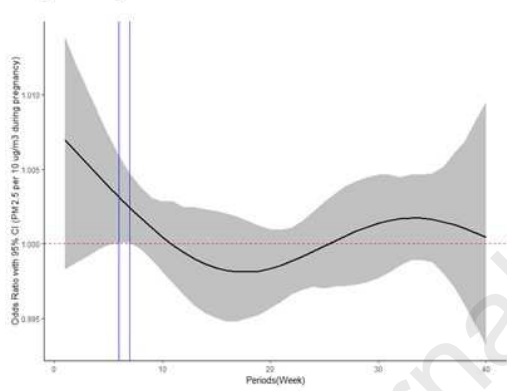
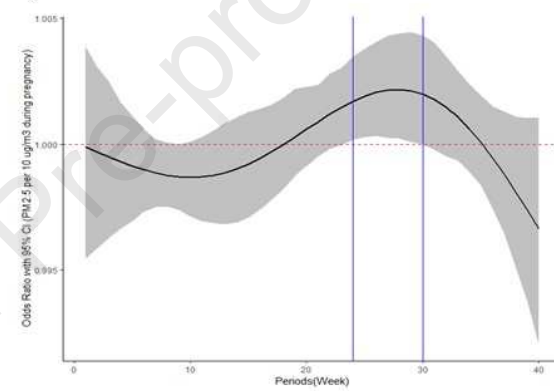
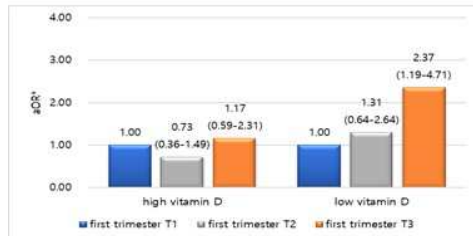
Figure 1.**a) Early Onset AD****b) Early Onset Transient AD****c) Early Onset Persistent AD****d) Late Onset AD**

Figure 2.

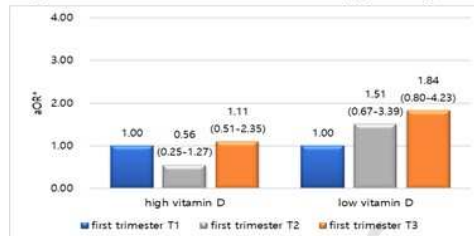
1) Early Onset AD

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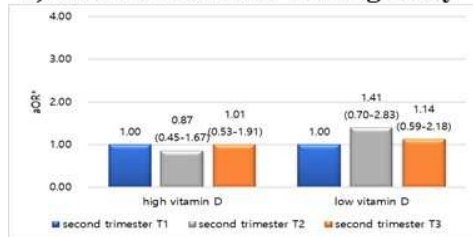


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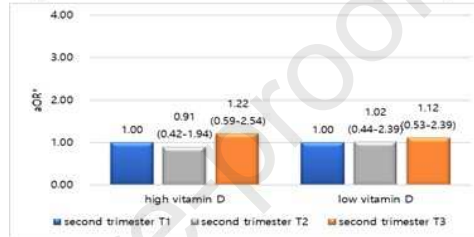
a) First Trimester of Pregnancy



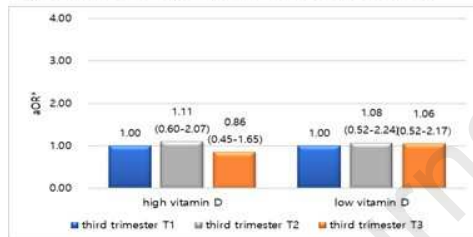
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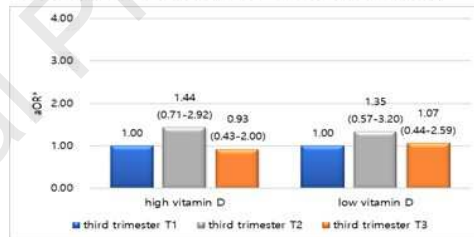
b) Second Trimester of Pregnancy



