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Epigenome-wide association study reveals methylation pathways associated with childhood allergic sensitization

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RESEARCH PAPER



Epigenome-wide association study reveals methylation pathways associated with childhood allergic sensitization

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ABSTRACT

Epigenetic mechanisms integrate both genetic variability and environmental exposures. However, comprehensive epigenome-wide analysis has not been performed across major childhood allergic phenotypes. We examined the association of epigenome-wide DNA methylation in mid-childhood peripheral blood (Illumina HumanMethyl450K) with mid-childhood atopic sensitization, environmental/inhalant and food allergen sensitization in 739 children in two birth cohorts (Project Viva–Boston, and the Generation R Study–Rotterdam). We performed covariate-adjusted epigenome-wide association meta-analysis and employed pathway and regional analyses of results. Seven-hundred and five methylation sites (505 genes) were significantly cross-sectionally associated with mid-childhood atopic sensitization, 1411 (905 genes) for environmental and 45 (36 genes) for food allergen sensitization (FDR<0.05). We observed differential methylation across multiple genes for all three phenotypes, including genes implicated previously in innate immunity (*DICER1*), eosinophilic esophagitis and sinusitis (*SIGLEC8*), the atopic march (*AP5B1*) and asthma (*EPX*, *IL4*, *IL5RA*, *PRG2*, *SIGLEC8*, *CLU*). In addition, most of the associated methylation marks for all three phenotypes occur in putative transcription factor binding motifs. Pathway analysis identified multiple methylation sites associated with atopic sensitization and environmental allergen sensitization located in/near genes involved in asthma, mTOR signaling, and inositol phosphate metabolism. We identified multiple differentially methylated regions associated with atopic sensitization (8 regions) and environmental allergen sensitization (26 regions). A number of nominally significant methylation sites in the cord blood analysis were epigenome-wide significant in the mid-childhood analysis, and we observed significant methylation – time interactions among a subset of sites examined. Our findings provide insights into epigenetic regulatory pathways as markers of childhood allergic sensitization.

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Introduction

The increase in prevalence of allergic diseases has continued on a global scale [1]. In the United States, allergy accounts for more than \$18 billion

of the annual healthcare cost, with more than 50 million people being affected each year [2]. Children suffer disproportionately from this increasing trend, and allergic conditions have

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become the third most common chronic disease of childhood [2].

Childhood allergy is a complex disease phenotype determined by both genetic predisposition and environmental exposures. The strong familial aggregation of allergy suggests a genetic basis and a number of genetic polymorphisms have been implicated [3–8]. In addition, prenatal and early life environmental exposures, such as tobacco smoking [9–12], air pollution [13–16] and dietary factors [17–21] – alone or through interactions with genetic predisposition – also alter the risk of childhood allergy. However, the molecular underpinnings of allergic disease susceptibility and severity are still not well-understood.

DNA methylation – through the addition of a methyl group to cytosine residues in the cytosine-phosphate-guanine (CpG) dinucleotide sequence – is as an epigenetic regulatory mechanism influenced by both genetic variability and environmental exposures, and has been postulated to be a biomarker of allergy. Previous epigenome-wide association studies (EWAS) have linked the blood DNA methylome with atopy [22], food allergy [23,24], serum IgE measured as a continuous trait [22,25,26] and asthma [27]. Further, the nasal [28,29] and bronchial mucosal tissue methylomes [30] have also been associated with atopy-related phenotypes. However, no comprehensive epigenome-wide analysis has been performed across major allergic phenotypes in the same subjects from birth to mid-childhood.

In this study, we sought to evaluate the association between epigenome-wide DNA methylation measured at two time points – birth and mid-childhood – with allergy-related phenotypes (atopic sensitization, and two intermediate phenotypes: environmental/inhalant and food allergen sensitization) measured during mid-childhood. We performed the cord blood and mid-childhood epigenome-wide analyses separately. For the cord blood analysis, we performed a meta-analysis of a total of 1071 newborns from two independent birth cohort – Project Viva (Boston, USA) (N = 232) and the Generation R Study (Rotterdam, the Netherlands) (N = 839); the mid-childhood analysis was conducted in 739 child blood samples from Project Viva (N = 396) and the Generation R Study (N = 343). We hypothesized that childhood allergen sensitization maybe associated with differential DNA methylation in cord blood and mid-childhood

peripheral blood. We anticipated finding associations for the mid-childhood analysis given the priming role of childhood environmental exposures, but considered that methylation patterns established during fetal development and measured at birth may set the epigenetic trajectories and mark the susceptibility for allergic sensitization in childhood.

Materials and methods

Study populations

This study included mother-child pairs from Project Viva (Boston, USA) and mother-child pairs from the Generation R Study (Rotterdam, the Netherlands) – both of which are population-based prospective longitudinal pre-birth cohorts. Project Viva enrolled 2128 pregnant women during 1999–2002 at their initial obstetrical visit at Atrius Harvard Vanguard Medical Associates in Boston Massachusetts; the Generation R Study enrolled 8,879 pregnant women with a delivery date between April 2002 and January 2006 from midwifery practices, hospitals and child health centers located in Rotterdam, the Netherlands. Detailed protocols have been published previously for both cohorts [31–33]. Seven hundred and thirty-nine mother-child pairs (Project Viva N = 396; the Generation R Study N = 343) had complete information on peripheral blood DNA methylation and allergy – both measured at mid-childhood. Two-hundred and thirty-two mother-child pairs in Project Viva had complete information on cord blood DNA methylation and allergy measures, and 839 mother-child pairs in the Generation R Study had complete information on cord blood DNA methylation and allergy measures. In our previous work, we showed that the subset of participants with DNA methylation and serum IgE measures had similar personal characteristics compared to the overall population in Project Viva [34]. Written informed consent was obtained for all children in both studies. All study protocols were reviewed and approved by the Institutional Review Boards of the participating institutions.

DNA methylation measurements

In Project Viva, we extracted cord blood and peripheral blood DNA using the Qiagen Puregene Kit

(Valencia, CA) and bisulfite converted using the EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA). We randomized samples by chips and plates and generated DNA methylation data using the Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA). In the sample quality control step, we removed samples that were technical replicates, samples with low quality, and samples with genotype or sex mismatches. We removed low quality probes (based on detection p-values >0.05), probes on sex chromosomes, SNP probes, probes within 10bp of a known SNP with minor allele frequency $\geq 1\%$, non-CpG probes, as well as non-specific and cross-reactive probes. We applied the normal-exponential out-of-band ('noob') method for background correction and dye bias adjustment, and we normalized our data using Beta Mixture Quantile Dilation (BMIQ) – a model based intra-array normalization strategy to reduce bias of type 2 probes, while controlling type 1 probe enrichment bias [35].

In the Generation R Study, DNA was extracted from blood samples taken at birth (cord blood) and the 9-year follow-up visit. Five hundred nanograms of DNA per sample underwent bisulfite conversion using the EZ-96 DNA Methylation kit (Shallow) (Zymo Research Corporation, Irvine, USA). Samples were plated randomly onto 96-well plates. Samples were processed with the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA). Preparation and normalization of the HumanMethylation450 BeadChip array data were performed according to the Control Probe Adjustment and reduction of global CORection (CPACOR) workflow [36] using the software package R [37]. Quality control of analyzed samples was performed using standardized criteria. Probes that had a detection p-value $\geq 1\text{E-}16$ were set to missing per array. Next, the intensity values were stratified by autosomal and non-autosomal probes and quantile normalized for each of the six probe type categories separately: type II red/green, type I methylated red/green and type I unmethylated red/green. Arrays with observed technical problems such as failed bisulfite conversion, hybridization or extension, as well as arrays with a mismatch between sex of the proband and sex determined by the chromosome X and Y probe intensities were removed from subsequent analyses. Additionally, only arrays with a call rate > 95% per sample were processed further.

For both cohorts, we reported methylation as beta values, which were expressed as the percentage of methylated (M) cytosine residuals over the sum of methylated (M) and unmethylated (U) cytosine residues at the 5 carbon position (i.e. beta value = $[M/(M + U)] \times 100\%$).

Allergy measures

We studied three allergy-related outcomes in mid-childhood: atopic sensitization; environmental and food allergen sensitization. In Project Viva, atopic sensitization was defined as any serum specific IgE level ≥ 0.35 IU/ml to common indoor allergens (dust mite, cat dander, dog dander, cockroach), mold (*Aspergillus*, *Alternaria tenuis*), outdoor allergens (rye grass, ragweed) or food allergens (egg white, milk, wheat, peanut, soybean), or total serum IgE greater than or equal to 100 IU/ml; environmental allergen sensitization was defined as any serum specific IgE level ≥ 0.35 IU/ml to common indoor allergens (dust mite, cat dander, dog dander, cockroach), mold (*Aspergillus*, *Alternaria tenuis*) or grass pollen (ryegrass, ragweed) [17]; and food allergen sensitization was defined as any serum specific IgE level ≥ 0.35 IU/ml to one of the food allergens listed (egg white, milk, wheat, peanut, soybean) [18]. Twenty-two children were sensitized to all three phenotypes; 56 children were sensitized to environmental allergen only and 24 children were sensitized to food allergen only.

In the Generation R Study, allergic sensitization was measured by skin prick tests on the volar surface of the left forearm with single-use sterile 1 mm-tip metal lancets (ALK-Abelló A/S, Hørsholm, Denmark). Histamine dihydrochloride (10 mg/mL) and a saline solution (NaCl 0.9%) served as 2 positive controls and 1 negative control, respectively. Environmental allergens comprised house dust mite (*Dermatophagoides pteronyssinus*), 5-grass mixture (*Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*), birch (*Betula verrucosa*), cat (*Felis catus*) and dog (*Canis familiaris*) (ALK-Abelló B.V., Almere, The Netherlands). Food allergens comprised hazelnut, cashew nut, peanut and peach extracts. For all food allergens, aliquots were stored at -20°C and were defrosted for 1 h prior to

the skin prick test. Skin responses were recorded 15 min after applying allergens to the skin by measuring the area of the wheal (in mm²) using the Precise Automated Area Measurement of Skin Test (PAAMOST) software and considered positive if the area of the wheal was $\geq 40\%$ of the histamine response (i.e. histamine equivalent prick index area ≥ 0.40) [38]. A skin prick test was not performed if children could not omit antihistamine intake or corticosteroid ointment ≤ 72 h and ≤ 48 h prior to the test, respectively, used oral prednisone ≥ 10 mg daily or had eczema on the volar surface of the left forearm. Data from 343 participants contributed to the mid-childhood analysis, 107 were not sensitized to environmental or food allergen, 105 participants were sensitized to environmental allergen only, and 18 participants were sensitized to food allergen only.

Statistical analysis

Peripheral blood analysis

We performed cohort-specific cross-sectional analyses of peripheral blood DNA methylation and allergic phenotypes – both measured at mid-childhood. We fit covariate-adjusted logistic regression models for each methylation site, and adjusted for maternal covariates [age at enrollment (continuous), smoking status (Project Viva: never, former, or during pregnancy; the Generation R Study: never, stopped in early pregnancy or continued throughout pregnancy), education (college/graduate level or not college/graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race/ethnicity (white, black or others), gestational age (continuous), age at mid-childhood (questionnaire based) (continuous)], and cell type proxies estimated for peripheral blood (Houseman estimation) (percentages of monocytes, CD8T cells, CD4T cells, NK cells, B cells) [39]. We also performed a sensitivity analyses adjusting for childhood asthma status. We meta-analyzed results from Project Viva and the Generation R Study using fixed-effect meta-analysis weighted by the inverse of the variance using *METAL* [40]. We reviewed Q-Q plots and reported genomic inflation factor (lambda) to examine whether our associations were subject to

systematic bias/inflation. We accounted for multiple comparisons using the false discovery rate (FDR) method of Benjamini and Hochberg [41]. We further compared the overlaps among FDR-significant methylation sites (FDR <0.05) identified for each phenotype (i.e. atopic sensitization, environmental allergen and food allergen sensitization). Using the Database for Annotation, Visualization and Integrated Discovery (DAVID 6.7) pathway analysis [42], we searched for functional enrichment of biological pathways of statistically significant methylation sites identified. Specifically, we selected methylation sites that showed FDR <0.05 based on the meta-analysis results, mapped each methylation site to its corresponding UCSC reference gene name and chose Kyoto Encyclopedia of Genes and Genomes (KEGG) database to perform pathway analysis with default settings, with a minimum of four genes in an annotated group.

After identification of overlapping methylation marks from the meta-analyses for all phenotypes, we assessed whether the 36 overlapping CpG associations for all phenotypes occur preferentially in transcription factor (TF) binding motifs. The DNA-binding domains (DBD) of the TFs recognize these short consensus sequence motifs (8–20 bp), represented as position weight matrices (PWM). We have downloaded human transcription factor motifs from the Catalogue of Inferred Sequence Binding Preferences (CIS-BP) [43], selected a subset that represent a non-redundant set of transcription factors [44], which were then mapped to the reference human genome (hg19) using Find Individual Motif Occurrence (FIMO) [45], retaining only significant matches ($P < 1 \times 10^{-5}$). We then used the intersect feature of BEDTools [46] to search all motifs occurrences within 20 base pairs of the selected CpG sites.

We also performed *comb-p*, a regional analysis method that combines meta-analysis p-values in sliding windows, while taking into account spatial correlation across the genome [47]. For *comb-p* analyses, we chose an initial seed of 10^{-7} , a step size of 800 bases and a p value cut-off of 0.10.

Cord blood analysis

For the cord blood associations, we first performed cohort-specific analysis using covariate-adjusted logistic regressions for each methylation site as

the predictor. We adjusted for the following covariates selected *a priori*: maternal covariates [age at enrollment (continuous), smoking status (Project Viva: never, former, or during pregnancy; the Generation R Study: never, stopped in early pregnancy or continued throughout pregnancy), education (college/graduate level or not college/graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race/ethnicity (white, black or others), gestational age (continuous)], and cell type proxies estimated for cord blood (Balkuski estimation) (percentages of monocytes, CD8T cells, CD4T cells, NK cells, B cells and nucleated red blood cells (nRBC)) [48]. We meta-analyzed results from the two cohorts using fixed-effect meta-analysis weighted by the inverse of the variance [40]. We evaluated Q-Q plots, computed the genomic inflation factor (lambda) and accounted for multiple comparisons using the false discovery rate (FDR) method of Benjamini and Hochberg [41].

Longitudinal trend in methylation trajectories

We reported the number of methylation sites that showed nominal significance ($p < 0.05$) in the cord blood meta-analysis, and became epigenome-wide significance ($FDR < 0.05$) in the mid-childhood cross-sectional meta-analysis, and plotted trajectory plots of a subset of these sites. We also calculated the difference in methylation values between mid-childhood and cord blood measurements (delta change for 211 subjects with two time points of methylation data), and evaluated the association between methylation change from cord till mid-childhood and allergic outcomes in mid-childhood in Project Viva for sites that showed nominal significance in the cord blood meta-analysis, and became epigenome wide significance in the mid-childhood meta-analysis. We adjusted for covariates reported in the section above; we additionally adjusted for cord blood (baseline) DNA methylation of the same sites. For selected methylation sites, we performed mixed effects models with repeated statement for binary outcomes. We introduced a methylation – time interaction to examine whether there is any time trend among 211

children Project Viva with both cord blood and mid-childhood DNA methylation measurements.

Sensitivity analysis

To evaluate whether the significant associations for the peripheral blood analysis primarily reflect childhood environmental exposures, we evaluated additional models adjusted for cord blood DNA methylation of the same CpG site when examining the association between peripheral blood DNA methylation and childhood allergic sensitization in Project Viva (211 children with methylation measures at both time points).

Using a reference methylation Quantitative Trait Loci (QTL) library, we evaluated the genetic control of methylation for the top 30 associated CpG sites identified from the mid-childhood meta-analysis for atopic sensitization, environmental and food allergen sensitization. We leveraged genetic-epigenetic information from the ARIES large-scale genome-mQTL analysis, which included 1,000 mother-child pairs (<http://www.mqtladb.org/>) [49]. The genetic influences on DNA methylation were studied across 5 different time points in blood in this reference library: maternal [pregnancy, middle-age]; child [at birth, childhood, adolescence]. We focused our mQTL comparisons in the *cis* position (i.e. a genetic variant within ± 500 Kb of a methylation locus) measured from peripheral blood in childhood, and with $MAF \geq 5\%$.

Eosinophils and neutrophils are central effector immune cells that often associate with allergic outcomes. To further ensure the associations we observed were due to changes in methylation levels, rather than shifts in eosinophil/neutrophil cell proportions, we applied a reference-free method based on principal component analysis (PCA) with low rank approximation (ReFACTOR) to control for cell-type heterogeneity [50]. Previous studies have shown that ReFACTOR components correlate well with measured eosinophil and neutrophil counts [50].

We also compared the FDR-significant methylation sites identified from a previously published epigenome-wide association study of IgE across various age groups [25].

All analyses were performed in SAS® version 9.4 (SAS Institute, Inc.) and R version 3.4.1.

Results

Population characteristics

This study includes a total of 739 mother-child pairs for the mid-childhood peripheral blood analysis (Project Viva N = 396; the Generation R Study N = 343), and 1071 mother-child pairs for the cord blood analysis (Project Viva N = 232; the Generation R Study N = 839) (Table 1). For the mid-childhood subset, mean maternal age was 32.3 (standard deviation (SD) = 5.5) years for Project Viva and 32.3 (SD = 3.9) years for the Generation R Study. The percentage of mothers who smoked actively during

pregnancy was comparable in both cohorts; however, a larger proportion of mothers were former smokers in Project Viva (Project Viva: 20.2% vs. the Generation R Study 12.2%). Approximately 39% of mothers in Project Viva and in the Generation R Study had a history of atopy (asthma, eczema and hay fever) reported during their pregnancy interviews. Children from the Generation R Study were slightly older when the allergy tests were conducted (age mean $\pm$ SD: Project Viva 7.7 $\pm$ 0.7; the Generation R Study 9.8 $\pm$ 0.3). Sixty-three percent of children were white in the Project Viva subset, while all children were white in the

Table 1. Characteristics of children and their mothers in project viva and the generation R study.

Participants characteristics	Viva (N = 232)	Cord blood	<i>p</i> -value	Viva (N = 396)	Mid-childhood	<i>p</i> -value
		GenR (N = 839)			GenR (N = 343)	
Maternal						
Age at enrollment (years), mean (SD)	32.4 (5.4)	32.1 (4.1)	0.43	32.3 (5.5)	32.3 (3.9)	1
Smoking status, N (%)						
Smoking during pregnancy	20 (8.6%)	87 (10.4%)	<0.0001	40 (10.1%)	32 (9.3%)	0.01
Former smoker	52 (22.4%)	80 (9.5%)		80 (20.2%)	42 (12.2%)	
Never smoker	160 (69.0%)	672 (80.1%)		276 (69.7%)	269 (78.4%)	
Education, N (%)						
College or graduate	154 (66.4%)	580 (69.1%)	0.47	262 (66.2%)	227 (66.2%)	1
Not college or graduate	78 (33.6%)	259 (30.9%)		134 (33.8%)	116 (33.8%)	
Atopy						
Yes	87 (37.5%)	328 (39.1%)	0.72	156 (39.4%)	134 (39.1%)	0.99
No	145 (62.5%)	511 (60.9%)		240 (60.6%)	209 (60.9%)	
Child						
Gest age (weeks), mean (SD)	39.7 (1.6)	40.2 (1.5)	<0.0001	39.6 (1.6)	40.1 (1.6)	<0.0001
Age (years), mean (SD)	–	–	<0.0001	7.7 (0.7)	9.8 (0.3)	<0.0001
Sex, N (%)						
Female	114 (49.1%)	439 (52.3%)	0.43	198 (50.0%)	170 (49.6%)	0.96
Male	118 (50.9%)	400 (47.7%)		198 (50.0%)	173 (50.4%)	
Race, N (%)						
White	157 (67.7%)	839 (100%)	<0.0001	248 (62.6%)	343 (100%)	<0.0001
Black	39 (16.8%)	–		76 (19.2%)	–	
Other	36 (15.5%)	–		72 (18.2%)	–	
Atopic sensitization*						
Yes	121 (52.2%)	264 (31.5%)	<0.0001	215 (54.3%)	107 (31.2%)	<0.0001
No	111 (47.8%)	575 (68.5%)		181 (45.7%)	236 (68.8%)	
Environ. allergen sensitization*						
Yes	100 (43.1%)	261 (31.1%)	<0.01	167 (42.2%)	105 (30.6%)	<0.01
No	132 (56.9%)	578 (68.9%)		229 (57.8%)	238 (69.4%)	
Food allergen sensitization*						
Yes	54 (23.3%)	54 (6.4%)	<0.0001	104 (26.3%)	18 (5.2%)	<0.0001
No	178 (76.7%)	785 (93.6%)		292 (73.7%)	325 (94.8%)	

*Atopic sensitization, environmental and food allergen sensitizations were measured at mid-childhood. In Project Viva, atopic sensitization was defined as any serum specific IgE level $\geq$ 0.35 IU/ml to common indoor allergens (dust mite, cat dander, dog dander, cockroach), mold (*Aspergillus*, *Alternaria tenuis*), outdoor allergens (rye grass, ragweed) or food allergens (egg white, milk, wheat, peanut, soybean), or total serum IgE greater than or equal to 100 IU/ml; environmental allergen sensitization was defined as any serum specific IgE level $\geq$ 0.35 IU/ml to common indoor allergens (dust mite, cat dander, dog dander, cockroach), mold

(*Aspergillus*, *Alternaria tenuis*) or grass pollen (ryegrass, ragweed); and food allergen sensitization was defined as any serum specific IgE level $\geq$ 0.35 IU/ml to one of the food allergens listed (egg white, milk, wheat, peanut, soybean). In the Generation R Study, allergic sensitization was measured by skin prick tests on the volar surface of the left forearm with single-use sterile 1 mm-tip metal lancets (ALK-Abelló A/S, Hørsholm, Denmark). Histamine dihydrochloride (10 mg/mL) and a saline solution (NaCl 0.9%) served as 2 positive controls and 1 negative control, respectively. Environmental allergens comprised house dust mite (*Dermatophagoides pteronyssinus*), 5-grass mixture (*Dactylis glomerata*, *Festuca pratensis*, *Lolium perenne*, *Phleum pratense*, *Poa pratensis*), birch (*Betula verrucosa*), cat (*Felis catus*) and dog (*Canis familiaris*) (ALK-Abelló B.V., Almere, The Netherlands). Food allergens comprised hazelnut, cashew nut, peanut and peach extracts.

Generation R Study subset. Higher proportions of children had positive allergic tests in Project Viva than in the Generation R Study (Project Viva: 54.3%, 42.2% and 26.3% for atopic sensitization, environmental allergen sensitization and food allergen sensitization, respectively; the Generation R Study: 31.2%, 30.6% and 5.2% for atopic sensitization, environmental allergen sensitization and food allergen sensitization, respectively). Participant characteristics were similar for the cord blood subset compared to the mid-childhood subset (Table 1).

Mid-childhood peripheral blood analysis

Our epigenome-wide meta-analysis identified 705 (representing 505 annotated genes), 1411 (representing 905 annotated genes), and 45 (representing 36 annotated genes) differentially methylated CpG sites in mid-childhood peripheral blood associated with mid-childhood atopic sensitization, environmental allergen sensitization and food allergen sensitization, respectively (FDR < 0.05) (Table 2; Table S1 – full list; Figure 1). Genomic inflation factor ranges from 1.16–1.26 were acceptable for the three allergic phenotypes, suggesting our findings were unlikely due to systematic bias (Figure S1). We observed differential methylation associations for all three phenotypes in multiple genes previously associated with asthma (EPX [51,52], IL4 [53], IL5RA [53], PRG2 [54], SIGLEC8 [55–57]) (Figure 2). Adjusting for current asthma status of the child did not change the results, as we observed similar magnitude of effect estimates and p values in models with and without the adjustment of asthma status (Table S2). For atopic sensitization, the biological processes suggested from the DAVID pathway analysis of the FDR significant methylation sites included mTOR signaling pathway (number of genes contributing to the pathway = 7, p value = 0.0029), lipid metabolism (number of genes contributing to the pathway = 5, p value = 0.015), Fc gamma R-mediated phagocytosis (number of genes contributing to the pathway = 8, p value = 0.016), endocytosis (number of genes contributing to the pathway = 11, p value = 0.031), and asthma (number of genes contributing to the pathway = 4, p value = 0.045). For environmental allergen sensitization, the biological processes implicated in the pathway analysis included inositol phosphate metabolism (number of genes contributing to the

pathway = 9, p value = 0.007), lysosome (number of genes contributing to the pathway = 14, p value = 0.0086), N-Glycan biosynthesis (number of genes contributing to the pathway = 8, p value = 0.0099), asthma (number of genes contributing to the pathway = 6, p value = 0.017), mTOR signaling pathway (number of genes contributing to the pathway = 8, p value = 0.019), type II diabetes mellitus (number of genes contributing to the pathway = 7, p value = 0.036), phosphatidylinositol signaling system (number of genes contributing to the pathway = 5, p value = 0.041). For food allergen sensitization, only the asthma-related pathway appeared to be significant (number of genes contributing to the pathway = 2, p value = 0.039). A list of genes contributing to each pathway is shown in Table S3a.

Thirty-six methylation sites with similar magnitude and direction of effect overlapped among the three analyses (atopic sensitization, environmental allergen sensitization and food allergen sensitization) (Table 3; Figure 3). This list (Table 3) includes multiple genes that have been implicated in innate immunity (*DICER1* [58], the atopic march (*AP5B1*) [59], eosinophilic esophagitis [60] and chronic sinusitis (*SIGLEC8*) [61] and asthma (*EPX* [51,52], *PRG2* [54], *SIGLEC8* [55–57], and *CLU* [62]). In addition, all but two of the CpG sites in the overlapping list occur in putative transcription factor binding motifs; motifs from TF families are present for more than one CpG association. For example, five CpG sites overlap putative GATA family transcription sites (GATA2-cg01901579, cg06391412 GATA3-cg08722695, GATA4-cg01901579, cg25854298, GATA6-cg13829849) and three overlap SMAD family motifs (SMAD1-cg26791242, SMAD2-cg25758167, SMAD4-cg25738116) (Supplemental Table S3b) suggesting the common aspects of allergy may include TF-mediated gene regulatory pathways.

In addition to the CpG-by-CpG analysis, we also performed regional analysis based on meta-analysis p-values using *comb-p*. We identified 8 methylation regions associated with mid-childhood atopic sensitization and 26 regions associated with environmental allergen sensitization (with at least two methylation sites) (Table 4). All methylation regions identified for atopic sensitization were significantly associated with environmental allergen sensitization (Table 4). Those overlapping regions were annotated to *VKORC1L1*

Table 2. Association between mid-childhood peripheral blood DNA methylation with mid-childhood atopic sensitization, environmental and food allergen sensitization (top 30 methylation sites are presented here sorted by meta-analysis p-values) .

Atopic sensitization									
CpG	CHR	Gene	Viva		GenR		Meta-analysis		
			Estimate	P value	Estimate	P value	Estimate	P value	FDR
cg04983687	16	ZFPM1	-9.67	3.36E-07	-8.44	1.76E-05	-9.08	2.87E-11	6.97E-06
cg02427831	19	SIGLEC8	-17.31	5.07E-06	-23.46	1.03E-06	-19.67	3.89E-11	6.97E-06
cg26791242	1	LOC339524	-21.08	5.23E-09	-12.94	9.65E-04	-17.35	6.49E-11	6.97E-06
cg11770323	13	NDFIP2	-15.39	3.88E-08	-16.51	4.63E-04	-15.68	7.33E-11	6.97E-06
cg02970679	17	EPX	-22.82	6.99E-08	-16.78	3.48E-04	-20.11	1.56E-10	1.19E-05
cg13628444	9	MED27	-19.84	3.66E-07	-18.20	4.31E-04	-19.24	6.41E-10	3.39E-05
cg21220721	1	ACOT7	-10.67	7.09E-09	-5.50	3.67E-03	-8.15	6.65E-10	3.39E-05
cg09212118	1	GUCA2A	-16.59	7.89E-06	-17.89	2.13E-05	-17.16	7.16E-10	3.39E-05
cg08698681	3	TNFIK	-24.72	7.21E-08	-17.25	1.71E-03	-21.66	8.01E-10	3.39E-05
cg10433549	17	-	-20.79	9.25E-08	-14.68	1.70E-03	-18.29	9.79E-10	3.72E-05
cg26396815	4	BANK1	-25.48	1.46E-06	-19.96	1.65E-04	-22.73	1.28E-09	3.78E-05
cg07948085	10	-	-30.67	5.47E-08	-16.57	1.04E-03	-22.84	1.29E-09	3.78E-05
cg04406620	16	CYB5B	-22.41	4.69E-08	-11.65	1.14E-03	-16.31	1.51E-09	3.78E-05
cg19434937	12	LPCAT3	-14.65	3.57E-07	-11.05	8.05E-04	-13.09	1.56E-09	3.78E-05
cg07819010	11	-	-19.61	4.16E-09	-9.16	1.15E-02	-14.81	1.58E-09	3.78E-05
cg24491618	7	KCNH2	-17.84	1.22E-07	-12.50	2.24E-03	-15.68	1.68E-09	3.78E-05
cg00114012	9	SLC2A8	-14.65	2.44E-06	-16.12	1.68E-04	-15.16	1.69E-09	3.78E-05
cg11988722	20	-	-10.46	1.12E-04	-19.77	7.74E-07	-13.39	2.38E-09	5.01E-05
cg09596645	3	-	-20.27	1.78E-06	-17.22	3.26E-04	-18.93	2.54E-09	5.01E-05
cg25173129	17	EPX	-18.99	4.56E-06	-17.30	1.41E-04	-18.22	2.65E-09	5.01E-05
cg06391412	3	FOXP1	-21.66	2.35E-08	-12.12	1.12E-02	-17.87	2.94E-09	5.01E-05
cg10704177	1	PATJ	-17.58	5.38E-08	-10.33	5.17E-03	-14.43	2.96E-09	5.01E-05
cg00944309	5	-	-15.98	5.00E-07	-13.26	1.43E-03	-14.98	3.03E-09	5.01E-05
cg04497992	16	NHLRC4	-15.50	4.05E-07	-11.13	1.38E-03	-13.59	3.27E-09	5.18E-05
cg01916918	8	CLU	-24.05	9.18E-07	-18.97	8.38E-04	-21.89	3.68E-09	5.28E-05
cg11456013	6	-	-17.49	1.25E-07	-12.10	5.21E-03	-15.50	3.71E-09	5.28E-05
cg14769121	7	SMURF1	-19.43	1.16E-06	-15.57	6.93E-04	-17.77	3.75E-09	5.28E-05
cg13054523	17	-	-29.77	1.58E-06	-13.38	4.50E-05	-16.96	4.87E-09	6.62E-05
cg20885063	17	ATPAF2	-19.50	1.17E-06	-13.85	7.66E-04	-16.75	5.54E-09	7.10E-05
cg06528816	2	TTC7A	-14.87	1.02E-06	-12.72	1.35E-03	-14.08	5.60E-09	7.10E-05
Environmental allergen sensitization									
CpG	CHR	Gene	Viva		GenR		Meta-analysis		
			Estimate	P value	Estimate	P value	Estimate	P value	FDR
cg02427831	19	SIGLEC8	-27.44	7.40E-11	-24.82	3.22E-07	-26.31	1.37E-16	5.21E-11
cg04983687	16	ZFPM1	-13.89	1.21E-11	-8.46	1.95E-05	-11.08	7.11E-15	1.35E-09
g11988722	20	-	-16.75	1.78E-08	-20.21	5.84E-07	-17.96	6.51E-14	7.45E-09
cg02475695	16	NHLRC4	-23.33	1.96E-10	-16.29	4.56E-05	-20.12	9.60E-14	7.45E-09
cg19434937	12	LPCAT3	-23.39	7.11E-13	-10.97	8.83E-04	-17.26	9.80E-14	7.45E-09
cg09212118	1	GUCA2A	-25.48	6.19E-10	-17.60	2.85E-05	-21.62	2.02E-13	9.98E-09
cg02970679	17	EPX	-30.45	1.50E-11	-16.82	3.51E-04	-23.92	2.06E-13	9.98E-09
cg26791242	1	LOC339524	-27.95	1.40E-12	-13.05	9.95E-04	-20.53	2.10E-13	9.98E-09
cg21220721	1	ACOT7	-15.29	3.83E-14	-5.42	4.07E-03	-10.02	3.76E-13	1.59E-08
cg13628444	9	MED27	-25.68	3.43E-10	-18.72	2.55E-04	-22.97	6.61E-13	2.35E-08
cg00114012	9	SLC2A8	-20.98	3.78E-10	-15.60	2.58E-04	-18.93	6.81E-13	2.35E-08
cg24491618	7	KCNH2	-25.12	6.04E-12	-12.44	2.30E-03	-19.48	8.17E-13	2.59E-08
cg04497992	16	NHLRC4	-23.49	4.89E-12	-10.90	1.70E-03	-17.33	9.92E-13	2.90E-08
cg25173129	17	EPX	-28.04	3.84E-10	-17.10	1.63E-04	-22.64	1.22E-12	3.31E-08
cg18666454	7	KCNH2	-24.13	4.05E-11	-12.33	5.31E-04	-18.07	1.36E-12	3.35E-08
cg07970948	7	ZNF862	-22.19	3.41E-11	-10.43	2.51E-04	-15.37	1.41E-12	3.35E-08
cg17988187	2	LOC100189589	-21.27	4.00E-10	-15.20	5.98E-04	-19.02	1.78E-12	3.98E-08
cg10433549	17	-	-27.98	5.51E-11	-15.02	1.58E-03	-22.20	2.76E-12	5.67E-08
cg10704177	1	PATJ	-24.75	2.47E-12	-10.28	5.39E-03	-17.83	2.84E-12	5.67E-08
cg00944309	5	-	-21.73	1.49E-10	-13.21	1.52E-03	-18.33	3.16E-12	5.67E-08
cg09596645	3	-	-27.51	5.28E-10	-17.01	4.00E-04	-22.69	3.26E-12	5.67E-08
cg12614529	4	MND1	-23.08	1.31E-11	-10.68	2.58E-03	-17.11	3.28E-12	5.67E-08
cg26396815	4	BANK1	-34.91	4.40E-10	-19.40	2.37E-04	-26.71	3.52E-12	5.75E-08
cg08698681	3	TNFIK	-31.66	6.67E-11	-17.09	1.90E-03	-25.29	3.63E-12	5.75E-08
cg10065736	12	FBXW8	-23.77	3.85E-12	-9.72	5.15E-03	-16.85	4.88E-12	7.14E-08
cg16263722	1	MECR	-22.84	9.35E-11	-12.65	2.36E-03	-18.58	4.95E-12	7.14E-08

(Continued)

Table 2. (Continued).