c) Third Trimester of Pregnancy

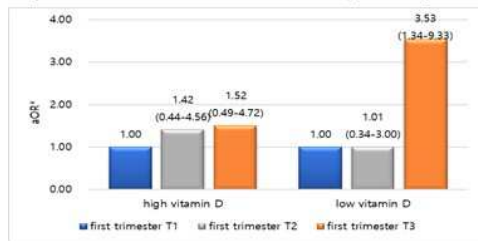


c) Third Trimester of Pregnancy

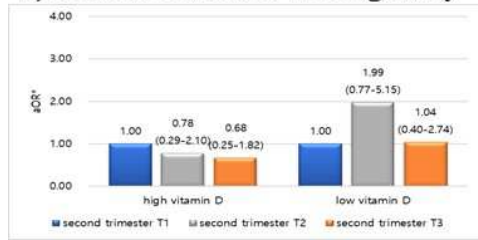


3) Early Onset Persistent AD

a) First Trimester of Pregnancy



b) Second Trimester of Pregnancy



c) Third Trimester of Pregnancy

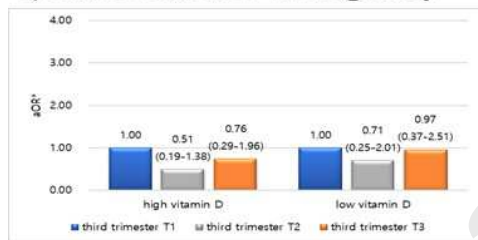
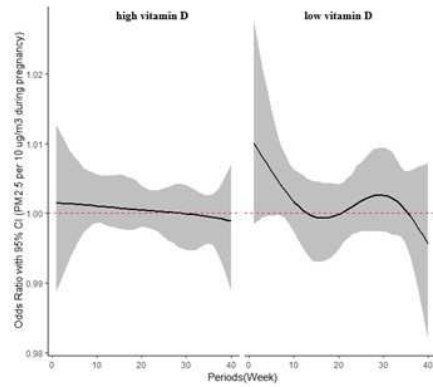
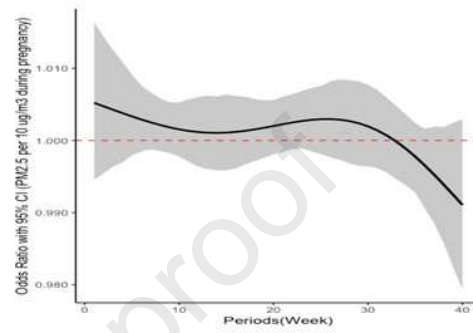


Figure 3.