Atopic sensitization									
CpG	CHR	Gene	Viva		GenR		Estimate	Meta-analysis	
			Estimate	P value	Estimate	P value		P value	FDR
cg06528816	2	<i>TTC7A</i>	−20.09	4.76E-10	−13.06	1.03E-03	−17.30	5.07E-12	7.14E-08
cg11456013	6	–	−24.21	2.12E-11	−11.74	6.60E-03	−19.08	5.99E-12	8.14E-08
cg18421167	17	<i>EPX</i>	−35.36	4.19E-10	−18.63	3.27E-04	−26.27	6.47E-12	8.49E-08
cg20151107	10	<i>FAM53B</i>	−26.45	3.87E-09	−17.13	1.54E-04	−21.83	7.57E-12	9.29E-08
Food allergen sensitization									
CpG	CHR	Gene	Viva		GenR		Estimate	Meta-analysis	
			Estimate	P value	Estimate	P value		P value	FDR
cg13054523	17	–	−20.37	2.28E-05	−19.31	1.11E-04	−19.86	9.86E-09	3.75E-03
cg06906869	13	<i>NEK3</i>	−19.45	1.24E-06	−25.18	4.75E-03	−20.41	2.40E-08	4.56E-03
cg13829849	9	<i>MAMDC2</i>	−55.37	3.64E-06	−44.50	2.61E-03	−51.07	3.93E-08	4.98E-03
cg23021241	1	<i>DMAP1</i>	66.23	1.87E-01	14.16	1.32E-07	14.31	9.32E-08	6.68E-03
cg21919729	8	<i>CTSB</i>	−11.70	2.05E-05	−15.41	1.24E-03	−12.62	1.15E-07	6.68E-03
cg11456013	6	–	−14.31	1.13E-05	−31.93	5.22E-04	−16.27	1.18E-07	6.68E-03
cg21220721	1	<i>ACOT7</i>	−7.65	8.55E-06	−11.30	2.79E-03	−8.27	1.23E-07	6.68E-03
cg01916918	8	<i>CLU</i>	−22.68	3.72E-06	−21.34	2.14E-02	−22.38	2.40E-07	1.14E-02
cg18372930	5	–	17.40	2.65E-05	19.74	4.44E-03	18.01	4.07E-07	1.56E-02
cg18543428	13	–	−14.64	1.73E-04	−20.23	4.95E-04	−16.37	4.21E-07	1.56E-02
cg26791242	1	<i>LOC339524</i>	−15.02	1.10E-05	−18.21	1.33E-02	−15.59	4.90E-07	1.56E-02
cg12198633	3	<i>KLHL24</i>	−30.03	1.00E-05	−20.66	1.03E-02	−26.13	4.93E-07	1.56E-02
cg12819873	11	<i>PRG2</i>	−16.15	3.65E-06	−14.58	6.13E-02	−15.88	6.02E-07	1.76E-02
cg26396815	4	<i>BANK1</i>	−16.91	5.47E-05	−29.02	1.64E-03	−18.98	6.49E-07	1.76E-02
cg17521120	19	<i>SIGLEC8</i>	−13.65	5.73E-05	−24.88	1.43E-03	−15.44	7.00E-07	1.77E-02
cg01901579	14	<i>DICER1</i>	−12.01	9.31E-05	−19.20	1.45E-03	−13.49	8.33E-07	1.98E-02
cg02970679	17	<i>EPX</i>	−13.75	5.11E-05	−26.47	1.99E-03	−15.47	9.36E-07	2.04E-02
cg13562011	9	<i>EXD3</i>	−15.63	4.13E-05	−21.43	5.74E-03	−16.76	9.67E-07	2.04E-02
cg25758167	1	<i>AGL</i>	−22.07	8.05E-03	−38.29	1.93E-05	−29.58	1.24E-06	2.36E-02
cg25738116	3	<i>PA2G4P4</i>	−13.46	7.82E-06	−13.73	7.65E-02	−13.50	1.52E-06	2.75E-02
cg08284993	16	<i>ANKRD11</i>	20.86	2.04E-06	10.60	2.17E-01	18.73	1.67E-06	2.89E-02
cg06391412	3	<i>FOXP1</i>	−14.57	1.87E-05	−18.88	3.09E-02	−15.14	1.83E-06	3.03E-02
cg23149249	6	–	−36.76	1.43E-03	−34.76	4.35E-04	−35.61	2.07E-06	3.14E-02
cg08818866	2	<i>LTBP1</i>	−27.55	3.65E-05	−29.58	1.95E-02	−27.99	2.12E-06	3.14E-02
cg14769121	7	<i>SMURF1</i>	−12.69	1.83E-04	−25.13	1.15E-03	−14.70	2.22E-06	3.14E-02
cg25488567	6	<i>CALHM6</i>	−17.32	6.08E-05	−18.61	1.23E-02	−17.65	2.31E-06	3.14E-02
cg15344640	5	<i>LMAN2</i>	−24.37	1.07E-04	−28.58	8.61E-03	−25.42	3.03E-06	3.97E-02
cg05300717	11	<i>AP5B1</i>	−18.35	2.78E-04	−17.91	3.68E-03	−18.17	3.28E-06	3.99E-02
cg08698681	3	<i>TNFK</i>	−16.27	5.33E-05	−23.50	1.63E-02	−17.32	3.31E-06	3.99E-02
cg14937228	10	<i>FAM107B</i>	−17.36	1.24E-03	−26.05	5.17E-04	−20.31	3.36E-06	3.99E-02

Models adjusted for maternal covariates[age at enrollment (continuous), smoking status (never, former, or during pregnancy), education (college/graduate level or not college/graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race/ethnicity (white, black or others), gestational age (continuous)], and cell type proxies estimated for peripheral blood (percentages of monocytes, CD8T cells, CD4T cells, NK cells, and B cells).

(7 sites), *TLDC2* (5 sites), *ACOT7* (4 sites), *LOC339524* (3 sites), *SIGLEC8* (3 sites), *ZFPM1* (3 sites), *NHLRC4* (2 sites), and *ZNF862* genes (2 sites). There were no significant associations for food allergen sensitization in this regional analyses.

Cord blood analysis

There were nominal p values of at least 10^{-6} for all three atopic phenotypes (Table S4), but we did not observe statistically significant (FDR<0.05) differentially methylated sites in cord blood DNA CpGs

associated with childhood atopic sensitization or environmental allergen sensitization at mid-childhood in the meta-analysis of cord blood methylation. For food allergen sensitization, 2 methylation sites reached epigenome-wide significance level (*RPS6KA2* and *CHIT1*). The significant associations at these sites were predominantly driven by the Generation R Study results (Table S4), and we observed heterogeneous associations for *CHIT1* between the two cohorts ($p_{\text{heterogeneity}} < 0.05$). The Q-Q plots indicate there is little inflation, and we did not observe systematic bias (Figure S1).

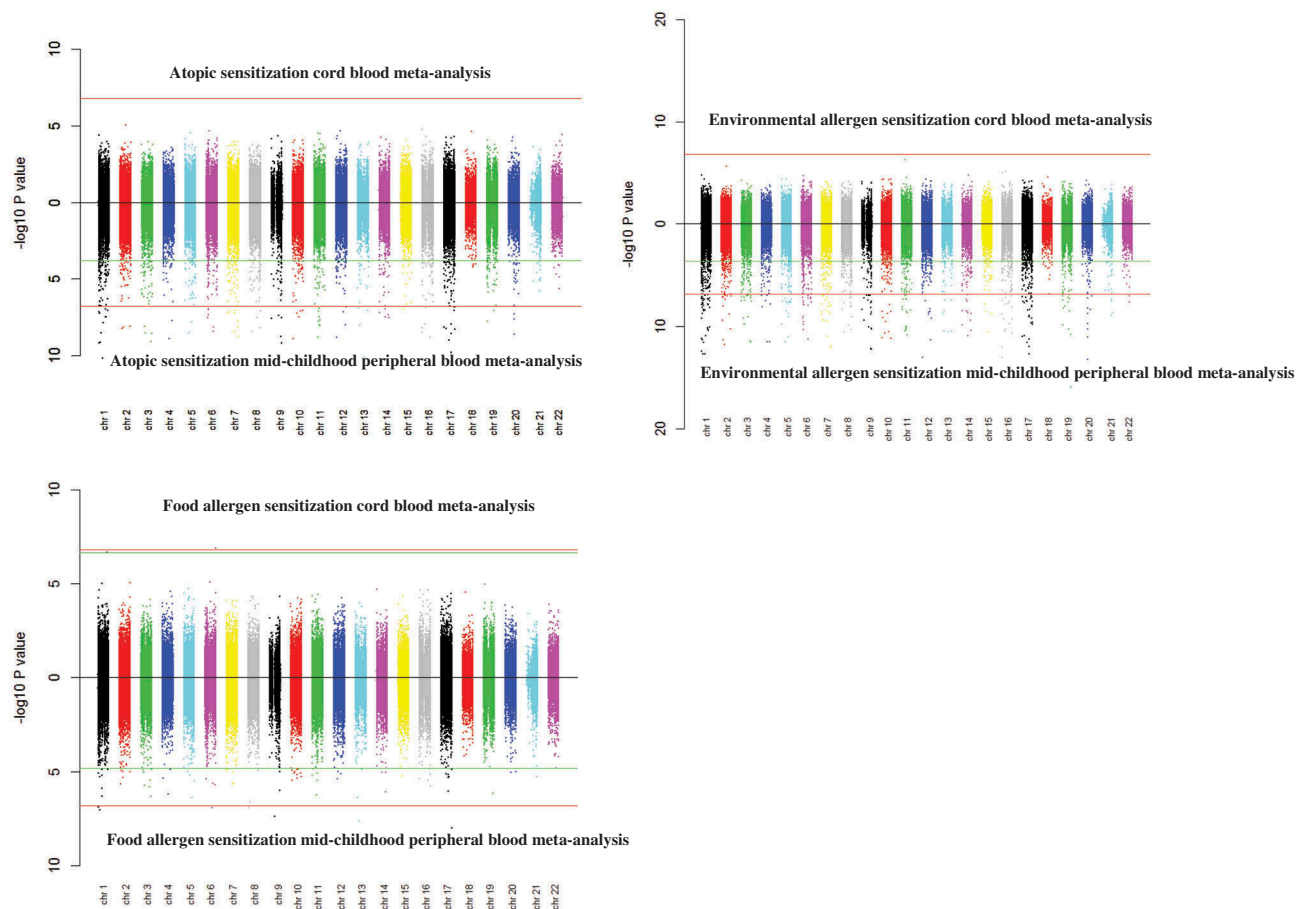


Figure 1. Miami plots. *P* values based on meta-analysis results of the associations between cord blood or mid-childhood peripheral blood DNA methylation with mid-childhood atopic sensitization, environmental and food allergen sensitization.

Redline – Bonferroni significance (Bonferroni-adjusted *p*-value < 0.05) Green line – FDR significance (FDR-adjusted *p*-value < 0.05)

Longitudinal trend

Next, we explored whether the differentially methylated CpG sites (FDR < 0.05) in the mid-childhood cross-sectional analysis showed nominal significance ($p < 0.05$) at birth. We identified 99 methylation sites that showed nominal significance at birth and were epigenome-wide significant at mid-childhood for atopic sensitization; 163 for environmental allergen sensitization and 3 for food allergen sensitization (Table S5). For atopic sensitization and environmental allergen sensitization, multiple sites are in/close to genes related to asthma pathway genes (*EPX*, *IL4*, *IL5RA*). Among the methylation sites that showed nominal significance ($p < 0.05$) in the cord blood analysis, and were epigenome-wide significance (FDR < 0.05) in the mid-childhood analysis, changes in methylation values from cord to mid-childhood were also nominally significantly associated with mid-childhood

allergy, with *p* values in the range of 10^{-5} to 10^{-6} (Table S5). In the mixed-effects model analysis, which included methylation – time interactions, we observed significant time trend among 18 out of 19 sites examined, where we selected the top 4 methylation sites (sorted by *p*-value significance) for the cord blood analysis, 4 methylation sites previously linked to asthma pathways for atopic sensitization and environmental allergen sensitization; and the only 3 methylation sites that met the *p*-value criteria for food allergen sensitization (Table S6). Trajectory plots are presented in Figure 4 (for atopic sensitization) and Figure S2 (for environmental and food allergen sensitization).

Sensitivity analysis

We additionally adjusted for cord blood DNA methylation when examining the association

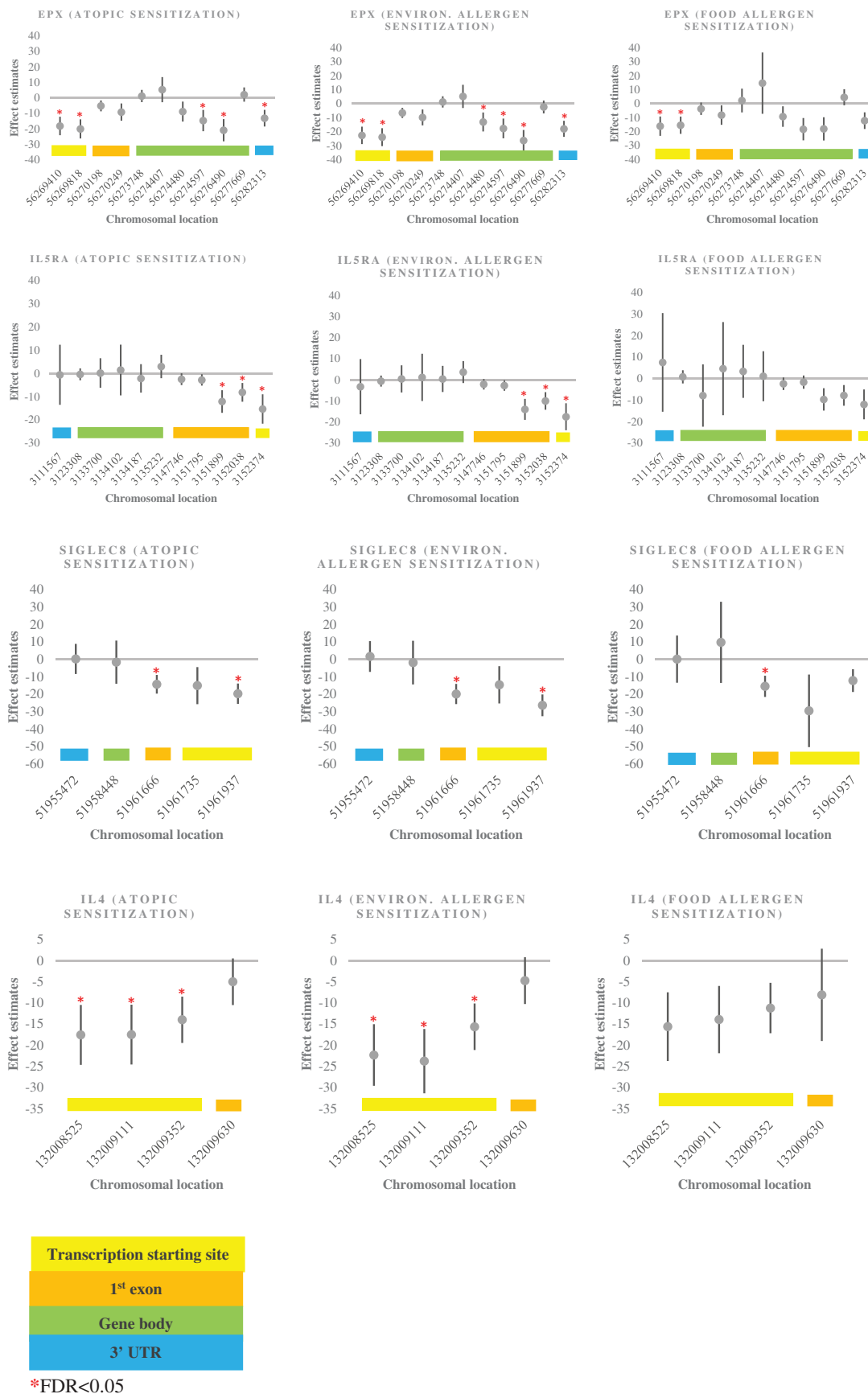


Figure 2. Plots for the associations between *EPX*, *IL5RA*, *SIGLEC8*, *IL4* DNA methylation in mid-childhood with mid-childhood atopy (sorted by chromosomal locations) .

Table 3. Overlapping results of all three phenotypes from the mid-childhood meta-analysis (i.e. methylation sites that met FDR<0.05 for all three phenotypes) .

Atopic sensitization					Environ. Sensitization		Food sensitization	
CpG	CHR	Gene	Estimate	P value	Estimate	P value	Estimate	P value
cg01901579	14	<i>DICER1</i>	-13.99	4.68E-08	-16.17	4.02E-10	-13.49	8.33E-07
cg01916918	8	<i>CLU</i>	-21.89	3.68E-09	-25.69	2.83E-11	-22.38	2.40E-07
cg02941852	7	<i>SRPK2</i>	-36.41	8.41E-08	-40.35	2.46E-09	-28.64	2.28E-06
cg02970679	17	<i>EPX</i>	-20.11	1.56E-10	-23.92	2.06E-13	-15.47	9.36E-07
cg04077085	4	<i>SLC2A9</i>	-22.09	8.78E-07	-26.45	9.07E-09	-24.06	4.36E-06
cg04497992	16	<i>NHLRC4</i>	-13.59	3.27E-09	-17.33	9.92E-13	-12.50	3.87E-06
cg05300717	11	<i>AP5B1</i>	-16.21	1.14E-07	-18.30	3.19E-09	-18.17	3.28E-06
cg06391412	3	<i>FOXP1</i>	-17.87	2.94E-09	-19.17	1.67E-10	-15.14	1.83E-06
cg06824199	1	<i>EFCAB14</i>	-14.51	2.82E-05	-16.86	1.65E-06	-17.71	5.31E-06
cg06906869	13	<i>NEK3</i>	-12.27	7.96E-05	-15.54	1.17E-06	-20.41	2.40E-08
cg08698681	3	<i>TNIN</i>	-21.66	8.01E-10	-25.29	3.63E-12	-17.32	3.31E-06
cg08722695	2	-	-28.13	1.07E-05	-30.02	2.43E-06	-36.39	4.77E-06
cg08818866	2	<i>LTBP1</i>	-20.71	3.03E-06	-24.20	8.10E-08	-27.99	2.12E-06
cg09335712	17	<i>TEX2</i>	-14.84	1.09E-06	-18.11	4.75E-09	-16.58	5.05E-06
cg11456013	6	-	-15.50	3.71E-09	-19.08	5.99E-12	-16.27	1.18E-07
cg11791710	10	<i>DOCK1</i>	-20.47	7.09E-06	-23.93	2.78E-07	-24.42	5.09E-06
cg12198633	3	<i>KLHL24</i>	-19.20	3.64E-06	-20.97	4.57E-07	-26.13	4.93E-07
cg12213680	6	<i>RIPOR2</i>	-16.75	2.89E-08	-18.97	6.34E-10	-15.89	4.14E-06
cg12402318	15	<i>TMOD3</i>	-15.71	4.55E-06	-16.38	9.35E-07	-18.23	5.82E-06
cg12819873	11	<i>PRG2</i>	-17.09	1.62E-08	-20.25	5.12E-11	-15.88	6.02E-07
cg13054523	17	-	-16.96	4.87E-09	-18.89	1.63E-10	-19.86	9.86E-09
cg13562011	9	<i>EXD3</i>	-17.28	2.92E-08	-21.20	5.64E-11	-16.76	9.67E-07
cg13829849	9	<i>MAMDC2</i>	-22.95	2.31E-05	-25.31	3.66E-06	-51.07	3.93E-08
cg14769121	7	<i>SMURF1</i>	-17.77	3.75E-09	-21.04	1.06E-11	-14.70	2.22E-06
cg15344640	5	<i>LMAN2</i>	-19.55	1.10E-05	-21.72	9.53E-07	-25.42	3.03E-06
cg17521120	19	<i>SIGLEC8</i>	-14.25	1.83E-07	-19.84	1.64E-11	-15.44	7.00E-07
cg18543428	13	-	-11.97	8.74E-06	-14.24	2.39E-07	-16.37	4.21E-07
cg21220721	1	<i>ACOT7</i>	-8.15	6.65E-10	-10.02	3.76E-13	-8.27	1.23E-07
cg21919729	8	<i>CTSB</i>	-8.94	1.98E-06	-11.43	4.51E-09	-12.62	1.15E-07
cg25173129	17	<i>EPX</i>	-18.22	2.65E-09	-22.64	1.22E-12	-16.15	4.97E-06
cg25488567	6	<i>CALHM6</i>	-13.64	3.58E-05	-17.12	4.75E-07	-17.65	2.31E-06
cg25738116	3	<i>PA2G4P4</i>	-10.36	1.29E-06	-10.18	2.47E-06	-13.50	1.52E-06
cg25758167	1	<i>AGL</i>	-22.64	2.27E-06	-25.60	1.22E-07	-29.58	1.24E-06
cg25854298	10	<i>ASCC1</i>	-20.23	3.34E-08	-22.20	1.95E-09	-21.77	4.36E-06
cg26396815	4	<i>BANK1</i>	-22.73	1.28E-09	-26.71	3.52E-12	-18.98	6.49E-07
cg26791242	1	<i>LOC339524</i>	-17.35	6.49E-11	-20.53	2.10E-13	-15.59	4.90E-07

Models adjusted for maternal covariates [age at enrollment (continuous), smoking status (never, former, or during pregnancy), education (college/graduate level or not college/graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race/ethnicity (white, black or others), gestational age (continuous)], and cell type proxies estimated for peripheral blood (percentages of monocytes, CD8T cells, CD4T cells, NK cells, and B cells).

between Project Viva peripheral blood DNA methylation and childhood allergy at the same sites (N = 211). Our mid-childhood results were of the same signs of directionality and with relatively comparable magnitude of effect estimates after the adjustment of cord blood DNA methylation (Table S7).

In the mid-childhood meta-analysis, we identified multiple methylation sites annotated to genes related to eosinophilic activities (such as *EPX*, *PRG2*, *SIGLEC8*). To further ensure the associations we observed were due to variability in methylation levels, rather than shifts in eosinophil cell proportions, we controlled for cell-type heterogeneity using ReFACTor. Our findings were

robust to the adjustment of cell proportions using ReFACTor (Table S8).

Among the top 30 methylation sites identified from the mid-childhood meta-analysis, most of them were not associated with known genetic variants in the *cis*-position (i.e. $\pm$ 500Kb) (Table S9). We identified 5 methylation loci with *cis*-mQTL in childhood peripheral blood for atopic sensitization, 3 for environmental allergen sensitization and 4 for food allergen sensitization, including cg26396815 (*BANK1*), cg07948085 (not annotated), cg24491618 (*KCNH2*), cg00114012 (*SLC2A8*), cg20885063 (*ATPAF2*), cg21919729 (*CTSB*), cg12198633 (*KLHL24*) and cg25738116 (*PA2G4P4*).

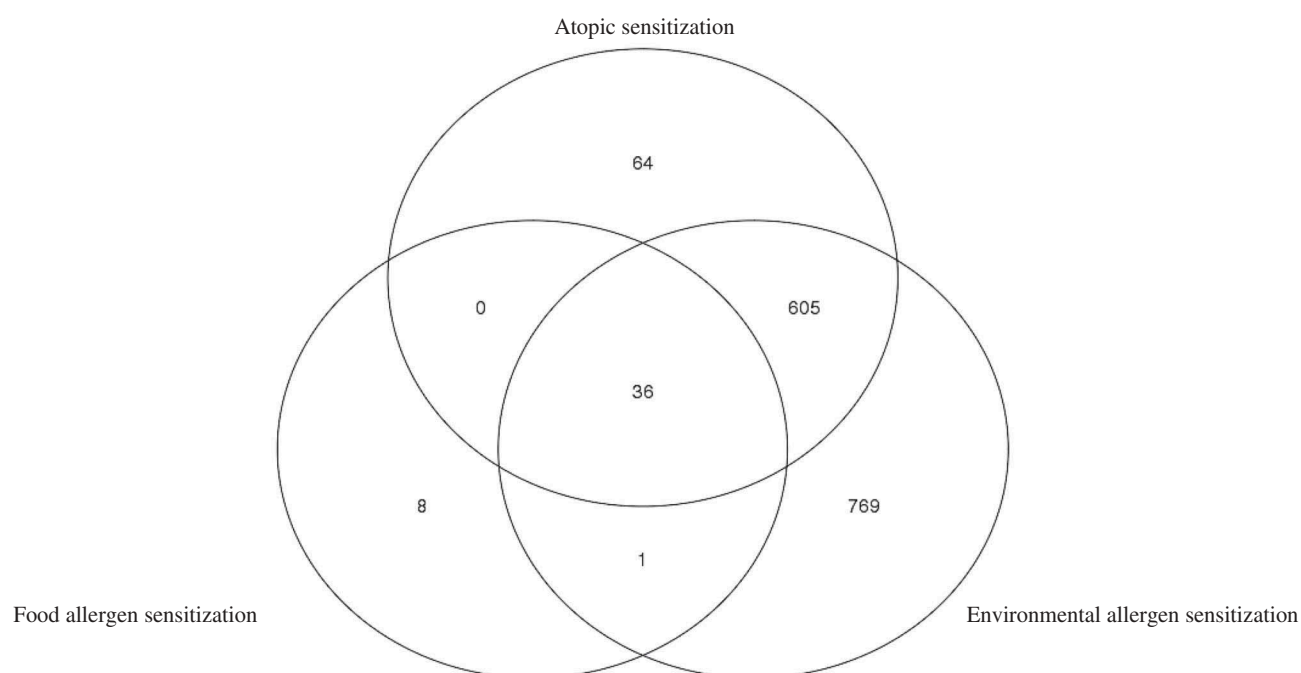


Figure 3. Venn diagram of overlapping FDR significant methylation sites (mid-childhood cross-sectional meta-analysis) among three phenotypes for the meta-analysis of Project Viva and The Generation R Study.

We replicated many of the FDR-significant methylation sites identified from a separate multi-ethnicity meta-analysis of IgE (Table S10) [25].

Discussion

We performed comprehensive epigenome-wide DNA methylation analyses across major allergic phenotypes (739 children at mid-childhood and 1071 newborns) from two birth cohorts from the United States and the Netherlands. Our meta-analysis demonstrated differential methylation in mid-childhood peripheral blood associated with mid-childhood atopic sensitization, environmental and food allergen sensitizations, and some of these had nominal associations in cord blood (14% for atopic sensitization, 12% for environmental allergen sensitization and 7% for food allergen sensitization) and demonstrated a significant longitudinal time trend. Multiple associated methylation sites were annotated to genes that have been implicated in asthma pathways, which were consistent across all three atopy phenotypes, including *EPX* (eosinophil peroxidase), *IL4* (interleukin 4), *IL5RA* (interleukin 5 receptor A) (*PRG2* (proteoglycan 2), and *SIGLEC8* (sialic acid-

binding, Ig-like lectin-8). The *EPX* (eosinophil peroxidase) gene encodes for a pre-protein in eosinophils, which functions as a potent oxidant [51,52]. The *IL4* and *IL5RA* are cytokine pathway genes associated with Th2 cell differentiation [53]. The *PRG2* (proteoglycan 2) gene product is the predominant constituent of the eosinophil granule, and displays anti-microbial activities against pathogens [54]. *SIGLEC8* (sialic acid-binding, Ig-like lectin-8) belongs to a family of single-pass transmembrane cell surface proteins on leukocytes; the *SIGLEC8* gene product has a role in apoptosis of eosinophils [56,57,63] and has been suggested recently as a therapeutic target [64]. We did not find epigenome-wide statistically significant differential methylation of cord blood CpGs associated with mid-childhood allergic phenotypes, although we did observe a longitudinal time trend for a subset, suggesting a predominant role for post-natal environment in guiding epigenetic trajectories associated with allergic diseases of childhood. We did identify a number of methylation sites with nominal significance in the cord blood associated with mid-childhood allergic phenotypes, and some of those associations remained epigenome-wide significant in mid-childhood,

Table 4. Regional analysis (*comb-p*) of the association between mid-childhood peripheral blood with mid-childhood atopic sensitization and environmental allergen sensitization.

Atopic sensitization						
CHR	Start	End	Gene	Min p value	No. of sites	Stouffer-Liptak-Kechris correction p value
7	65,419,185	65,419,289	<i>VKORC1L1</i>	2.56E-10	7	1.34E-14
20	35,504,198	35,504,554	<i>TLDC2</i>	1.37E-14	5	1.11E-19
1	6,341,140	6,341,328	<i>ACOT7</i>	8.25E-08	4	6.51E-12
16	88,558,065	88,558,238	<i>ZFPM1</i>	6.18E-11	3	1.55E-15
1	87,596,820	87,596,935	<i>LOC339524</i>	1.27E-08	3	8.34E-13
19	51,961,666	51,961,938	<i>SIGLEC8</i>	6.18E-11	3	1.79E-15
16	616,212	616,221	<i>NHLRC4</i>	5.55E-11	2	7.30E-16
7	149,543,165	149,543,178	<i>ZNF862</i>	1.22E-09	2	7.05E-14
Environmental allergen sensitization						
CHR	Start	End	Gene	Min p value	No. of sites	Stouffer-Liptak-Kechris correction p value
20	35,503,983	35,504,554	<i>TLDC2</i>	4.56E-25	7	2.09E-34
7	65,419,185	65,419,289	<i>VKORC1L1</i>	1.99E-18	7	7.86E-23
1	6,341,140	6,341,328	<i>ACOT7</i>	4.51E-12	4	4.03E-16
2	74,612,144	74,612,813	<i>LOC100189589</i>	6.53E-13	4	2.36E-27
11	1,297,066	1,297,251	<i>TOLLIP</i>	9.16E-10	3	2.90E-13
15	43,513,306	43,513,564	<i>EPB42</i>	5.93E-08	3	1.56E-11
16	88,540,241	88,540,397	<i>ZFPM1</i>	1.73E-08	3	3.19E-12
16	88,558,065	88,558,238	<i>ZFPM1</i>	3.90E-19	3	6.89E-24
17	76,564,972	76,565,079	<i>DNAH17</i>	4.33E-09	3	5.81E-13
1	87,596,820	87,596,935	<i>LOC339524</i>	2.83E-12	3	2.08E-16
19	51,961,666	51,961,938	<i>SIGLEC8</i>	7.73E-18	3	3.66E-22
3	3,151,795	3,152,039	<i>IL5RA</i>	7.85E-08	3	2.13E-11
6	158,507,753	158,508,189	<i>SYNJ2</i>	8.83E-13	3	8.06E-17
10	129,172,535	129,172,574	<i>DOCK1</i>	3.94E-08	2	8.90E-12
1	150,780,684	150,780,719	<i>CTSK</i>	4.51E-12	2	4.02E-16
1	27,241,853	27,241,898	<i>NR0B2</i>	3.13E-09	2	3.95E-13
14	100,610,071	100,610,187	<i>EVL</i>	5.93E-08	2	1.50E-11
16	616,212	616,221	<i>NHLRC4</i>	3.90E-19	2	8.21E-24
16	85,551,478	85,551,515	<i>ATP1</i>	6.54E-09	2	1.02E-12
17	56,269,410	56,269,819	<i>EPX</i>	1.87E-09	2	7.05E-24
17	73,631,460	73,631,587	<i>RECQL5</i>	1.96E-11	2	1.91E-15
5	132,009,111	132,009,353	<i>IL4</i>	8.22E-12	2	7.78E-16
6	25,754,190	25,754,220	<i>SLC17A4</i>	2.69E-09	2	3.11E-13
7	100,240,094	100,240,342	<i>TFR2</i>	3.13E-09	2	3.83E-13
7	149,543,165	149,543,178	<i>ZNF862</i>	2.30E-14	2	1.33E-18
9	130,859,454	130,859,607	<i>SLC25A25</i>	5.93E-08	2	1.53E-11

Models adjusted for maternal covariates [age at enrollment (continuous), smoking status (never, former, or during pregnancy), education (college/graduate level or not college/graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race/ethnicity (white, black or others), gestational age (continuous)], and cell type proxies estimated for peripheral blood (percentages of monocytes, CD8T cells, CD4T cells, NK cells, and B cells).

suggesting that methylation patterns established at birth may mark allergic sensitization susceptibility in childhood.

In addition to the asthma pathway, we further identified multiple methylation sites associated with mTOR signaling and inositol phosphate metabolism for the mid-childhood cross-sectional analysis for atopic sensitization and environmental allergen sensitization. The mTOR pathway plays a central role in directing immune responses toward allergy, immune-disturbance of Th17/Treg and Th1/Th2 balance, and has been implicated in asthma onset, remission, and allergy [65]; additionally mTOR

inhibition through drugs such as rapamycin have been proposed as therapeutic options for allergic diseases [66], suggesting further investigation of epigenetic plasticity of the mTOR pathway may have therapeutic potential. Inositol phosphate production is also involved in eosinophil degranulation and increased tracheal smooth muscle contractions; delineating mechanistic underpinnings of airway smooth muscle constriction may provide new therapeutic targets associated with the inhibition of bronchoconstriction in asthma [67].

Recently, transcription factors have been implicated in the pathogenesis of allergic diseases and

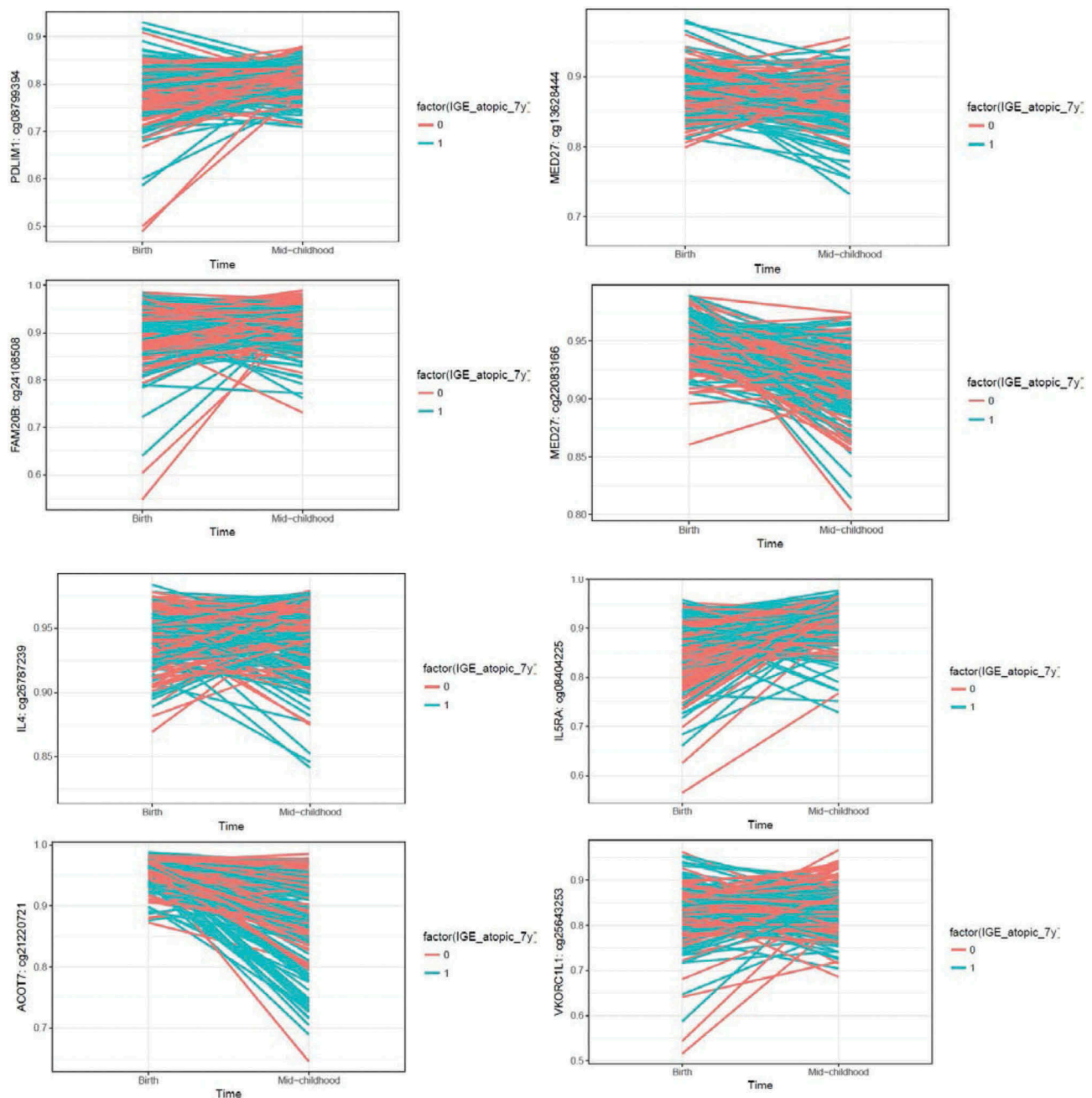


Figure 4. Trajectory plots of methylation changes by atopic status. Methylation sites were selected as they showed nominal significance in the cord blood analysis, and epigenome-wide significance in the mid-childhood analysis. Top 4 plots: top 4 methylation sites sorted by cord blood p-values based on the criteria described above; bottom 4 plots: methylation sites previously shown to be associated with asthma.

asthma in part through impacting the differentiation of Th1/Th2 cells and promoting inflammation through regulation of inflammatory pathways [68]. One functional feature of variable DNA methylation that associates with allergy may be through DNA methylation of transcription factors or DNA methylation within transcription factor binding sites, with downstream impacts on gene expression. Our

evaluation of overlapping methylation marks for the allergic phenotypes (Table 3) revealed that the majority of the CpGs were located within putative TF binding sequences, including multiple sequences that occur in the GATA and SMAD families of transcription factors. Both of these families of transcription factors have been implicated in allergy. For example, GATA1 and GATA2 have been implicated

as transcription factors for eosinophil lineage commitment [69,70]; in addition, GATA pathways have been suggested as potential therapeutic targets [71]. The SMAD family members are key regulators of TGF beta signaling [72], with SMAD3 [4,73] and low SMAD2 expression were associated with both increased asthma susceptibility and exacerbations [74]. Although this is an *in silico* analysis it highlights the potential ways that EWAS identified targets may have coordinated impact on resulting disease phenotypes, and offers targets to consider for functional evaluation and therapeutic development.

We performed regional analysis based on meta-analysis p-values obtained from the mid-childhood cross-sectional analysis (*comb-p*) [47]. We identified 8 methylation regions associated with mid-childhood atopic sensitization and 26 regions associated with environmental allergen sensitization. All 8 regions associated with atopic sensitization overlapped with environmental allergen sensitization and were annotated to *VKORC1L1*, *TLDC2*, *ACOT7*, *LOC339524*, *SIGLEC8*, *ZFPM1*, *NHLRC4*, and *ZNF862*. Interestingly, several methylation sites identified from the regional analysis also showed nominal significance in the cord blood, which included methylation sites annotated to *ACOT7* and *VKORC1L1*. The *VKORC1L1* gene encodes for an enzyme in the vitamin K cycle, which catalyzes the de-epoxidation of vitamin K. The *VKORC1L1* gene product protects cell membrane proteins, and upregulation of this gene is involved in oxidative damage [75]. *ACOT7* is a hub gene involved in macrophage activation [76]. *SIGLEC8* and *ZFPM1* influence eosinophil apoptosis and production [56,57,63,77].

Previous epigenome-wide association studies have linked blood DNA methylation with childhood food allergy. In a case-control study of 12 allergic children and 12 non-allergic controls, multiple genes (*CACNA1B*, *DUSP3*, *RPS6KA2*, *ZAK*) involved in MAPK signaling pathway were identified in purified CD4 + T cells [23]. A separate study of 2,197 children of European ancestry showed differential DNA methylation of the *HLA-DQB1* and *HLA-DRB1* gene partially mediates the genetic risk associated with peanut allergy [24]. Further, an epigenome-wide association study of cow's milk allergy also demonstrated multiple

hypo-methylated sites in allergic children in comparison to their matched controls [78]. Top significant differentially methylated sites were mapped to *NDFIP2*, *EVL*, *TRAPPC9*, *RPS6KA2*, *IL1RL1*, *IL5RA*, *STAT4*, *IL4*, *CLL18* in the discovery dataset. However, those associations were less robust in the replication dataset [78]. To our knowledge, no epigenome-wide association studies have been performed for environmental allergy. Candidate gene studies identified *ORMDL1* and *STAT6* hypomethylation and *RAD50* and *IL13* hypermethylation in children whose mothers were living on or running a farm with livestock during pregnancy compared to children whose mothers were living in rural areas but not being exposed to farms or livestock during pregnancy [79], indicating that maternal exposures to farm environment may be associated with differential methylation in distinct genes in children. Early life microbial exposure has also been shown to be related to interferon gamma gene demethylation in naïve T-cells, and associated with subsequent risk for allergic diseases [80].