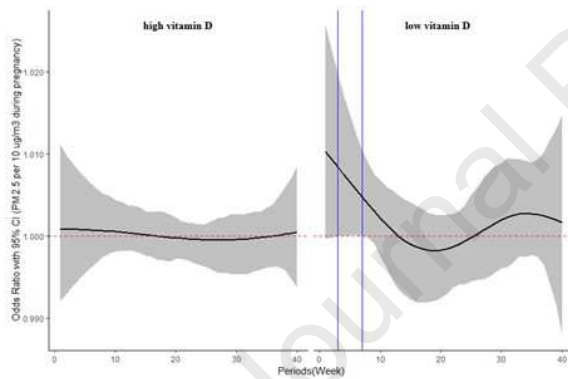
a) Early Onset AD



b) Early Onset Transient AD



c) Early Onset Persistent AD



d) Late Onset AD

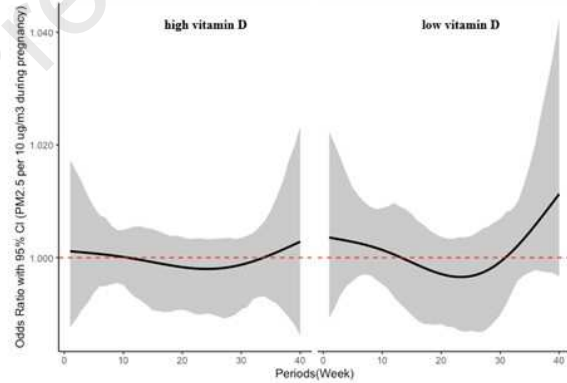
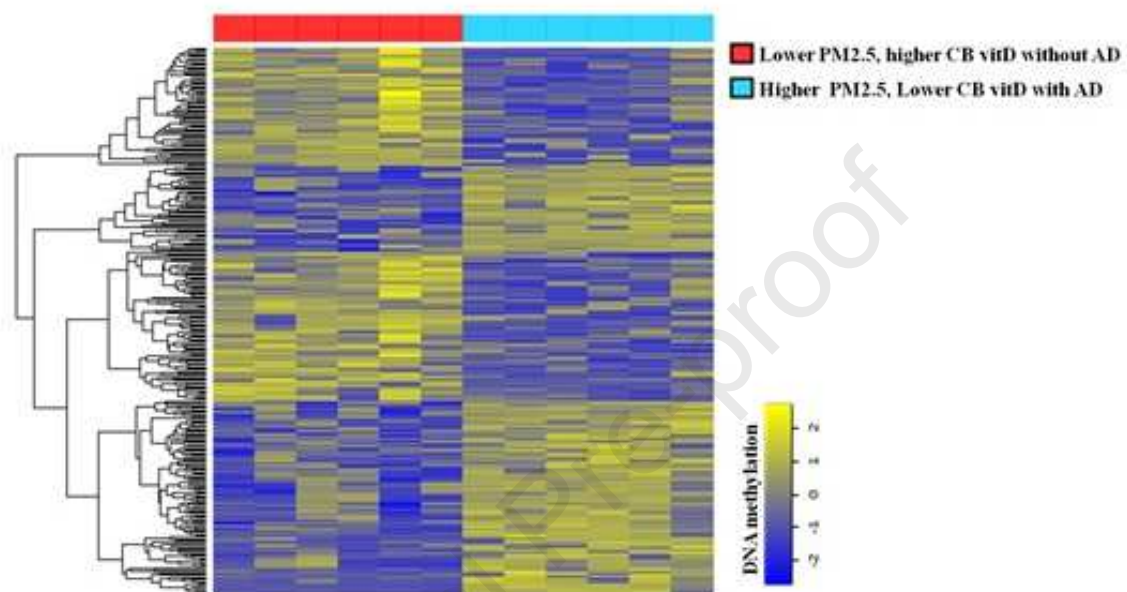
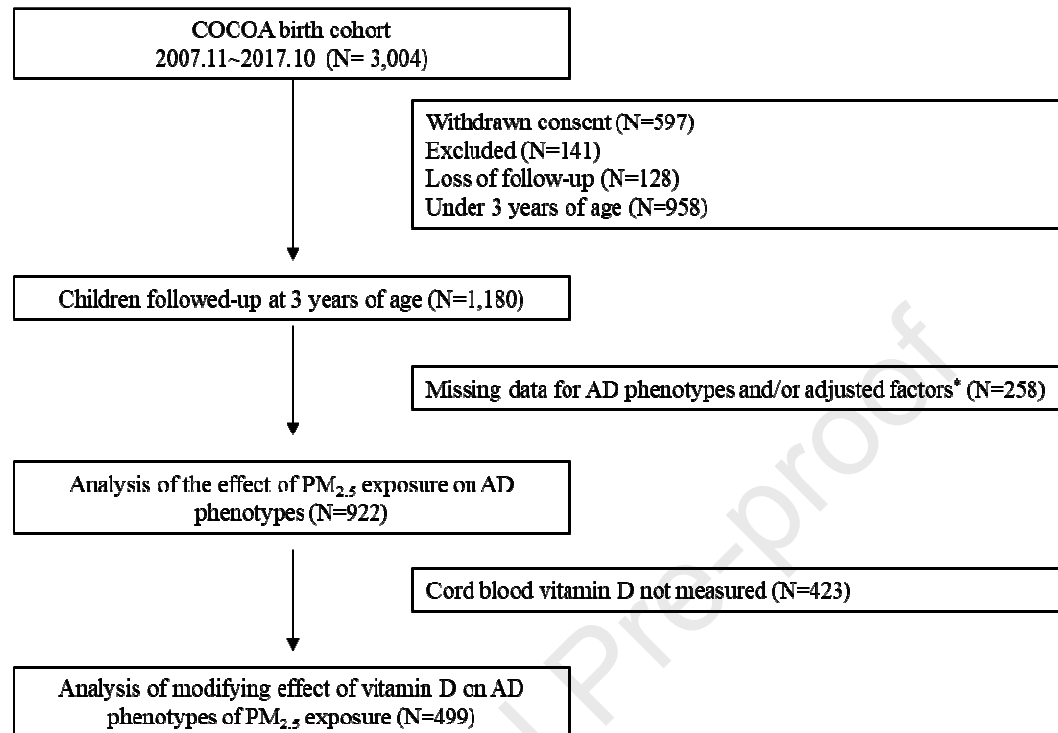