Neither the cohort-specific nor the meta-analysis results identified differential methylation in cord blood associated with childhood atopic sensitization and environmental allergen sensitization at a stringent statistical threshold, although there were suggestive nominal associations. Among those nominal associations, a number of methylation sites encode genes that are involved in cell migration and adhesion. For example, *ITGAL* (integrin subunit alpha L) is a lymphocyte co-stimulatory protein and plays a key role in cellular adhesion [81]. *ACTN3* (alpha actinin-3) is an actin-binding protein responsible for myofibrillar actin filament anchoring [82]. *APC2* (Adenomatous polyposis coli 2) downregulates beta-catenin and interacts with E-cadherin [83]. Adenomatous polyposis coli proteins are also known to regulate cell polarization, attachment and migration [83]. Allergen-associated airway remodeling has been repeatedly observed in animal experiments, *in vitro* studies and *in silico* models [84–87]. Although airway remodeling is thought to have a genetic component [88], to our knowledge, no study to date has reported differential methylation sites in cord blood associated with genes involved in airway remodeling. At nominal significance level, our study identified a number of methylation sites

that regulate cytoskeletal remodeling, cell adhesion and polarization across the three allergy phenotypes, which may suggest that epigenetic marks in cord blood DNA – reflecting the *in utero* environment – may serve as early biomarkers of allergen-associated airway remodeling susceptibility in childhood.

We observed multiple methylation sites that showed nominal significance in the cord blood analysis and that became epigenome-wide significant in the mid-childhood analysis. Among those sites, changes in methylation values from cord till mid-childhood were also nominally significantly associated with mid-childhood allergy. We observed significant methylation – time interaction among sites related to allergic and asthma pathways. Although under powered, these pieces of evidence suggest that methylation patterns established during fetal development and measured at birth may set the epigenetic trajectory and mark the susceptibility for allergic sensitization in childhood. Further longitudinal studies with larger sample sizes are warranted to further verify our hypothesis, as these may be targets for both diagnostics and therapeutics for allergic processes that start in childhood.

Our study has a number of strengths [1]. For the mid-childhood concurrent analysis, our top associations were consistent in magnitude and direction across two relatively large birth cohorts from the United States and Europe, which suggests that our findings were unlikely to be cohort specific effects [2]. We identified methylation sites implicated in several pathways (asthma, focal adhesion, mTOR signaling, and inositol phosphate metabolism), and these may highlight plastic pathways to leverage for prevention and therapeutics [3]. Our results were robust to the adjustment by cell counts using the Houseman's method [39] or by a stringent reference-free method, ReFACTor [50] – a modified PCA method to control for cellular heterogeneity [4]. Many of the FDR-significant methylation sites identified from a separate multi-ethnicity meta-analysis of IgE also replicated in our cohort (Table S10) [25] [5]. We observed longitudinal time trends for a number of methylation sites that were nominally significant at birth and became epigenome-wide significant in mid-childhood, supporting the hypothesis that both *in utero* and postnatal environment are important for the development of

allergy and the allergic march. Lastly, although exploratory, most of the overlapping methylation marks occur within predicted transcription factor motifs; this observation may provide a mechanistic link for the EWAS results for these allergic phenotypes.

Our study also has several limitations [1]. Peripheral blood childhood DNA methylation and mid-childhood allergic outcomes are measured at the same time. Hence, it is difficult to disentangle the temporal relationships between DNA methylation and allergic sensitizations, and our results may be impacted by reverse causation; however this does not limit these findings as biomarkers of atopic disease. On the other hand, our longitudinal prospective cord blood analysis identified nominal significant methylation sites (in the range of 10^{-6} to 10^{-5}) associated with mid-childhood allergy. We also identified a number of methylation sites that showed nominal significance ($p < 0.05$) at birth and epigenome-wide significance in mid-childhood, and a suggestive longitudinal time trend, suggesting that methylation patterns at birth may mark allergic vulnerability later in childhood. Larger longitudinal studies would be informative. (2 [2] We analyzed atopic sensitization, and two composite intermediate phenotypes (environmental and food allergy). These phenotypes are correlated and hence not three independent sets of EWAS findings [3]. Project Viva and the Generation R Study used different methods to assess allergy sensitizations. In Project Viva, atopic sensitization was defined using serum specific IgEs, while the Generation R Study used skin prick testing. Serum specific IgE testing is sensitive but not very specific for true atopy, whereas skin testing is more specific [89,90]. A more sensitive, but less specific test will yield more false positive but less false negative case diagnosis, while a less sensitive, but more specific test will yield less false positive but more false negative case diagnosis. The differences in allergic sensitization definitions may result in heterogeneous findings [4]. However, we contend that the genes and pathways we have identified are robust despite this concern for contributions of phenotypic heterogeneity. DNA methylation variability does not always translate to phenotypic differences; functional analyses are needed to examine

whether the methylation sites we identified influence gene expression or chromatin accessibility; this is a direction for future work.

In summary, our study identified differential methylation in mid-childhood peripheral blood DNA associated with mid-childhood atopic sensitization, environmental allergen and food allergen sensitization. Multiple DNA methylation sites here associated with atopy have been implicated in allergy and asthma. Further, we observed that epigenetic perturbations in focal adhesion, mTOR signaling, and inositol phosphate metabolism pathways are likely relevant to allergy and the allergic march, and that mechanistic implications may include gene regulatory pathway perturbations due to variable methylation in regions of transcription factor binding. Most compelling is the suggestion that methylation patterns established during fetal development may set the epigenetic trajectory and mark the susceptibility for allergic sensitization in childhood. Our study provides evidence for the epigenetic regulation as a marker of childhood allergy, and may inform future efforts for prevention and treatment of childhood allergy that leverage the plasticity of the epigenome.

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Disclosure statement

None of the authors has any actual or potential competing financial interests. The views expressed in this article do not necessarily represent the views of the US Government, the Department of Health and Human Services or the National Institutes of Health.

Data availability statement

The datasets for the current project are not publicly available because this study began in 1999, and we did not obtain consent for such public release of genetic data. Data are available from the responding author or Project Viva study team (project_viva@hphc.org) upon request.

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Table S2. Association between mid-childhood peripheral blood DNA methylation with mid-childhood atopic sensitization, environmental and food allergen sensitization (top 30 methylation sites are presented here sorted by meta-analysis p-values).

CpG	CHR	Gene	Model 1		Model 2	
			Estimate	P value	Estimate	P value
cg04983687	16	<i>ZFPM1</i>	-9.08	2.87E-11	-9.14	5.96E-11
cg02427831	19	<i>SIGLEC8</i>	-19.67	3.89E-11	-19.05	4.53E-10
cg26791242	1	<i>LOC339524</i>	-17.35	6.49E-11	-17.46	1.21E-10
cg11770323	13	<i>NDFIP2</i>	-15.68	7.33E-11	-16.3	2.38E-11
cg02970679	17	<i>EPX</i>	-20.11	1.56E-10	-20.18	2.25E-10
cg13628444	9	<i>MED27</i>	-19.24	6.41E-10	-19.65	6.90E-10
cg21220721	1	<i>ACOT7</i>	-8.15	6.65E-10	-8.21	1.48E-09
cg09212118	1	<i>GUCA2A</i>	-17.16	7.16E-10	-16.99	2.58E-09
cg08698681	3	<i>TNIK</i>	-21.66	8.01E-10	-21.43	4.02E-09
cg10433549	17	--	-18.29	9.79E-10	-19.76	7.46E-11
cg26396815	4	<i>BANK1</i>	-22.73	1.28E-09	-23.48	5.40E-10
cg07948085	10	--	-22.84	1.29E-09	-22.82	5.28E-09
cg04406620	16	<i>CYB5B</i>	-16.31	1.51E-09	-16.32	3.97E-09
cg19434937	12	<i>LPCAT3</i>	-13.09	1.56E-09	-12.96	6.42E-09
cg07819010	11	--	-14.81	1.58E-09	-15.18	1.04E-09
cg24491618	7	<i>KCNH2</i>	-15.68	1.68E-09	-16.29	8.99E-10
cg00114012	9	<i>SLC2A8</i>	-15.16	1.69E-09	-15.81	6.31E-10
cg11988722	20	--	-13.39	2.38E-09	-13.39	5.48E-09
cg09596645	3	--	-18.93	2.54E-09	-19.18	2.49E-09
cg25173129	17	<i>EPX</i>	-18.22	2.65E-09	-18.61	2.62E-09
cg06391412	3	<i>FOXP1</i>	-17.87	2.94E-09	-18.56	1.04E-09
cg10704177	1	<i>PATJ</i>	-14.43	2.96E-09	-14.82	3.60E-09
cg00944309	5	--	-14.98	3.03E-09	-14.99	5.04E-09
cg04497992	16	<i>NHLRC4</i>	-13.59	3.27E-09	-13.72	8.46E-09
cg01916918	8	<i>CLU</i>	-21.89	3.68E-09	-22.77	3.70E-09
cg11456013	6	--	-15.5	3.71E-09	-15.25	1.20E-08
cg14769121	7	<i>SMURF1</i>	-17.77	3.75E-09	-17.61	5.23E-09
cg13054523	17	--	-16.96	4.87E-09	-16.98	1.89E-08
cg20885063	17	<i>ATPAF2</i>	-16.75	5.54E-09	-16.95	6.55E-09
cg06528816	2	<i>TTC7A</i>	-14.08	5.60E-09	-13.87	2.23E-08

CpG	CHR	Gene	Model 1		Model 2	
			Estimate	P value	Estimate	P value
cg02427831	19	<i>SIGLEC8</i>	-26.31	1.37E-16	-26.23	1.97E-15
cg04983687	16	<i>ZFPM1</i>	-11.08	7.11E-15	-11.43	9.38E-15
cg11988722	20	--	-17.96	6.51E-14	-18.08	2.53E-13
cg02475695	16	<i>NHLRC4</i>	-20.12	9.60E-14	-20.5	4.06E-13
cg19434937	12	<i>LPCAT3</i>	-17.26	9.80E-14	-17.75	2.30E-13
cg09212118	1	<i>GUCA2A</i>	-21.62	2.02E-13	-21.93	4.43E-13

cg02970679	17	EPX	-23.92	2.06E-13	-24.45	2.45E-13
cg26791242	1	LOC339524	-20.53	2.10E-13	-21.09	2.10E-13
cg21220721	1	ACOT7	-10.02	3.76E-13	-10.4	3.89E-13
cg13628444	9	MED27	-22.97	6.61E-13	-23.94	4.45E-13
cg00114012	9	SLC2A8	-18.93	6.81E-13	-20.01	1.52E-13
cg24491618	7	KCNH2	-19.48	8.17E-13	-20.62	2.83E-13
cg04497992	16	NHLRC4	-17.33	9.92E-13	-17.85	2.19E-12
cg25173129	17	EPX	-22.64	1.22E-12	-23.49	6.38E-13
cg18666454	7	KCNH2	-18.07	1.36E-12	-18.02	1.07E-11
cg07970948	7	ZNF862	-15.37	1.41E-12	-15.72	1.38E-12
cg17988187	2	LOC100189589	-19.02	1.78E-12	-19.39	2.46E-12
cg10433549	17	--	-22.2	2.76E-12	-24.08	1.14E-13
cg10704177	1	PATJ	-17.83	2.84E-12	-18.68	2.47E-12
cg00944309	5	--	-18.33	3.16E-12	-18.55	4.78E-12
cg09596645	3	--	-22.69	3.26E-12	-23.12	3.96E-12
cg12614529	4	MND1	-17.11	3.28E-12	-17.99	2.83E-12
cg26396815	4	BANK1	-26.71	3.52E-12	-27.89	1.01E-12
cg08698681	3	TNIK	-25.29	3.63E-12	-25.58	1.57E-11
cg10065736	12	FBXW8	-16.85	4.88E-12	-18.08	1.58E-12
cg16263722	1	MECR	-18.58	4.95E-12	-19.98	7.18E-13
cg06528816	2	TTC7A	-17.3	5.07E-12	-17.55	1.74E-11
cg11456013	6	--	-19.08	5.99E-12	-19.12	1.96E-11
cg18421167	17	EPX	-26.27	6.47E-12	-26.55	7.37E-11
cg20151107	10	FAM53B	-21.83	7.57E-12	-22.57	1.08E-11

CpG	CHR	Gene	Model 1		Model 2	
			Estimate	P value	Estimate	P value
cg13054523	17	--	-19.86	9.86E-09	-19.28	5.05E-08
cg06906869	13	NEK3	-20.41	2.40E-08	-20.17	3.60E-08
cg13829849	9	MAMDC2	-51.07	3.93E-08	-51.33	3.42E-08
cg23021241	1	DMAP1	14.31	9.32E-08	14.49	2.59E-08
cg21919729	8	CTSB	-12.62	1.15E-07	-12.5	2.80E-07
cg11456013	6	--	-16.27	1.18E-07	-15.81	3.80E-07
cg21220721	1	ACOT7	-8.27	1.23E-07	-8.04	4.29E-07
cg01916918	8	CLU	-22.38	2.40E-07	-21.72	7.67E-07
cg18372930	5	--	18.01	4.07E-07	18.41	5.35E-07
cg18543428	13	--	-16.37	4.21E-07	-15.88	7.16E-07
cg26791242	1	LOC339524	-15.59	4.90E-07	-15.1	1.28E-06
cg12198633	3	KLHL24	-26.13	4.93E-07	-25.99	8.39E-07
cg12819873	11	PRG2	-15.88	6.02E-07	-15.52	1.38E-06
cg26396815	4	BANK1	-18.98	6.49E-07	-18.83	9.78E-07
cg17521120	19	SIGLEC8	-15.44	7.00E-07	-14.96	1.79E-06
cg01901579	14	DICER1	-13.49	8.33E-07	-12.8	4.69E-06
cg02970679	17	EPX	-15.47	9.36E-07	-15.07	2.43E-06

cg13562011	9	<i>EXD3</i>	-16.76	9.67E-07	-16.26	2.77E-06
cg25758167	1	<i>AGL</i>	-29.58	1.24E-06	-29.49	6.78E-06
cg25738116	3	<i>PA2G4P4</i>	-13.5	1.52E-06	-13.82	1.09E-06
cg08284993	16	<i>ANKRD11</i>	18.73	1.67E-06	17.68	9.60E-06
cg06391412	3	<i>FOXP1</i>	-15.14	1.83E-06	-14.94	2.87E-06
cg23149249	6	--	-35.61	2.07E-06	-35.55	3.09E-06
cg08818866	2	<i>LTBP1</i>	-27.99	2.12E-06	-26.8	7.55E-06
cg14769121	7	<i>SMURF1</i>	-14.7	2.22E-06	-14.14	6.25E-06
cg25488567	6	<i>CALHM6</i>	-17.65	2.31E-06	-17.16	4.73E-06
cg15344640	5	<i>LMAN2</i>	-25.42	3.03E-06	-25.24	5.22E-06
cg05300717	11	<i>AP5B1</i>	-18.17	3.28E-06	-17.38	1.37E-05
cg08698681	3	<i>TNIK</i>	-17.32	3.31E-06	-16.56	1.13E-05
cg14937228	10	<i>FAM107B</i>	-20.31	3.36E-06	-19.02	1.24E-05

Model 1 adjusted for maternal covariates[age at enrollment (continuous), smoking status (never, former, or during pregnancy), education (college / graduate level or not college / graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race / ethnicity (white, black or others), gestational age (continuous)], and cell type proxies estimated for peripheral blood (percentages of monocytes, CD8T cells, CD4T cells, NK cells, and B cells).

Model 2 adjusted for maternal covariates[age at enrollment (continuous), smoking status (never, former, or during pregnancy), education (college / graduate level or not college / graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race / ethnicity (white, black or others), gestational age (continuous), current asthma (yes / no)], and cell type proxies estimated for peripheral blood (percentages of monocytes, CD8T cells, CD4T cells, NK cells, and B cells).

Table S4. Association between cord blood DNA methylation with mid-childhood atopic sensitization, environmental and food allergen sensitization (top 30 methylation sites are presented here sorted by p-values).

CpG	CHR	Gene	Atopic sensitization		GenR		Meta-analysis		
			Viva		GenR		Meta-analysis		
			Estimate	P value	Estimate	P value	Estimate	P value	FDR
cg18049299	2	--	21.35	1.70E-03	10.91	6.23E-04	12.79	9.44E-06	7.56E-01
cg14444710	16	<i>PDPK1</i>	19.71	3.53E-01	19.76	2.85E-05	19.76	1.81E-05	7.56E-01
cg18426014	12	<i>SMUG1</i>	-14.22	6.84E-01	-22.30	2.36E-05	-22.12	2.23E-05	7.56E-01
cg09823543	6	<i>SRF</i>	12.41	1.60E-02	9.71	4.48E-04	10.32	2.31E-05	7.56E-01
cg23746577	18	<i>ZBTB7C</i>	32.58	6.08E-03	13.15	3.59E-04	14.86	2.43E-05	7.56E-01
cg12991050	5	<i>LINC00461</i>	2.32	9.50E-01	14.41	2.79E-05	14.31	2.95E-05	7.56E-01
cg23377395	11	--	4.27	9.40E-02	6.81	9.88E-05	6.00	3.21E-05	7.56E-01
cg21855205	11	<i>PPP1CA</i>	19.44	7.02E-03	10.36	8.21E-04	11.77	3.51E-05	7.56E-01
cg04240548	22	<i>TTL8</i>	-6.56	1.22E-01	-8.44	1.32E-04	-8.04	4.03E-05	7.56E-01
cg09069072	1	<i>TMEM51</i>	3.57	2.61E-01	6.07	6.20E-05	5.61	4.16E-05	7.56E-01
cg25881344	12	<i>ETNK1</i>	-22.84	8.09E-01	38.02	3.96E-05	37.44	4.77E-05	7.56E-01
cg24033122	16	<i>ITGAL</i>	-1.82	4.89E-01	-5.98	2.38E-05	-5.05	5.16E-05	7.56E-01
cg19846318	17	<i>PYCR1</i>	17.60	4.51E-01	18.67	7.44E-05	18.62	5.52E-05	7.56E-01
cg14127347	20	<i>NAPB</i>	-19.45	8.30E-01	-42.86	5.78E-05	-42.54	5.84E-05	7.56E-01
cg11617818	17	<i>B9D1</i>	9.38	1.51E-01	11.69	1.71E-04	11.26	6.04E-05	7.56E-01
cg13160528	6	--	15.23	8.72E-02	13.74	3.22E-04	13.97	6.90E-05	7.56E-01
cg08805241	17	--	29.10	8.93E-02	17.14	2.52E-04	17.97	6.92E-05	7.56E-01
cg13814693	12	<i>YBX3</i>	-4.75	9.15E-01	-20.76	6.81E-05	-20.55	7.24E-05	7.56E-01
cg13875807	9	<i>KDM4C</i>	22.90	3.91E-01	-24.42	2.34E-05	-22.31	7.71E-05	7.56E-01
cg26558749	5	<i>ANKRD33B</i>	-184.55	4.75E-02	-62.01	2.56E-04	-65.94	7.75E-05	7.56E-01
cg18275445	14	<i>ELMSAN1</i>	-159.77	3.39E-02	-47.06	2.68E-04	-50.28	7.80E-05	7.56E-01
cg06684321	11	<i>ACTN3</i>	7.63	1.58E-01	9.75	2.33E-04	9.34	8.63E-05	7.56E-01
cg00782707	19	<i>APC2</i>	4.38	9.12E-01	16.26	8.54E-05	16.14	8.89E-05	7.56E-01
cg04813787	6	--	9.07	4.77E-02	9.05	7.25E-04	9.05	8.97E-05	7.56E-01
cg15286905	10	<i>ACBD5</i>	-21.52	5.14E-01	-16.60	1.12E-04	-16.68	9.00E-05	7.56E-01
cg07304186	6	<i>ZFAND3</i>	24.39	1.57E-01	19.12	2.53E-04	19.56	9.16E-05	7.56E-01
cg06139794	10	--	-13.33	3.71E-01	-9.19	1.36E-04	-9.30	9.27E-05	7.56E-01
cg07084345	15	--	2.41	1.28E-03	1.36	1.30E-02	1.73	9.40E-05	7.56E-01
cg22112918	6	<i>KIFC1</i>	-30.84	1.75E-03	-12.81	4.13E-03	-15.88	9.45E-05	7.56E-01
cg10215507	20	<i>LINC00261</i>	-14.79	2.04E-02	-7.91	9.92E-04	-8.76	9.66E-05	7.56E-01

CpG	CHR	Gene	Environmental allergen sensitization		GenR		Meta-analysis		
			Viva		GenR		Meta-analysis		
			Estimate	P value	Estimate	P value	Estimate	P value	FDR
cg23377395	11	--	9.65	1.02E-03	6.83	1.10E-04	7.58	5.54E-07	1.79E-01
cg18049299	2	--	23.01	8.33E-04	11.74	2.57E-04	13.76	2.30E-06	3.72E-01

cg24033122	16	<i>ITGAL</i>	-3.08	2.48E-01	-6.29	8.72E-06	-5.59	7.82E-06	6.10E-01
cg14444710	16	<i>PDPK1</i>	32.90	1.28E-01	19.94	2.87E-05	20.55	1.02E-05	6.10E-01
cg09069072	1	<i>TMEM51</i>	4.45	1.70E-01	6.33	3.78E-05	5.99	1.62E-05	6.10E-01
cg18275445	14	<i>ELMSAN1</i>	-91.02	2.12E-01	-51.86	3.31E-05	-52.98	1.69E-05	6.10E-01
cg23768510	6	<i>TAPBP</i>	-89.99	1.28E-03	-31.83	6.02E-04	-37.60	1.95E-05	6.10E-01
cg23746577	18	<i>ZBTB7C</i>	33.03	6.86E-03	13.37	3.53E-04	15.06	2.58E-05	6.10E-01
cg06684321	11	<i>ACTN3</i>	6.06	2.67E-01	10.65	4.18E-05	9.80	2.96E-05	6.10E-01
cg06139794	10	--	-26.43	7.85E-02	-9.38	1.05E-04	-9.81	3.99E-05	6.10E-01
cg18167921	1	<i>DEPDC1</i>	6.84	8.06E-03	4.08	1.04E-03	4.60	4.05E-05	6.10E-01
cg26558749	5	<i>ANKRD33B</i>	-180.11	5.79E-02	-65.43	1.32E-04	-69.04	4.15E-05	6.10E-01
cg18426014	12	<i>SMUG1</i>	3.47	9.21E-01	-21.82	3.22E-05	-21.27	4.19E-05	6.10E-01
cg15286905	10	<i>ACBD5</i>	-9.75	7.72E-01	-17.64	4.30E-05	-17.51	4.24E-05	6.10E-01
cg12991050	5	<i>LINC00461</i>	-4.11	9.12E-01	14.22	3.88E-05	14.06	4.37E-05	6.10E-01
cg08799394	10	<i>PDLIM1</i>	13.88	1.10E-03	8.36	8.09E-03	10.32	4.66E-05	6.10E-01
cg14330737	10	<i>PFKP</i>	-38.31	3.35E-01	23.73	2.19E-05	22.53	4.73E-05	6.10E-01
cg09823543	6	<i>SRF</i>	11.85	1.75E-02	9.20	8.78E-04	9.82	4.88E-05	6.10E-01
cg02117884	6	<i>LY6G6D</i>	22.93	6.18E-04	11.31	9.76E-03	14.79	5.43E-05	6.10E-01
cg25482786	3	<i>CHL1</i>	-180.52	4.41E-02	-40.79	1.43E-04	-42.76	5.94E-05	6.10E-01
cg19466160	17	<i>DLG4</i>	5.55	8.25E-03	4.58	2.34E-03	4.91	6.03E-05	6.10E-01
cg14127347	20	<i>NAPB</i>	12.02	8.95E-01	-43.06	5.09E-05	-42.33	6.09E-05	6.10E-01
cg19846318	17	<i>PYCR1</i>	28.05	2.46E-01	18.17	1.17E-04	18.53	6.23E-05	6.10E-01
cg03926166	12	<i>HRK</i>	58.03	3.36E-01	40.91	1.02E-04	41.42	6.50E-05	6.10E-01
cg07784986	12	<i>DNAH10</i>	18.26	2.18E-02	10.07	6.90E-04	11.07	6.85E-05	6.10E-01
cg21349849	8	--	50.68	5.73E-04	13.66	1.83E-03	16.67	7.19E-05	6.10E-01
cg08805241	17	--	38.94	2.93E-02	16.60	4.18E-04	18.05	7.28E-05	6.10E-01
cg13875807	9	<i>KDM4C</i>	32.37	2.26E-01	-25.19	1.55E-05	-22.58	7.36E-05	6.10E-01
cg00782707	19	<i>APC2</i>	-3.79	9.24E-01	16.49	6.56E-05	16.28	7.49E-05	6.10E-01
cg04117455	14	<i>ZFYVE21</i>	7.75	8.24E-01	20.24	7.26E-05	19.97	7.56E-05	6.10E-01

CpG	CHR	Gene	Food allergen sensitization						
			Viva		GenR		Meta-analysis		
			Estimate	P value	Estimate	P value	Estimate	P value	FDR
cg05068730	6	<i>RPS6KA2</i>	11.38	7.21E-01	46.51	7.35E-08	44.10	1.24E-07	3.54E-02
cg05767015	1	<i>CHIT1</i>	10.71	4.10E-01	-15.87	3.95E-08	-14.62	2.19E-07	3.54E-02
cg27359629	6	--	-13.49	1.23E-01	-23.54	1.76E-05	-20.71	8.31E-06	5.15E-01
cg04288241	2	<i>AGAP1</i>	-29.70	3.82E-01	-37.14	1.36E-05	-36.70	9.34E-06	5.15E-01
cg24395801	1	<i>COL24A1</i>	-16.20	8.61E-01	77.32	5.71E-06	74.23	9.44E-06	5.15E-01
cg15169257	19	--	-1.49	8.94E-01	-24.77	1.73E-06	-20.64	1.11E-05	5.15E-01
cg22737917	5	<i>ENC1</i>	-40.47	5.08E-01	35.64	1.02E-05	34.33	1.79E-05	5.15E-01
cg08209104	14	<i>MYH7</i>	20.17	1.66E-03	14.88	3.15E-03	16.90	1.99E-05	5.15E-01
cg10782019	1	--	-6.24	5.48E-03	-7.50	1.16E-03	-6.85	2.08E-05	5.15E-01
cg07316263	16	<i>TRADD;FBXL8</i>	9.90	8.53E-01	48.62	1.65E-05	46.97	2.11E-05	5.15E-01

cg00731458	16	--	21.59	1.10E-01	27.36	7.80E-05	26.16	2.19E-05	5.15E-01
cg27607583	16	<i>TRAP1</i>	-23.23	2.60E-01	-40.34	3.25E-05	-37.23	2.25E-05	5.15E-01
cg20115508	4	--	53.88	4.63E-01	-10.58	2.32E-05	-10.50	2.62E-05	5.15E-01
cg06952751	18	<i>RMC1</i>	-21.29	7.90E-01	48.80	1.89E-05	47.40	2.71E-05	5.15E-01
cg19873602	6	--	23.65	5.57E-02	22.45	2.19E-04	22.68	3.16E-05	5.15E-01
cg21393885	17	--	22.66	4.66E-02	26.26	2.58E-04	25.24	3.29E-05	5.15E-01
cg05458842	5	--	10.50	7.32E-01	-41.11	9.05E-06	-36.80	3.31E-05	5.15E-01
cg01908690	16	<i>ZNF646</i>	-93.97	4.31E-01	12.57	3.40E-05	12.50	3.74E-05	5.15E-01
cg18503234	11	<i>TPCN2</i>	-7.42	4.69E-02	-12.28	1.87E-04	-10.16	3.84E-05	5.15E-01
cg03950590	15	<i>CCNB2</i>	-34.42	2.92E-01	-36.10	7.62E-05	-35.98	4.24E-05	5.15E-01
cg13330341	11	<i>ASCL2</i>	24.91	3.95E-01	40.46	5.67E-05	38.82	4.42E-05	5.15E-01
cg12251075	17	<i>NME2</i>	7.52	2.28E-01	12.74	7.52E-05	11.64	4.67E-05	5.15E-01
cg10571951	5	<i>MEF2C</i>	-87.47	9.90E-02	-46.45	1.46E-04	-48.52	4.68E-05	5.15E-01
cg08303283	9	--	25.61	6.02E-05	10.54	5.55E-02	16.97	4.69E-05	5.15E-01
cg14477767	4	--	-8.69	5.15E-03	-6.89	2.77E-03	-7.53	4.70E-05	5.15E-01
cg13267264	8	<i>PRDM14</i>	-15.44	7.19E-01	-48.34	3.78E-05	-46.06	4.70E-05	5.15E-01
cg16743070	8	<i>KAT6A</i>	-99.01	4.96E-02	-63.52	2.90E-04	-67.35	4.75E-05	5.15E-01
cg14940705	8	<i>SGK3</i>	-52.67	3.10E-01	-69.63	7.86E-05	-67.87	4.80E-05	5.15E-01
cg01222168	2	<i>D2HGDH</i>	-16.70	1.10E-01	-16.37	1.99E-04	-16.42	5.16E-05	5.15E-01
cg00093058	16	<i>RHOT2</i>	-15.41	5.98E-01	-53.00	3.09E-05	-47.03	5.54E-05	5.15E-01

Models adjusted for maternal covariates [age at enrollment (continuous), smoking status (never, former, or during pregnancy), education (college / graduate level or not college / graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race / ethnicity (white, black or others), gestational age (continuous)], and cell type proxies estimated for cord blood (percentages of monocytes, CD8T cells, CD4T cells, NK cells, B cells and nucleated red blood cells (nRBC)).

Table S6. Among methylation sites that showed nominal significance ($p < 0.05$) in the cord blood analysis, and epigenome-wide significance ($FDR < 0.05$) in the mid-childhood analysis, we selected (1) top differentially methylation sites sorted by cord blood p-values; (2) methylation sites previously shown to be associated with asthma. We performed mixed-effects models and introduced a methylation—time interaction.

Atopic sensitization						
CpG	CHR	Gene	Main effect		Time interaction	
			Effect estimate (95% CI)	P-value	Effect estimate (95% CI)	P-value
cg08799394	10	<i>PDLIM1</i>	0.0004 (-0.0001; 0.0008)	0.096	-0.0003 (-0.0004; -0.0002)	<0.0001
cg24108508	1	<i>FAM20B</i>	0.0003 (-0.0001; 0.0006)	0.151	-0.0002 (-0.0003; -0.0001)	<0.0001
cg13628444	9	<i>MED27</i>	0.0009 (-0.0001; 0.0019)	0.088	-0.0004 (-0.0005; -0.0002)	<0.0001
cg22083166	17	<i>EPN2</i>	0.0017 (0.0003; 0.0031)	0.018	-0.0003 (-0.0006; -0.0001)	0.008
cg26787239	5	<i>IL4</i>	0.0012 (-0.0003; 0.0027)	0.113	-0.0005 (-0.0007; -0.0002)	0.0003
cg21220721	1	<i>ACOT7</i>	0.0009 (-0.0004; 0.0022)	0.175	-0.0003 (-0.0004; -0.0001)	0.005
cg08404225	3	<i>IL5RA</i>	0.0006 (0.0002; 0.0011)	0.010	-0.0002 (-0.0003; -0.0001)	0.003
cg25643253	7	<i>VKORC1L1</i>	0 (-0.0004; 0.0004)	0.978	-0.0001 (-0.0002; -0.0001)	0.0006

Environmental allergen sensitization						
CpG	CHR	Gene	Main effect		Time interaction	
			Effect estimate (95% CI)	P-value	Effect estimate (95% CI)	P-value
cg08799394	10	<i>PDLIM1</i>	0.0003 (-0.0001; 0.0008)	0.155	-0.0003 (-0.0004; -0.0002)	<0.0001
cg15134174	4	<i>SH3RF1</i>	0.0003 (-0.0001; 0.0006)	0.135	-0.0003 (-0.0005; -0.0002)	<0.0001
cg07819010	11	--	0.0002 (-0.0002; 0.0006)	0.449	-0.0002 (-0.0003; -0.0001)	0.0004
cg24108508	1	<i>FAM20B</i>	0.0002 (-0.0001; 0.0006)	0.196	-0.0002 (-0.0003; -0.0001)	<0.0001
cg26787239	5	<i>IL4</i>	0.0009 (-0.0007; 0.0024)	0.268	-0.0005 (-0.0008; -0.0003)	0.0001
cg21220721	1	<i>ACOT7</i>	0.0005 (-0.0008; 0.0019)	0.438	-0.0002 (-0.0004; -0.0001)	0.017
cg08404225	3	<i>IL5RA</i>	0.0007 (0.0002; 0.0011)	0.008	-0.0002 (-0.0004; -0.0001)	0.0003
cg25643253	7	<i>VKORC1L1</i>	-0.0001 (-0.0005; 0.0003)	0.634	-0.0001 (-0.0002; -0.0001)	0.0002

Food allergen sensitization						
CpG	CHR	Gene	Main effect		Time interaction	
			Effect estimate (95% CI)	P-value	Effect estimate (95% CI)	P-value
cg25488567	6	<i>FAM26F</i>	-0.0013 (-0.0029; 0.0004)	0.129	0 (-0.0002; 0.0003)	0.922
cg08818866	2	<i>LTBP1</i>	0.0013 (0; 0.0026)	0.050	-0.006 (-0.0010; -0.0002)	0.006
cg13562011	9	<i>EXD3</i>	0.0007 (-0.0009; 0.0023)	0.414	-0.0003 (-0.0005; 0)	0.021

Models adjusted for maternal covariates [age at enrollment (continuous), smoking status (never, former, or during pregnancy), education (college / graduate level or not college / graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race / ethnicity (white, black or others), gestational age (continuous)], and cell type proxies estimated for cord blood (percentages of monocytes, CD8T cells, CD4T cells, NK cells, B cells and nucleated red blood cells (nRBC)).

Table S7. Association between mid-childhood peripheral blood DNA methylation with mid-childhood atopic sensitization, environmental and food allergen sensitization (top 30 methylation sites are presented here sorted by p-values). Model 1: Project Viva mid-childhood analysis (N=396); Model 2: Project Viva mid-childhood analysis, with additional adjustment for cord blood methylation (N=211).