Figure 4.

eTable 1. Clinical characteristics of subjects selected for genome-wide methylation microarray

	Low PM _{2.5}				High PM _{2.5}			
	High CB* vitamin D		Low CB vitamin D		High CB vitamin D		Low CB vitamin D	
	AD† (-)	AD (+)	AD (-)	AD (+)	AD (-)	AD (+)	AD (-)	AD (+)
Number of subjects	6	6	6	6	6	6	6	6
Sex (male: female)	3:3	3:3	3:3	3:3	3:3	3:3	3:3	3:3
Birth weight (kg)	3.23± 0.16	3.36±0.03	3.59±0.21	3.15±0.12	3.27±0.14	2.98±0.08	2.96±0.13	3.20±0.00
Maternal age at delivery (years)	34.67±0.84	34.67±1.05	36.5±2.23	34.83±1.68	35.5±2.11	31.33±1.12	33.5±1.43	34.83±0.75
Parental history of allergic disease (number, %)	2 (33.3)	5 (83.3)	4 (66.7)	4 (66.7)	1 (16.7)	4 (66.7)	4 (66.7)	3 (50.0)
Maternal secondhand smoking exposure during pregnancy (number, %)	4 (66.7)	3 (50.0)	4 (66.7)	3 (50.0)	3 (50.0)	2 (33.3)	1 (16.7)	3 (50.0)
Cord blood vitamin D (ng/ml)	26.64±2.93	22.87±2.18	8.36±0.73	9.41±0.81	105.90±75.02	45.31±15.11	9023±0.83	0.51±1.21
Entire pregnancy PM _{2.5} (µg/m ³)	20.44± 0.9	20.26±0.46	20.06±0.76	23.76±0.81	29.52±2.70	32.57±2.32	33.40±0.98	29.7±2.68
First trimester PM _{2.5} (µg/m ³)	16.89±0.88	18.65±1.66	17.74±0.87	18.83±1.12	32.36±1.24	37.26±2.15	37.93±1.19	31.24±0.48
Second trimester PM _{2.5} (µg/m ³)	19.27± 1.48	19.51±1.22	18.44±1.57	23.40±1.16	29.55±4.44	35.87±2.86	34.13±1.69	27.82±3.42
Third trimester PM _{2.5} (µg/m ³)	26.26±0.67	23.18±2.73	24.80±1.33	29.29±1.93	26.48±3.79	25.53±2.65	26.76±3.10	30.00±5.80

* CB: cord blood, †AD: atopic dermatitis

eFigure 1. Study population

*Adjusted for sex, parental history of allergic disease, maternal age at delivery, maternal education level, and maternal exposure to secondhand smoke during pregnancy.