Atopic sensitization				
CpG	CHR	Gene	Model 1 estimate	Model 2 estimate
cg04983687	16	<i>ZFPM1</i>	-9.67	-10.72
cg02427831	19	<i>SIGLEC8</i>	-17.31	-23.52
cg26791242	1	<i>LOC339524</i>	-21.08	-16.18
cg11770323	13	<i>NDFIP2</i>	-15.39	-14.40
cg02970679	17	<i>EPX</i>	-22.82	-32.81
cg13628444	9	<i>MED27</i>	-19.84	-25.08
cg21220721	1	<i>ACOT7</i>	-10.67	-12.13
cg09212118	1	<i>GUCA2A</i>	-16.59	-16.65
cg08698681	3	<i>TNIK</i>	-24.72	-29.27
cg10433549	17	--	-20.79	-22.85
cg26396815	4	<i>BANK1</i>	-25.48	-44.75
cg07948085	10	--	-30.67	-31.76
cg04406620	16	<i>CYB5B</i>	-22.41	-14.01
cg19434937	12	<i>LPCAT3</i>	-14.65	-18.57
cg07819010	11	--	-19.61	-14.31
cg24491618	7	<i>KCNH2</i>	-17.84	-23.31
cg00114012	9	<i>SLC2A8</i>	-14.65	-16.08
cg11988722	20	--	-10.46	-16.35
cg09596645	3	--	-20.27	-37.96
cg25173129	17	<i>EPX</i>	-18.99	-19.31
cg06391412	3	<i>FOXP1</i>	-21.66	-27.93
cg10704177	1	<i>PATJ</i>	-17.58	-23.99
cg00944309	5	--	-15.98	-23.88
cg04497992	16	<i>NHLRC4</i>	-15.50	-18.84
cg01916918	8	<i>CLU</i>	-24.05	-25.59
cg11456013	6	--	-17.49	-23.75
cg14769121	7	<i>SMURF1</i>	-19.43	-23.18
cg13054523	17	--	-29.77	-46.14
cg20885063	17	<i>ATPAF2</i>	-19.50	-36.71
cg06528816	2	<i>TTC7A</i>	-14.87	-17.06

Environmental allergen sensitization				
CpG	CHR	Gene	Model 1 estimate	Model 2 estimate
cg02427831	19	<i>SIGLEC8</i>	-27.44	-33.46
cg04983687	16	<i>ZFPM1</i>	-13.89	-14.44
cg11988722	20	--	-16.75	-18.57

cg02475695	16	<i>NHLRC4</i>	-23.33	-24.38
cg19434937	12	<i>LPCAT3</i>	-23.39	-23.91
cg09212118	1	<i>GUCA2A</i>	-25.48	-20.55
cg02970679	17	<i>EPX</i>	-30.45	-39.95
cg26791242	1	<i>LOC339524</i>	-27.95	-17.97
cg21220721	1	<i>ACOT7</i>	-15.29	-14.93
cg13628444	9	<i>MED27</i>	-25.68	-25.08
cg00114012	9	<i>SLC2A8</i>	-20.98	-20.49
cg24491618	7	<i>KCNH2</i>	-25.12	-30.22
cg04497992	16	<i>NHLRC4</i>	-23.49	-23.65
cg25173129	17	<i>EPX</i>	-28.04	-25.40
cg18666454	7	<i>KCNH2</i>	-24.13	-25.10
cg07970948	7	<i>ZNF862</i>	-22.19	-29.05
cg17988187	2	<i>LOC100189589</i>	-21.27	-23.40
cg10433549	17	--	-27.98	-23.51
cg10704177	1	<i>PATJ</i>	-24.75	-28.08
cg00944309	5	--	-21.73	-27.13
cg09596645	3	--	-27.51	-46.67
cg12614529	4	<i>MND1</i>	-23.08	-13.04
cg26396815	4	<i>BANK1</i>	-34.91	-49.07
cg08698681	3	<i>TNIK</i>	-31.66	-33.08
cg10065736	12	<i>FBXW8</i>	-23.77	-27.74
cg16263722	1	<i>MECR</i>	-22.84	-22.02
cg06528816	2	<i>TTC7A</i>	-20.09	-21.26
cg11456013	6	--	-24.21	-24.91
cg18421167	17	<i>EPX</i>	-35.36	-42.68
cg20151107	10	<i>FAM53B</i>	-26.45	-23.21

Food allergen sensitization				
CpG	CHR	Gene	Model 1 estimate	Model 2 estimate
cg13054523	17	--	-20.37	-26.74
cg06906869	13	<i>NEK3</i>	-19.45	-22.83
cg13829849	9	<i>MAMDC2</i>	-55.37	-81.56
cg23021241	1	<i>DMAP1</i>	66.23	144.21
cg21919729	8	<i>CTSB</i>	-11.70	-14.38
cg11456013	6	--	-14.31	-15.82
cg21220721	1	<i>ACOT7</i>	-7.65	-7.21
cg01916918	8	<i>CLU</i>	-22.68	-18.86
cg18372930	5	--	17.40	9.77
cg18543428	13	--	-14.64	-9.42
cg26791242	1	<i>LOC339524</i>	-15.02	-12.09
cg12198633	3	<i>KLHL24</i>	-30.03	-19.82

cg12819873	11	<i>PRG2</i>	-16.15	-16.98
cg26396815	4	<i>BANK1</i>	-16.91	-25.18
cg17521120	19	<i>SIGLEC8</i>	-13.65	-13.60
cg01901579	14	<i>DICER1</i>	-12.01	-13.96
cg02970679	17	<i>EPX</i>	-13.75	-17.14
cg13562011	9	<i>EXD3</i>	-15.63	-12.17
cg25758167	1	<i>AGL</i>	-22.07	-17.37
cg25738116	3	<i>PA2G4P4</i>	-13.46	-5.75
cg08284993	16	<i>ANKRD11</i>	20.86	13.88
cg06391412	3	<i>FOXP1</i>	-14.57	-14.78
cg23149249	6	--	-36.76	-50.37
cg08818866	2	<i>LTBP1</i>	-27.55	-27.34
cg14769121	7	<i>SMURF1</i>	-12.69	-12.27
cg25488567	6	<i>CALHM6</i>	-17.32	-12.37
cg15344640	5	<i>LMAN2</i>	-24.37	-28.01
cg05300717	11	<i>AP5B1</i>	-18.35	-19.44
cg08698681	3	<i>TNIK</i>	-16.27	-17.67
cg14937228	10	<i>FAM107B</i>	-17.36	-16.54

Models adjusted for maternal covariates [age at enrollment (continuous), smoking status (never, former, or during pregnancy), education (college / graduate level or not college / graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race / ethnicity (white, black or others), gestational age (continuous)], and cell type proxies estimated for peripheral blood (percentages of monocytes, CD8T cells, CD4T cells, NK cells, and B cells). Model 2 additionally adjusted for cord blood DNA methylation of the same site.

Table S8. Association between mid-childhood peripheral blood DNA methylation with mid-childhood atopic sensitization, environmental and food allergen sensitization (top 30 methylation sites are presented here sorted by meta-analysis p-values). We compared analysis using two methods to adjust for cellular heterogeneity (left column—Houseman estimation; right column—ReFACTor, a PCA based method).

Atopic sensitization						
CpG	CHR	Gene	Houseman adjustment		ReFACTor adjustment	
			Estimate	P value	Estimate	P value
cg04983687	16	<i>ZFPM1</i>	-9.67	3.36E-07	-11.91	1.84E-08
cg02427831	19	<i>SIGLEC8</i>	-17.31	5.07E-06	-17.94	7.33E-06
cg26791242	1	<i>LOC339524</i>	-21.08	5.23E-09	-22.79	8.87E-09
cg11770323	13	<i>NDFIP2</i>	-15.39	3.88E-08	-16.91	1.63E-08
cg02970679	17	<i>EPX</i>	-22.82	6.99E-08	-23.78	7.72E-08
cg13628444	9	<i>MED27</i>	-19.84	3.66E-07	-20.60	9.43E-07
cg21220721	1	<i>ACOT7</i>	-10.67	7.09E-09	-11.70	1.83E-09
cg09212118	1	<i>GUCA2A</i>	-16.59	7.89E-06	-17.78	5.99E-06
cg08698681	3	<i>TNIK</i>	-24.72	7.21E-08	-26.25	7.80E-08
cg10433549	17	--	-20.79	9.25E-08	-26.01	7.80E-09
cg26396815	4	<i>BANK1</i>	-25.48	1.46E-06	-25.65	4.82E-06
cg07948085	10	--	-30.67	5.47E-08	-31.26	3.95E-07
cg04406620	16	<i>CYB5B</i>	-22.41	4.69E-08	-20.15	1.09E-06
cg19434937	12	<i>LPCAT3</i>	-14.65	3.57E-07	-15.42	7.94E-07
cg07819010	11	--	-19.61	4.16E-09	-22.14	3.29E-09
cg24491618	7	<i>KCNH2</i>	-17.84	1.22E-07	-20.39	1.50E-08
cg00114012	9	<i>SLC2A8</i>	-14.65	2.44E-06	-15.43	3.34E-06
cg11988722	20	--	-10.46	1.12E-04	-10.97	1.28E-04
cg09596645	3	--	-20.27	1.78E-06	-19.41	9.15E-06
cg25173129	17	<i>EPX</i>	-18.99	4.56E-06	-21.89	7.63E-07
cg06391412	3	<i>FOXP1</i>	-21.66	2.35E-08	-24.97	1.98E-08
cg10704177	1	<i>PATJ</i>	-17.58	5.38E-08	-18.48	1.02E-07
cg00944309	5	--	-15.98	5.00E-07	-16.30	3.54E-06
cg04497992	16	<i>NHLRC4</i>	-15.50	4.05E-07	-18.57	4.81E-08
cg01916918	8	<i>CLU</i>	-24.05	9.18E-07	-25.24	1.94E-06
cg11456013	6	--	-17.49	1.25E-07	-18.08	4.77E-07
cg14769121	7	<i>SMURF1</i>	-19.43	1.16E-06	-19.26	7.40E-06
cg13054523	17	--	-29.77	1.58E-06	-34.52	4.94E-07
cg20885063	17	<i>ATPAF2</i>	-19.50	1.17E-06	-19.55	2.38E-06
cg06528816	2	<i>TTC7A</i>	-14.87	1.02E-06	-18.23	9.48E-08

Environmental allergen sensitization						
CpG	CHR	Gene	Houseman adjustment		ReFACTor adjustment	
			Estimate	P value	Estimate	P value
cg02427831	19	<i>SIGLEC8</i>	-27.44	7.40E-11	-26.87	5.66E-10

cg04983687	16	<i>ZFPM1</i>	-13.89	1.21E-11	-16.13	2.05E-12
cg11988722	20	--	-16.75	1.78E-08	-16.94	5.09E-08
cg02475695	16	<i>NHLRC4</i>	-23.33	1.96E-10	-26.27	7.24E-11
cg19434937	12	<i>LPCAT3</i>	-23.39	7.11E-13	-24.69	2.91E-12
cg09212118	1	<i>GUCA2A</i>	-25.48	6.19E-10	-25.40	2.16E-09
cg02970679	17	<i>EPX</i>	-30.45	1.50E-11	-30.90	2.94E-11
cg26791242	1	<i>LOC339524</i>	-27.95	1.40E-12	-28.44	1.18E-11
cg21220721	1	<i>ACOT7</i>	-15.29	3.83E-14	-16.54	1.68E-14
cg13628444	9	<i>MED27</i>	-25.68	3.43E-10	-26.12	1.90E-09
cg00114012	9	<i>SLC2A8</i>	-20.98	3.78E-10	-21.35	1.18E-09
cg24491618	7	<i>KCNH2</i>	-25.12	6.04E-12	-27.14	2.46E-12
cg04497992	16	<i>NHLRC4</i>	-23.49	4.89E-12	-26.16	2.45E-12
cg25173129	17	<i>EPX</i>	-28.04	3.84E-10	-29.71	2.45E-10
cg18666454	7	<i>KCNH2</i>	-24.13	4.05E-11	-24.39	1.32E-10
cg07970948	7	<i>ZNF862</i>	-22.19	3.41E-11	-23.43	2.56E-11
cg17988187	2	<i>LOC100189589</i>	-21.27	4.00E-10	-22.41	4.74E-10
cg10433549	17	--	-27.98	5.51E-11	-32.36	1.55E-11
cg10704177	1	<i>PATJ</i>	-24.75	2.47E-12	-25.46	8.96E-12
cg00944309	5	--	-21.73	1.49E-10	-22.49	1.45E-09
cg09596645	3	--	-27.51	5.28E-10	-27.22	2.90E-09
cg12614529	4	<i>MND1</i>	-23.08	1.31E-11	-26.00	2.07E-12
cg26396815	4	<i>BANK1</i>	-34.91	4.40E-10	-35.18	2.07E-09
cg08698681	3	<i>TNIK</i>	-31.66	6.67E-11	-32.67	1.39E-10
cg10065736	12	<i>FBXW8</i>	-23.77	3.85E-12	-27.11	1.70E-12
cg16263722	1	<i>MECR</i>	-22.84	9.35E-11	-24.07	1.82E-10
cg06528816	2	<i>TTC7A</i>	-20.09	4.76E-10	-22.81	1.67E-10
cg11456013	6	--	-24.21	2.12E-11	-24.50	1.84E-10
cg18421167	17	<i>EPX</i>	-35.36	4.19E-10	-34.06	5.45E-09
cg20151107	10	<i>FAM53B</i>	-26.45	3.87E-09	-26.53	2.27E-08

Food allergen sensitization						
CpG	CHR	Gene	Houseman adjustment		ReFACTor adjustment	
			Estimate	P value	Estimate	P value
cg13054523	17	--	-20.37	2.28E-05	-21.94	1.02E-05
cg06906869	13	<i>NEK3</i>	-19.45	1.24E-06	-20.77	5.71E-07
cg13829849	9	<i>MAMDC2</i>	-55.37	3.64E-06	-52.92	7.88E-06
cg23021241	1	<i>DMAP1</i>	66.23	1.87E-01	32.99	5.33E-01
cg21919729	8	<i>CTSB</i>	-11.70	2.05E-05	-12.70	9.53E-06
cg11456013	6	--	-14.31	1.13E-05	-14.54	2.32E-05
cg21220721	1	<i>ACOT7</i>	-7.65	8.55E-06	-8.25	2.57E-06
cg01916918	8	<i>CLU</i>	-22.68	3.72E-06	-23.86	4.20E-06
cg18372930	5	--	17.40	2.65E-05	11.39	9.31E-03

cg18543428	13	--	-14.64	1.73E-04	-13.68	7.54E-04
cg26791242	1	<i>LOC339524</i>	-15.02	1.10E-05	-15.85	1.12E-05
cg12198633	3	<i>KLHL24</i>	-30.03	1.00E-05	-29.06	1.45E-05
cg12819873	11	<i>PRG2</i>	-16.15	3.65E-06	-16.46	3.21E-06
cg26396815	4	<i>BANK1</i>	-16.91	5.47E-05	-16.96	1.14E-04
cg17521120	19	<i>SIGLEC8</i>	-13.65	5.73E-05	-15.17	1.31E-05
cg01901579	14	<i>DICER1</i>	-12.01	9.31E-05	-12.78	1.38E-04
cg02970679	17	<i>EPX</i>	-13.75	5.11E-05	-13.91	4.47E-05
cg13562011	9	<i>EXD3</i>	-15.63	4.13E-05	-16.80	1.87E-05
cg25758167	1	<i>AGL</i>	-22.07	8.05E-03	-23.85	7.73E-03
cg25738116	3	<i>PA2G4P4</i>	-13.46	7.82E-06	-12.77	5.32E-05
cg08284993	16	<i>ANKRD11</i>	20.86	2.04E-06	14.74	9.91E-04
cg06391412	3	<i>FOXP1</i>	-14.57	1.87E-05	-17.14	7.09E-06
cg23149249	6	--	-36.76	1.43E-03	-28.63	1.35E-02
cg08818866	2	<i>LTBP1</i>	-27.55	3.65E-05	-29.37	6.85E-05
cg14769121	7	<i>SMURF1</i>	-12.69	1.83E-04	-13.03	3.44E-04
cg25488567	6	<i>CALHM6</i>	-17.32	6.08E-05	-17.32	1.53E-04
cg15344640	5	<i>LMAN2</i>	-24.37	1.07E-04	-26.40	8.81E-05
cg05300717	11	<i>AP5B1</i>	-18.35	2.78E-04	-19.42	1.49E-04
cg08698681	3	<i>TNIK</i>	-16.27	5.33E-05	-17.29	3.62E-05
cg14937228	10	<i>FAM107B</i>	-17.36	1.24E-03	-17.83	1.17E-03

Houseman adjusted for maternal covariates [age at enrollment (continuous), smoking status (never, former, or during pregnancy), education (college / graduate level or not college / graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race / ethnicity (white, black or others), gestational age (continuous)], and cell type proxies estimated for peripheral blood (percentages of monocytes, CD8T cells, CD4T cells, NK cells, and B cells).

ReFACTor adjusted for maternal covariates[age at enrollment (continuous), smoking status (never, former, or during pregnancy), education (college / graduate level or not college / graduate level), atopy history (asthma, eczema or hay fever)], child covariates [sex (female or male), race / ethnicity (white, black or others), gestational age (continuous)], and the first five components estimated from ReFACTor.

Table S9. Genetic control of DNA methylation of top 30 sites identified from the mid-childhood meta-analysis—results from the ARIES mQTL database (1,000 mother-child pairs), where DNA methylation was measured using peripheral blood collected at mid-childhood. We restricted to SNPs with MAF >5% and within 500Kb away from the methylation site.

Atopic sensitization				
CpG	CHR	Gene	cis	No. of SNPs
cg04983687	16	<i>ZFPM1</i>	--	--
cg02427831	19	<i>SIGLEC8</i>	--	--
cg26791242	1	<i>LOC339524</i>	--	--
cg11770323	13	<i>NDFIP2</i>	--	--
cg02970679	17	<i>EPX</i>	--	--
cg13628444	9	<i>MED27</i>	--	--
cg21220721	1	<i>ACOT7</i>	--	--
cg09212118	1	<i>GUCA2A</i>	--	--
cg08698681	3	<i>TNIK</i>	--	--
cg10433549	17	--	--	--
cg26396815	4	<i>BANK1</i>	Yes	29
cg07948085	10	--	Yes	1
cg04406620	16	<i>CYB5B</i>	--	--
cg19434937	12	<i>LPCAT3</i>	--	--
cg07819010	11	--	--	--
cg24491618	7	<i>KCNH2</i>	Yes	1
cg00114012	9	<i>SLC2A8</i>	Yes	2
cg11988722	20	--	--	--
cg09596645	3	--	--	--
cg25173129	17	<i>EPX</i>	--	--
cg06391412	3	<i>FOXP1</i>	--	--
cg10704177	1	<i>PATJ</i>	--	--
cg00944309	5	--	--	--
cg04497992	16	<i>NHLRC4</i>	--	--
cg01916918	8	<i>CLU</i>	--	--
cg11456013	6	--	--	--
cg14769121	7	<i>SMURF1</i>	--	--
cg13054523	17	--	--	--
cg20885063	17	<i>ATPAF2</i>	Yes	4
cg06528816	2	<i>TTC7A</i>	--	--
Environmental allergen sensitization				
CpG	CHR	Gene	cis	No. of SNPs
cg02427831	19	<i>SIGLEC8</i>	--	--
cg04983687	16	<i>ZFPM1</i>	--	--
cg11988722	20	--	--	--

cg02475695	16	<i>NHLRC4</i>	--	--
cg19434937	12	<i>LPCAT3</i>	--	--
cg09212118	1	<i>GUCA2A</i>	--	--
cg02970679	17	<i>EPX</i>	--	--
cg26791242	1	<i>LOC339524</i>	--	--
cg21220721	1	<i>ACOT7</i>	--	--
cg13628444	9	<i>MED27</i>	--	--
cg00114012	9	<i>SLC2A8</i>	Yes	2
cg24491618	7	<i>KCNH2</i>	Yes	1
cg04497992	16	<i>NHLRC4</i>	--	--
cg25173129	17	<i>EPX</i>	--	--
cg18666454	7	<i>KCNH2</i>	--	--
cg07970948	7	<i>ZNF862</i>	--	--
cg17988187	2	<i>LOC100189589</i>	--	--
cg10433549	17	--	--	--
cg10704177	1	<i>PATJ</i>	--	--
cg00944309	5	--	--	--
cg09596645	3	--	--	--
cg12614529	4	<i>MND1</i>	--	--
cg26396815	4	<i>BANK1</i>	Yes	29
cg08698681	3	<i>TNIK</i>	--	--
cg10065736	12	<i>FBXW8</i>	--	--
cg16263722	1	<i>MECR</i>	--	--
cg06528816	2	<i>TTC7A</i>	--	--
cg11456013	6	--	--	--
cg18421167	17	<i>EPX</i>	--	--
cg20151107	10	<i>FAM53B</i>	--	--

Food allergen sensitization				
CpG	CHR	Gene	cis	No. of SNPs
cg13054523	17	--	--	--
cg06906869	13	<i>NEK3</i>	--	--
cg13829849	9	<i>MAMDC2</i>	--	--
cg23021241	1	<i>DMAP1</i>	--	--
cg21919729	8	<i>CTSB</i>	Yes	88
cg11456013	6	--	--	--
cg21220721	1	<i>ACOT7</i>	--	--
cg01916918	8	<i>CLU</i>	--	--
cg18372930	5	--	--	--
cg18543428	13	--	--	--
cg26791242	1	<i>LOC339524</i>	--	--
cg12198633	3	<i>KLHL24</i>	Yes	1

cg12819873	11	<i>PRG2</i>	--	--
cg26396815	4	<i>BANK1</i>	Yes	29
cg17521120	19	<i>SIGLEC8</i>	--	--
cg01901579	14	<i>DICER1</i>	--	--
cg02970679	17	<i>EPX</i>	--	--
cg13562011	9	<i>EXD3</i>	--	--
cg25758167	1	<i>AGL</i>	--	--
cg25738116	3	<i>PA2G4P4</i>	Yes	99
cg08284993	16	<i>ANKRD11</i>	--	--
cg06391412	3	<i>FOXP1</i>	--	--
cg23149249	6	--	--	--
cg08818866	2	<i>LTBP1</i>	--	--
cg14769121	7	<i>SMURF1</i>	--	--
cg25488567	6	<i>CALHM6</i>	--	--
cg15344640	5	<i>LMAN2</i>	--	--
cg05300717	11	<i>AP5B1</i>	--	--
cg08698681	3	<i>TNIK</i>	--	--
cg14937228	10	<i>FAM107B</i>	--	--

Table S10. FDR significant methylation sites from Liang *et al.* (2015) and their corresponding effect estimates and p values in the concurrent meta-analysis.

CpG	CHR	Gene	Atopic sensitization		Environ. sensitization		Food sensitization	
			Estimate	P value	Estimate	P value	Estimate	P value
cg00002426	3	<i>SLMAP</i>	-15.63	3.10E-04	-17.09	7.86E-05	-10.24	9.86E-02
cg00079056	9	<i>SUGT1P1;SPINK4</i>	-15.55	3.54E-03	-19.66	3.27E-04	-8.44	1.94E-01
cg01614759	10	<i>C10orf25;ZNF22</i>	-23.21	3.94E-07	-32.81	2.42E-11	-16.09	8.13E-06
cg01770400	1	<i>SERPINC1</i>	-18.16	3.88E-04	-20.22	9.29E-05	-27.37	1.14E-04
cg01998785	16	<i>LPCAT2</i>	-11.72	1.21E-06	-13.95	1.95E-08	-11.38	6.35E-05
cg02643667	21	<i>TFF1</i>	-1.31	8.48E-01	-3.00	6.68E-01	-19.63	1.28E-01
cg03580247	17	<i>SLC4A1</i>	-1.37	4.70E-01	-2.46	2.24E-01	-2.75	1.99E-01
cg03693099	9	<i>CEL</i>	-13.57	1.03E-06	-18.94	7.30E-11	-10.62	2.06E-03
cg04111761	3	<i>CCR3</i>	-8.18	4.16E-04	-10.20	1.69E-05	-7.49	8.06E-03
cg04523589	3	<i>CAMP</i>	-7.87	4.41E-03	-10.84	1.48E-04	-10.89	2.06E-03
cg05154390	1	<i>MRPS15</i>	-2.21	5.74E-01	-2.65	5.07E-01	2.33	7.60E-01
cg05869585	16	<i>TMEM186;PMM2</i>	-3.90	3.80E-01	-3.64	4.16E-01	-1.10	8.63E-01
cg06690548	4	<i>SLC7A11</i>	-17.72	2.53E-04	-21.19	2.01E-05	-14.87	3.63E-02
cg06736444	16	<i>LOC100128788;SRRM2</i>	-11.49	6.38E-06	-14.67	4.20E-08	-6.52	2.96E-02
cg07374928	11	<i>FAM118B</i>	-3.01	3.39E-02	-0.84	5.45E-01	-1.91	2.26E-01
cg07689731	1	--	-0.04	9.77E-01	-0.68	5.86E-01	-0.25	8.76E-01
cg08377000	4	<i>TIGD2</i>	-3.22	1.08E-01	-4.43	2.76E-02	-5.14	4.78E-02
cg08404225	3	<i>IL5RA</i>	-12.10	1.13E-06	-14.02	3.42E-08	-9.70	2.62E-04
cg09447105	12	<i>PDE6H</i>	-10.57	3.10E-04	-14.42	2.05E-06	-8.31	1.71E-02
cg09676390	21	<i>ADARB1; SSR4P1</i>	-4.72	7.66E-03	-5.31	2.98E-03	-5.63	1.09E-02
cg09793866	8	<i>STAR</i>	-6.03	1.04E-02	-9.35	1.12E-04	-1.82	5.15E-01
cg09914444	1	<i>DMBX1</i>	-4.51	1.34E-02	-4.90	8.04E-03	-1.91	4.00E-01
cg10414946	11	<i>MS4A2</i>	-20.20	2.65E-06	-23.01	1.26E-07	-17.08	7.05E-04
cg10805676	16	<i>TMEM8A;MRPL28</i>	-6.84	1.58E-02	-12.22	3.54E-05	-7.77	3.20E-02
cg11136251	15	<i>ZWILCH;RPL4</i>	-16.23	6.54E-02	-17.05	5.80E-02	6.96	6.38E-01
cg11398517	20	<i>GTSF1L</i>	-5.51	3.11E-02	-10.18	1.32E-04	-4.44	1.73E-01
cg12818699	6	<i>RIPOR2</i>	-17.23	2.83E-06	-20.22	7.32E-08	-15.87	2.02E-04
cg12866859	17	<i>HEXIM1</i>	-5.85	5.70E-02	-7.69	1.48E-02	-3.81	3.23E-01
cg13221796	13	<i>RB1</i>	-11.10	4.64E-06	-13.74	3.79E-08	-8.42	3.59E-03
cg15357945	11	<i>PRG2</i>	-7.68	1.71E-03	-11.75	4.47E-06	-4.28	1.59E-01
cg15996947	14	<i>ATP5S</i>	-22.49	4.17E-03	-21.63	5.77E-03	-41.34	4.46E-03
cg15998761	2	<i>MFSD6</i>	-16.76	5.79E-07	-20.90	1.58E-09	-12.27	6.96E-04
cg16050349	3	<i>PIK3CB</i>	-13.87	3.60E-03	-14.05	4.39E-03	-30.80	1.71E-04
cg16386158	2	<i>IL1RL1</i>	-7.25	3.02E-02	-11.94	6.29E-04	-7.94	3.77E-02
cg16396488	12	<i>PLA2G1B</i>	-8.48	7.38E-05	-10.37	2.47E-06	-5.41	3.08E-02
cg17582777	1	<i>EFNA3</i>	-21.31	3.65E-06	-25.61	5.35E-08	-18.93	3.18E-04
cg17749520	17	<i>ITGA2B</i>	-20.47	2.49E-05	-22.40	5.60E-06	-24.69	2.92E-05
cg17784922	7	<i>KEL</i>	-16.38	2.10E-03	-20.03	2.02E-04	-23.01	8.42E-04

cg17890764	3	<i>ITIH4</i>	-5.89	1.60E-01	-5.42	1.99E-01	-13.65	1.37E-01
cg18254848	19	<i>CLC</i>	-13.40	2.16E-04	-15.19	3.24E-05	-15.20	5.85E-03
cg18783781	1	<i>SLC25A33</i>	-15.13	1.70E-07	-18.36	6.34E-10	-10.19	1.15E-03
cg19881895	11	<i>SLC43A3</i>	-10.97	1.08E-01	-14.09	4.05E-02	-13.43	2.80E-01
cg20503329	9	<i>COL15A1</i>	-27.84	2.23E-06	-31.64	1.09E-07	-35.83	2.06E-04
cg20967028	12	<i>ART4</i>	10.31	1.07E-01	9.40	1.41E-01	14.05	4.40E-01
cg21627181	6	<i>SLC17A4</i>	-18.69	4.57E-08	-22.41	1.57E-10	-16.20	3.74E-05
cg21631409	11	<i>ALDH3B2</i>	-6.33	1.81E-01	-6.45	1.80E-01	-20.00	5.23E-02
cg21682902	12	<i>HAL</i>	-6.84	1.36E-02	-9.22	1.06E-03	-5.10	1.34E-01
cg23064554	1	<i>CTRC</i>	-16.23	4.09E-02	-16.92	3.30E-02	-24.29	3.74E-02
cg23759710	2	<i>OXER1</i>	-10.53	3.38E-02	-10.95	2.86E-02	-11.62	2.40E-01
cg24459209	11	<i>PRG3</i>	-19.13	5.06E-06	-23.24	5.68E-08	-14.46	1.23E-03
cg25494227	12	<i>TMEM52B</i>	-11.53	1.85E-04	-13.22	2.70E-05	-7.30	5.17E-02
cg25636075	3	<i>TMEM41A</i>	-14.90	5.77E-04	-19.34	1.18E-05	-15.83	2.40E-03
cg26136776	19	<i>KLF1</i>	-13.81	4.25E-05	-14.73	1.27E-05	-7.27	7.19E-02
cg26251865	19	<i>IRGC</i>	-4.08	3.02E-02	-5.48	4.39E-03	-1.24	5.88E-01
cg26457013	19	<i>TMEM86B</i>	-10.02	9.75E-04	-16.24	3.92E-07	-2.42	5.25E-01
cg26787239	5	<i>IL4</i>	-17.55	1.14E-06	-22.30	1.82E-09	-15.60	1.65E-04
cg27653134	12	<i>A2ML1</i>	-22.56	5.27E-06	-25.95	1.58E-07	-21.50	3.88E-04

Figure S1. Q-Q plots of meta-analysis results of the association between mid-childhood peripheral blood DNA methylation with mid-childhood allergic phenotypes.

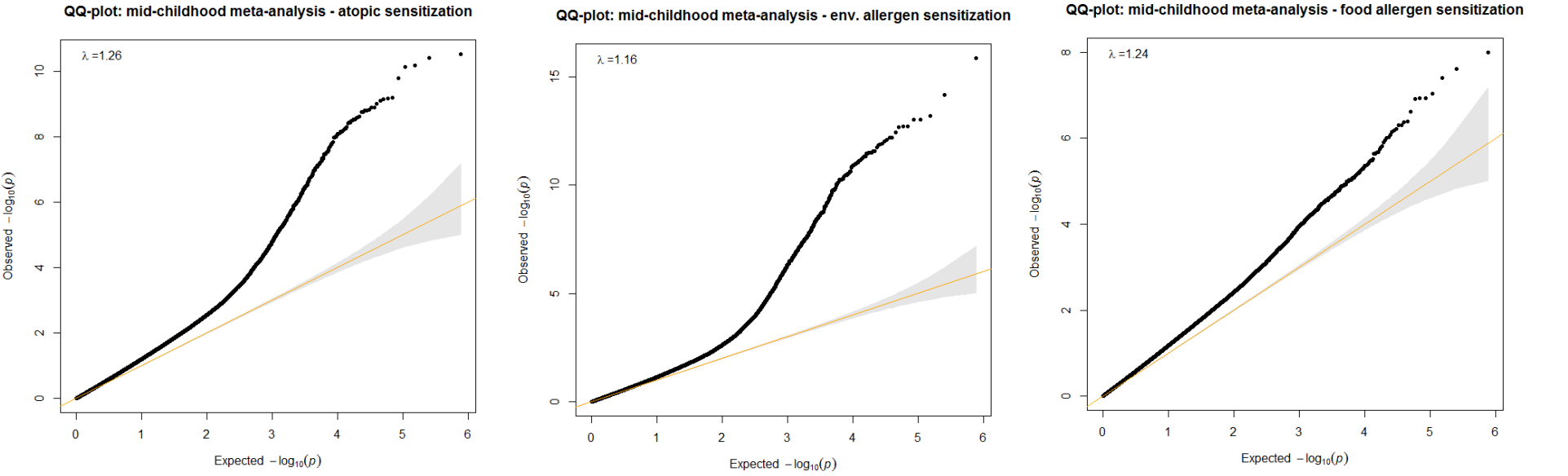


Figure S1 continued. Q-Q plots of meta-analysis results of the association between cord blood DNA methylation with mid-childhood allergic phenotypes.

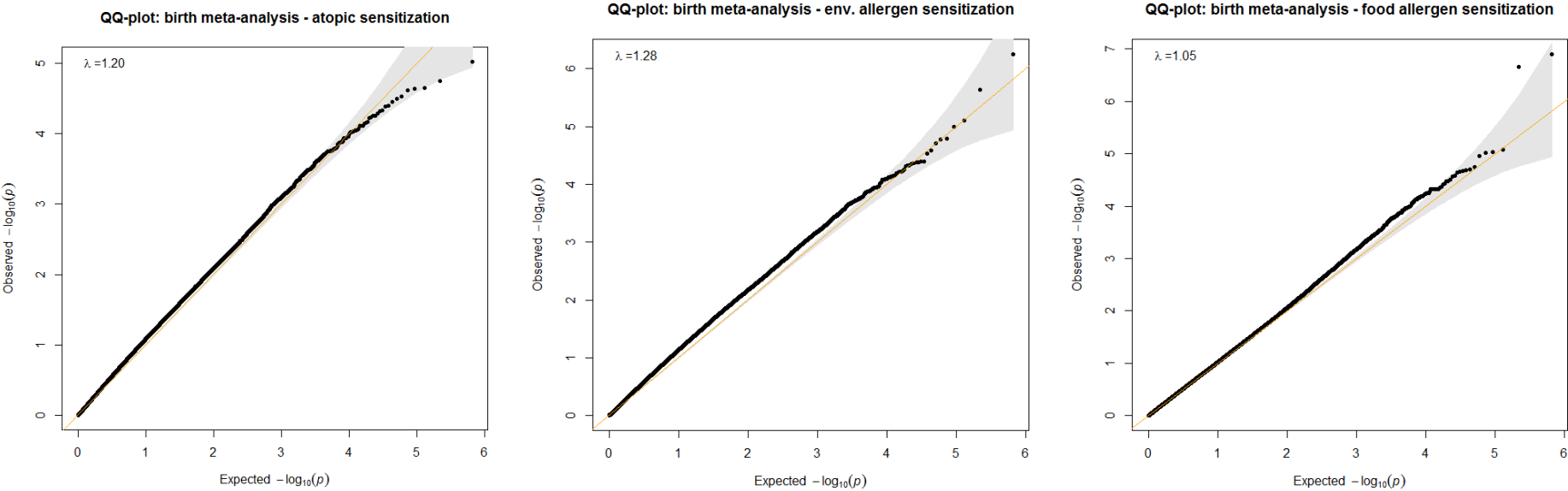


Figure S2. Trajectory plots of methylation changes by environmental allergen sensitization status. Methylation sites were selected as they showed nominal significance in the cord blood analysis, and epigenome-wide significance in the mid-childhood analysis. Top 4 plots: top 4 methylation sites sorted by cord blood p-values based on the criteria described above; bottom 4 plots: methylation sites previously shown to be associated with asthma.

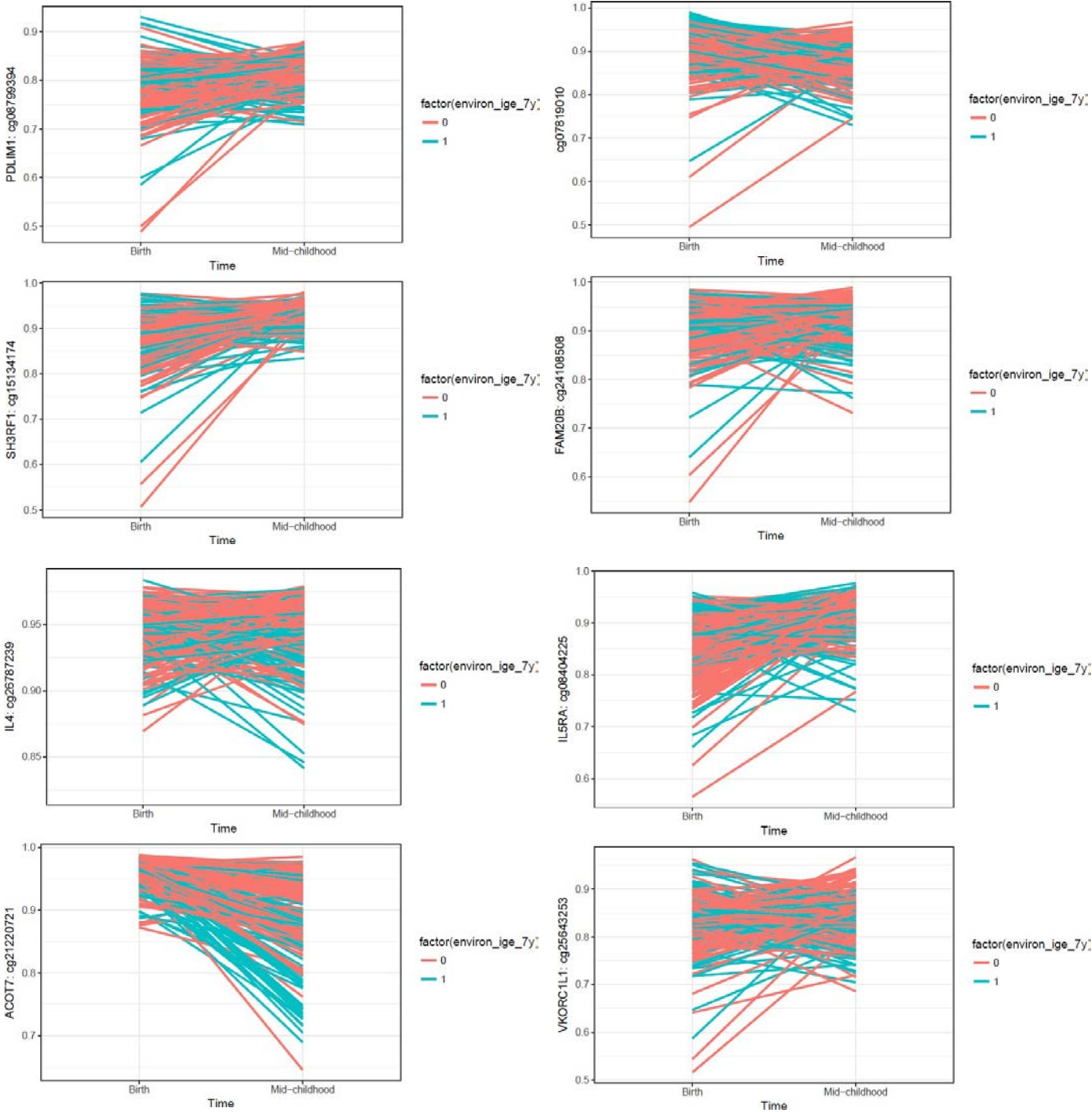


Figure S2 continued. Trajectory plots of methylation changes by food allergen sensitization status. Methylation sites were selected as they showed nominal significance in the cord blood analysis, and epigenome-wide significance in the mid-childhood analysis.

